

Microbiology for laboratory course

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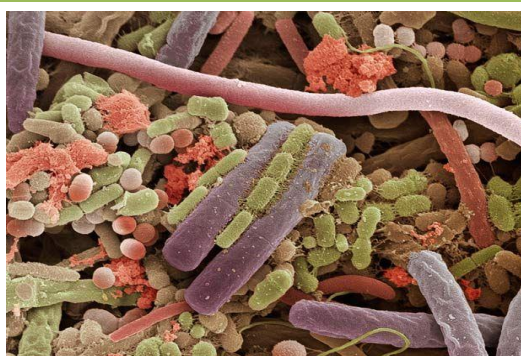
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منهاج دورة المختبرات التأهيلية لفرع الاحياء المجهرية

أشرف

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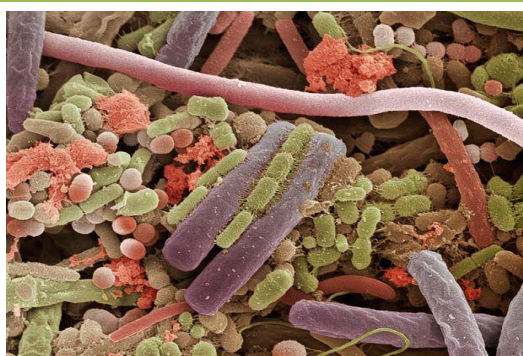
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المقدمة :

من أجل النهوض بالواقع الصحي وتطوير الدورات المختبرية التأهيلية وحرصا منا على أعداد كوادر مختبرية كفوءة في العمل المختبري بما يضمن الارتقاء بمستوى مختبراتنا الطبية الحكومية والاهلية وتطوير العاملين فيها لضمان العمل المختبري الدقيق لإعطاء أفضل النتائج وتقديم افضل الخدمات للمرضى وبما يتناسب مع المعايير القياسية المعتمدة عالميا ، لا يسعنا الى أن نضع بين أيديكم المنهاج الكامل لمادة الاحياء المجهرية وشرح وافى ومفصل ليكون دليل مرجعي لفهم المادة واستيعابها أملين أن تقدم لكم الفائدة العلمية والعملية وسائلين الله تعالى بالموفقة للجميع .

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Introductory Lecture-Part One

Microorganisms can be found in every ecosystem and in close association with every type of multicellular organism.

They populate the healthy human body by the billions as benign passengers (normal flora) and even as participants in bodily functions. Those relatively few species of microorganisms that are harmful to humans, either by production of toxic compounds or by direct infection, are characterized as pathogens.

Most infectious disease is initiated by colonization (the establishment of proliferating microorganisms on the skin or mucous membranes). The major exceptions are diseases caused by introduction of organisms directly into the bloodstream or internal organs.

Microbial colonization may result in:

- 1- elimination of the microorganism without affecting the host
- 2- infection in which the organisms multiply and cause the host to react by making an immune or other type of response
- 3- Transient or prolonged carrier state.

Infectious disease occurs when the organism causes tissue damage and impairment of body function.

All prokaryotic organisms are classified as bacteria, whereas eukaryotic organisms include fungi, protozoa, and helminths as well as humans.

Prokaryotic organisms are divided into two major groups: the ***eubacteria***, which include all bacteria of medical importance, and the ***archaeobacteria***, a collection of evolutionarily distinct organisms.

Most bacteria have shapes that can be described as a rod, sphere, or corkscrew. Prokaryotic cells are smaller than eukaryotic cells. Nearly all bacteria, with the exception of the *Mycoplasma*, have a rigid cell wall surrounding the cell membrane that determines the shape of the organism.

The cell wall also determines whether the bacterium is classified as gram positive or gram negative. External to the cell wall may be flagella, pili, capsule.

Bacterial cells divide by binary fission. However, many bacteria exchange genetic information carried on plasmids (small, specialized genetic elements capable of self-replication) including the information necessary for establishment of antibiotic resistance.

Atypical bacteria include groups of organisms such as *Mycoplasma*, *Chlamydia*, and *Rickettsiae* that, although prokaryotic, lack significant characteristic structural components or metabolic capabilities that separate them from the larger group of typical bacteria.

Fungi are nonphotosynthetic, generally saprophytic, eukaryotic organisms. Some fungi are filamentous and are commonly called ***molds***, whereas others (that is, the ***yeasts***) are unicellular.

Fungal reproduction may be asexual, sexual, or both, and all fungi produce spores. Pathogenic fungi can cause diseases, ranging from skin infections (***superficial mycoses***) to serious, systemic infections (***deep mycoses***).

Protozoa are single-celled, nonphotosynthetic, eukaryotic organisms that come in various shapes and sizes. Many protozoa are free living, but others are among the most clinically important parasites of humans. Members of this group infect all major tissues and organs of the body.

They can be intracellular parasites, or extracellular parasites in the blood, urogenital region, or intestine. Transmission is generally by ingestion of an infective stage of the parasite or by insect bite.

Helminths are groups of worms that live as parasites. They are multicellular, eukaryotic organisms with complex body organization. They are divided into three main groups: **tapeworms** (Cestodes), **flukes** (Trematodes), and **roundworms** (Nematodes).

Helminths are parasitic, receiving nutrients by ingesting or absorbing digestive contents or ingesting or absorbing body fluids or tissues. Almost any organ in the body can be parasitized.

Viruses are obligate intracellular parasites that do not have a cellular structure. Rather, a virus consists of molecule(s) of DNA (**DNA virus**) or RNA (**RNA virus**), **but not both**, surrounded by a protein coat.

A virus may also have an **envelope** derived from the plasma membrane of the host cell from which the virus is released. Viruses contain the genetic information necessary for directing their own replication but require the host's cellular structures and enzymatic machinery to complete the process of their own reproduction.

The fate of the host cell following viral infection ranges from **rapid lysis** and release of many progeny virions to **gradual prolonged release** of viral particles.

The human body is continuously inhabited by many different microorganisms (mostly bacteria, but also fungi and other microorganisms), which, under normal circumstances in a healthy individual, are harmless and may even be beneficial.

These microorganisms are termed "**normal flora**." The normal flora is also termed **commensals**, which literally means "organisms that dine together." Except for occasional transient invaders, **the internal organs and systems are sterile**, including the spleen, pancreas, liver, bladder, central nervous system, and blood.

A healthy newborn enters the world in essentially sterile condition, but, after birth, it rapidly acquires normal flora from food and the environment, including from other humans.

The human microbiome is the total number and diversity of microbes found in and on the human body. In the past, the ability to cultivate organisms from tissues and clinical samples was the gold standard for identification of normal flora and bacterial pathogens.

However, the recent application of culture-independent molecular detection methods based on DNA sequencing indicates that the human body contains a far greater bacterial diversity than previously recognized.

Unlike classic microbiologic culture methods, molecular detection requires neither prior knowledge of an organism nor the ability to culture it. Thus, molecular methods are capable of detecting fastidious and nonculturable species. Even using advanced molecular techniques, it is difficult to define the human microbiome because microbial species present vary from individual to individual as a result of physiologic differences, diet, age, and geographic habitat. Despite these limitations, it is useful to be aware of the dominant types and distribution of resident flora, because such knowledge provides an understanding of the possible infections that result from injury to a particular body site.

Microbial Flora

The most common sites of the body inhabited by normal flora are, as might be expected, those in contact or communication with the outside world, namely, the skin, eye, and mouth as well as the upper respiratory, gastrointestinal, and urogenital tracts.

I- Skin can acquire any bacteria that happen to be in the immediate environment, but this transient flora either dies or is removable by washing. Nevertheless, the skin supports a permanent bacterial population (**resident flora**), residing in multiple layers of the skin. The resident flora regenerates even after vigorous scrubbing.

Estimate of the skin microbiome using classical culture techniques: ***Staphylococcus epidermidis*** and other **coagulase-negative staphylococci (CON)** that reside in the outer layers of the skin appear to account for some 90 % of the skin aerobes. Anaerobic organisms, such as ***Propionibacterium acnes***, reside in deeper skin layers, hair follicles, and sweat and sebaceous glands.

Skin inhabitants are generally harmless, although ***S. epidermidis*** can attach to and colonize plastic catheters and medical devices that penetrate the skin, sometimes resulting in serious bloodstream infections.

While estimate of the skin microbiome using molecular sequencing techniques: The estimate of the number of species present on skin bacteria has been radically changed by the use of the **16S ribosomal RNA gene sequence** to identify bacterial species present on skin samples directly from their genetic material.

Previously, such identification had depended upon microbiological culture, upon which many varieties of bacteria did not grow and so were not detected. ***Staphylococcus epidermidis*** and ***Staphylococcus aureus*** were thought from culture-based research to be dominant. However DNA analysis research finds that, while common, these species make up only 5 % of skin bacteria. The skin apparently provides a rich and diverse habitat for bacteria.

II- Eye

The conjunctiva of the eye is colonized primarily by ***S. epidermidis***, followed by ***S. aureus***, aerobic ***Corynebacteria*** (diphtheroids), and ***Streptococcus pneumoniae***. Tears, which contain the antimicrobial enzyme **lysozyme**, help, limit the bacterial population of the conjunctiva.

III- Buccal cavity and Nasal passages

The mouth and nose harbor many microorganisms, both aerobic and anaerobic. Among the most common are diphtheroids (aerobic ***Corynebacterium*** species), ***S. aureus***, and ***S. epidermidis***. In addition, the teeth and surrounding gingival tissue are colonized by their own particular species, such as ***Streptococcus mutans***.

S. mutans can enter the bloodstream following dental surgery and colonize damaged or prosthetic heart valves, leading to potentially fatal infective endocarditis.

Some normal residents of the **nasopharynx** can also cause disease like ***S. pneumoniae***, found in the nasopharynx of many healthy individuals, can cause acute **bacterial pneumonia**, especially in older adults and those whose resistance is impaired (***Pneumonia is frequently preceded by an upper or middle***

respiratory viral infection, which predisposes the individual to S. pneumoniae infection of the pulmonary parenchyma))

IV- Gastrointestinal tract

In an adult, the density of microorganisms in the stomach is relatively low (10^3 to 10^5 per gram of contents) due to gastric enzymes and acidic pH.

The density of organisms increases along the alimentary canal, reaching 10^8 to 10^{10} bacteria per gram of contents in the ileum and 10^{11} per gram of contents in the large intestine.

Some 20 % of the fecal mass consists of many different species of bacteria, more than 99 % of which are anaerobes. **Bacteroides** species constitute a significant percentage of bacteria in the large intestine. **Escherichia coli**, a facultative anaerobic organism, constitutes less than 0.1 % of the total population of bacteria in the intestinal tract. However, this **endogenous E. coli** is a major cause of **urinary tract infections (UTIs)**.

V- Urogenital tract

The low pH of the adult vagina is maintained by the presence of **Lactobacillus** species, which are the primary components of normal flora. If the Lactobacillus population in the vagina is decreased (for example, by antibiotic therapy), the pH rises, and potential pathogens can overgrow.

The most common example of such overgrowth is the yeast-like fungus, **Candida albicans** which itself is a minor member of the normal flora of the vagina, mouth, and small intestine. **The urine in the kidney and bladder is sterile** but can become contaminated in the lower urethra by the same organisms that inhabit the outer layer of the skin and perineum.

Normal flora benefits

Normal flora can provide some definite benefits to the host:

- ✚ First, the sheer number of harmless bacteria in the lower bowel and mouth make it unlikely that, in a healthy person, an invading pathogen could compete for nutrients and receptor sites.
- ✚ Second, some bacteria of the bowel produce antimicrobial substances to which the producers themselves are not susceptible.
- ✚ Third, bacterial colonization of a newborn infant acts as a powerful stimulus for the development of the immune system.
- ✚ Fourth, bacteria of the gut provide important nutrients, such as vitamin K, and aid in digestion and absorption of nutrients; although humans can obtain vitamin K from food sources, bacteria can be an important supplemental source if nutrition is impaired.

Microbial flora problems

Clinical problems caused by normal flora arise in the following ways:

- ✚ The organisms are displaced from their normal site in the body to an abnormal site. An example is the introduction of the normal skin bacterium, **S. epidermidis**, into the bloodstream where it can colonize catheters and heart valves, resulting in bacterial endocarditis.

✚ Potential pathogens gain a competitive advantage due to diminished populations of harmless competitors; when normal bowel flora are depleted by antibiotic therapy leading to overgrowth by the resistant *Clostridium difficile*, which can cause severe colitis.

✚ Harmless, commonly ingested food substances are converted into carcinogenic derivatives by bacteria in the colon; like the conversion by bacterial sulfatases of the sweetener cyclamate into the bladder carcinogen cyclohexamine.

✚ When individuals are immunocompromised, normal flora can overgrow and become pathogenic ((Colonization by normal, but potentially harmful, flora should be distinguished from the carrier state in which a true pathogen is carried by a healthy (asymptomatic) individual and passed to other individuals where it results in disease)). ***Typhoid fever is an example of a disease that can be acquired from a carrier***

Microorganism's pathogenesis

A pathogenic microorganism is defined as one that is capable of causing disease. Some microorganisms are unequivocally pathogenic, whereas others (the majority) are generally harmless.

An organism may invade an individual without causing infectious disease when the host's defense mechanisms are successful.

The occurrence of such asymptomatic infections can be recognized by the presence of antibody against the organism in the patient's serum. Some infections result in a ***latent state***, meaning that the organism is dormant but may be reactivated with the recurrence of symptoms. Moreover, some pathogens cause disease only under certain conditions (for example, being introduced into a normally sterile body site or infection of an immunocompromised host).

Bacterial mediated pathogenesis

The mechanism of infectious process may vary among bacteria, the methods by which bacteria cause disease can, in general, be divided into several stages. Pathogenicity of a microorganism depends on its success in completing some or all of these stages. The terms “***virulence***” and “pathogenicity” are often used interchangeably. However, virulence can be quantified by how many organisms are required to cause disease in 50 percent of those exposed to the pathogen (ID₅₀, where I = Infectious and D = Dose), or to kill 50 percent of test animals (LD₅₀, where L = Lethal).

The number of organisms required to cause disease varies greatly among pathogenic bacteria, as less than 100 *Shigella* cause diarrhea by infecting the gastrointestinal (GI) tract, whereas the infectious dose of *Salmonella* is approximately 100,000 organisms.

The infectious dose of a bacterium depends primarily on its virulence factors. The probability that an infectious disease occurs is influenced by both the number and virulence of the infecting organisms and the strength of the host immune response opposing infection.

Virulence factors are those characteristics of a bacterium that enhance its pathogenicity, that is, properties that enable a microorganism to establish itself and replicate on or within a specific host; they are as follows:

1. *Entry into the host*

The first step of the infectious process is the entry of the microorganism into the host by one of several ports: by the respiratory, GI, or urogenital tract or through skin that has been cut, punctured, or burned.

Once entry is achieved, the pathogen must overcome diverse host defenses before it can establish itself. These include phagocytosis; the acidic environments of the stomach and urogenital tract; and various hydrolytic and proteolytic enzymes found in the saliva, stomach, and small intestine.

Bacteria that have an outer **polysaccharide capsule** (for example, *Streptococcus pneumoniae* and *Neisseria meningitidis*) have a better chance of surviving these primary host defenses.

2. *Adherence to host cells*

Some bacteria (e.g. *Escherichia coli*) use pili to adhere to the surface of host cells. Group A *Streptococci* have similar structures (fimbriae). Other bacteria have cell surface adhesion molecules or particularly hydrophobic cell walls that allow them to adhere to the host cell membrane.

The adherence enhances virulence by preventing the bacteria from being carried away by mucus or washed from organs with significant fluid flow, such as the urinary and the GI tracts. Adherence also allows each attached bacterial cell to form a **microcolony**. An example of the importance of adhesion is that of *Neisseria gonorrhoeae* in which strains that lack pili are not pathogenic.

3. *Invasiveness*

Invasive bacteria are those that can enter host cells or penetrate mucosal surfaces, spreading from the initial site of infection. Invasiveness is facilitated by several bacterial enzymes, the most notable of which are **collagenase** and **hyaluronidase**. These enzymes degrade components of the extracellular matrix, providing the bacteria with easier access to host cell surfaces.

Many bacterial pathogens express membrane proteins known as "**invasins**" that interact with host cell receptors, thereby eliciting signaling cascades that result in bacterial uptake by induced phagocytosis. Invasion is followed by inflammation, which can be either **pyogenic** (involving pus formation) or **granulomatous** (having nodular inflammatory lesions), depending on the organism.

The pus of pyogenic inflammations contains mostly neutrophils, whereas granulomatous lesions contain fibroblasts, lymphocytes, and macrophages.

4. *Iron sequestering*

Iron is an essential nutrient for most bacteria. To obtain the iron required for growth, bacteria produce iron-binding compounds, called **siderophores**. These compounds capture iron from the host by chelation, and then the ferrated siderophores binds to specific receptors on the bacterial surface. Iron is actively transported into the bacterium, where it is incorporated into essential compounds such as cytochromes.

The pathogenic *Neisseria* species are exceptions in that they do not produce siderophores but instead utilize host iron-binding proteins, such as **transferrin** and **lactoferrin**, as iron sources.

Virulence factors that inhibit phagocytosis

1. The most important antiphagocytic structure is the capsule external to the cell wall, such as in *S. pneumoniae* and *N. meningitidis*.

2. The second group of antiphagocytic factors is the cell wall proteins of gram-positive cocci, such as protein A of *Staphylococcus* and M protein of group A *Streptococci*.

5- Bacterial toxins

Some bacteria cause disease by producing toxic substances, of which there are two general types: **exotoxins** and **endotoxin**. Exotoxins, which are proteins, are secreted by both gram-positive and gram-negative bacteria.

In contrast, endotoxin, which is **lipopolysaccharide (LPS)**, is not secreted but instead is an integral component of the cell walls of gram-negative bacteria.

a. Exotoxins: These include some of the most poisonous substances known. It is estimated that as little as 1 µg of **tetanus exotoxin** can kill an adult human. Exotoxin proteins generally have two polypeptide components. One is responsible for binding the protein to the host cell, and one is responsible for the toxic effect.

In several cases, the precise target for the toxin has been identified. For example, **diphtheria toxin** is an enzyme that blocks protein synthesis. It does so by attaching an adenosine diphosphate-ribosyl group to human protein elongation factor EF-2, thereby inactivating it. Most exotoxins are rapidly inactivated by moderate heating (60° C), notable exceptions being *Staphylococcal enterotoxin* and *E. coli* heat-stable toxin (ST).

Also, treatment with dilute formaldehyde destroys the toxic activity of most exotoxins but does not affect their antigenicity.

Formaldehyde-inactivated toxins, called **toxoids**, are useful in preparing **vaccines**.

Exotoxin proteins are, in many cases, encoded by genes carried on plasmids or temperate **bacteriophages**. An example is the diphtheria exotoxin that is encoded by the **tox gene** of a temperate bacteriophage that can lysogenize *Corynebacterium diphtheriae*. Strains of *C. diphtheriae* that carry this phage are pathogenic, whereas those that lack the phage are nonpathogenic.

b. Endotoxins: These are heat-stable, LPS components of the outer membranes of gram-negative (but not gram-positive) bacteria. They are released into the host's circulation following bacterial cell lysis.

LPS consists of polysaccharide composed of repeating sugar subunits (**O antigen**), which protrudes from the exterior cell surface; a core polysaccharide; and a lipid component called **lipid A** that is integrated into the outer leaflet of the outer membrane.

The lipid A is responsible for the toxicity of this molecule. The main physiologic effects of LPS endotoxin are fever, shock, hypotension, and thrombosis, collectively referred to as **septic shock**. These effects are produced indirectly by macrophage activation, with the release of **cytokines**, activation of complement, and activation of the coagulation cascade. Death can result from multiple organ failure.

Elimination of the causative bacteria with antibiotics can initially exacerbate the symptoms by causing sudden massive release of endotoxin into the circulation. Although gram-positive bacteria do not contain LPS, their cell wall **peptidoglycan and teichoic acids** can elicit a shock syndrome similar to that caused by LPS but usually not as severe.

Host mediated pathogenesis

The pathogenesis of many bacterial infections is caused by the host response rather than by bacterial factors. Examples of host response-mediated pathogenesis are seen in diseases such as ***gram-negative bacteria sepsis, tuberculosis, and tuberculoid leprosy***.

The tissue damage in these infections is caused by various cytokines released from the lymphocytes, macrophages, and polymorphonuclear leukocytes at the site of infection or in the bloodstream. Often the host response is so intense that host tissues are destroyed, allowing remaining bacteria to proliferate.

Another microbial virulence factor is the ***antigenic variation*** as a successful pathogen must evade the host's immune system that recognizes bacterial surface antigens. One important evasive strategy for the pathogen is to change its surface antigens.

This is accomplished by several mechanisms. One mechanism, called ***phase variation***, is the genetically reversible ability of certain bacteria to turn off and turn on the expression of genes coding for surface antigens. The second mechanism, called ***antigenic variation***, involves the modification of the gene for an expressed surface antigen by genetic recombination with one of many variable unexpressed DNA sequences. In this manner, the expressed surface antigen can assume many different antigenic structures.

Introductory Lecture-Part Two

Identifying the organism causing an infectious process is usually essential for effective antimicrobial and supportive therapy.

Initial treatment may be empiric, based on the microbiologic epidemiology of the infection and the patient's symptoms. Definitive microbiologic diagnosis of an infectious disease usually involves one or more of the following **five basic laboratory techniques**:

- 1- Direct microscopic visualization of the organism
- 2- Cultivation and identification of the organism
- 3- Detection of microbial antigens
- 4- Detection of microbial DNA or RNA
- 5- Detection of an inflammatory or host immune response to the microorganism

A clinical history is the most important part of patient evaluation. For example, a history of cough points to the possibility of respiratory tract infection, whereas dysuria (painful or difficult urination) suggests urinary tract infection.

A history of travel to developing countries may implicate exotic organisms. For example, a patient who recently swam in the Nile has an increased risk of ***schistosomiasis***. Patient occupations may suggest exposure to certain pathogens, such as ***brucellosis*** in a butcher or ***anthrax*** in farmers. Even the age of the patient can sometimes guide the clinician in predicting the identity of pathogens. For example, a gram positive coccus in the spinal fluid of a newborn infant is unlikely to be ***Streptococcus pneumoniae*** (***Pneumococcus***) but most likely to be ***Streptococcus agalactiae*** (group B) which is sensitive to penicillin G.

By contrast, a gram-positive coccus in the spinal fluid of a 40-year-old patient is most likely to be ***S. pneumoniae***. This organism is frequently resistant to penicillin G and requires treatment with a third generation cephalosporin (such as Cefotaxime or ceftriaxone) or vancomycin.

In many infectious diseases, pathogenic organisms (excluding viruses) can often be directly visualized by microscopic examination of patient specimens, such as sputum, urine, and CSF.

The organism's microscopic morphology and staining characteristics can provide the first screening step in arriving at a specific identification. The organisms to be examined do not need to be alive or able to multiply. Microscopy yields rapid and inexpensive results and may allow the clinician to initiate treatment without waiting for the results of a culture, as in the spinal fluid specimens.

Gram stain

As unstained bacteria are difficult to detect with the light microscope, most patient material is stained prior to microscopic evaluation. The most common and useful staining procedure is the Gram stain, which separates bacteria into two classifications according to their cell wall composition. If a clinical specimen on a microscope slide is treated with a solution of crystal violet and then iodine, the bacterial cells will stain purple. If the stained cells are then treated with a solvent, such as alcohol or acetone, gram positive organisms retain the stain, whereas gram-negative species lose the stain, becoming colorless.

Addition of the counter stain safranin stains the clear, gram-negative bacteria pink or red. Most, but not all, bacteria are stainable and fall into one of these two groups ((microorganisms that lack cell walls, such as *Mycoplasma*, cannot be identified using the Gram stain)).

Gram stain applications

The Gram stain is important therapeutically because gram-positive and gram-negative bacteria differ in their susceptibility to various antibiotics, and the Gram stain may, therefore, be used to guide initial therapy until the microorganism can be definitively identified.

In addition, the morphology of the stained bacteria can sometimes be diagnostic. For example, **gram-negative intracellular diplococci** in urethral pus provide a **presumptive** diagnosis of **gonorrhea**. Gram stains of specimens submitted for culture are often invaluable aids in the interpretation of culture results. For example, a specimen may show organisms under the microscope but appear sterile in culture media. This discrepancy may suggest the presence of either **fastidious organisms (bacteria with complex nutrient requirements)** that are unable to grow on the culture media employed or fragile organisms, such as **gonococcus** or **anaerobic organisms**, which may not survive transport.

In these cases, direct visualization with the Gram stain may provide the only clue to the nature, variety, and relative number of infecting organisms.

Gram stain limitation is due to the need for high number of microorganisms required to be of benefit; as visualization with the Gram stain requires greater than 10^4 organisms /ml of the specimen.

Liquid samples with low numbers of microorganisms (for example, in CSF), require centrifugation to concentrate the pathogens, the sediment is then examined after staining.

Acid-fast stain

Stains such as **Ziehl-Neelsen** (the classic acid-fast stain) are used to identify organisms that have **waxy material (mycolic acids)** in their cell walls. Most bacteria that have been stained with carbol- fuchsin can be decolorized by washing with acidic alcohol. Certain acid-fast bacteria retain the carbol-fuchsin stain after being washed with an acidic solution. The most clinically important acid-fast bacterium is **Mycobacterium tuberculosis**, which appears pink, often beaded, and slightly curved. Acid fast staining is reserved for clinical samples from patients suspected of having **Mycobacterium infection**.

Indian ink stain

This is one of the simplest microscopic methods. It is useful in detecting **Cryptococcus neoformans** in CSF. One drop of centrifuged CSF is mixed with one drop of India ink on a microscope slide beneath a glass cover slip. **Cryptococcus** is identified by their large, transparent capsules that displace the India ink particles.

Potassium hydroxide preparation

Treatment with potassium hydroxide (KOH) dissolves host cells and bacteria, sparing fungi. One drop of sputum or skin scraping is treated with 10 % KOH, and the specimen is examined for fungal forms.

Bacterial Culturing

Culturing is routine for most bacterial and fungal infections but is rarely used to identify helminths or protozoa. Culturing of many pathogens is straight forward, for example, streaking a throat swab onto a blood agar plate in search of *group A β -hemolytic Streptococcus*.

Certain pathogens are very slow growing (for example, *M. tuberculosis*). Microorganisms isolated in culture are identified using such characteristics as *colony size, shape, color, and Gram stain, hemolytic reactions* on solid media, *odor*, and *metabolic properties*, also pure cultures provide samples for *antimicrobial susceptibility testing*.

The success of culturing depends on *appropriate collection* and *transport techniques* and on *selection of appropriate culture media*, because some organisms may require special nutrients. A. *Specimen collection*
Many organisms are fragile and must be transported to the laboratory with minimal delay. For example, *Gonococci* and *Pneumococci* are very sensitive to heating and drying. Samples must be cultured promptly, or, if this is not possible, transport media must be used to extend the viability of the organism to be cultured. When anaerobic organisms are suspected, the patient's specimen must be protected from the toxic effect of oxygen.

All clinically important bacteria are *heterotrophs* (that is, they require organic carbon for growth). Heterotrophs may have complex or simple requirements for organic molecules ((Organisms that can reduce carbon dioxide and, therefore, do not require organic compounds for cell growth, are called *autotrophs*)).

Most bacteria require varying numbers of growth factors, which are organic compounds required by the cell to grow, but which the organism cannot itself synthesize (for example, vitamins). Organisms that require either a large number of growth factors or must be supplied with very specific ones are referred to as *fastidious*.

Bacteria can be categorized according to their growth responses in the presence and absence of oxygen. *Strict aerobes* cannot survive in the absence of oxygen and produce energy only by *oxidative phosphorylation*.

Strict anaerobes generate energy by fermentation or by anaerobic respiration and are killed in the presence of oxygen.

Facultative anaerobes can grow in the absence of oxygen but grow better in its presence. *Aerotolerant anaerobes* have mechanisms to protect themselves from oxygen (therefore, being able to grow in its presence or absence) but do not use oxygen in their metabolism.

Microaerophiles require oxygen for their metabolism but cannot survive at atmospheric levels of oxygen, microaerophiles are found in lakes and wet soil where the oxygen concentration is within an acceptable range.

Two general strategies are used to isolate pathogenic bacteria, depending on the nature of the clinical sample:

- 1-First method uses *enriched media* to promote the nonselective growth of any bacteria that may be present
- 2-Second approach employs *selective media* that only allow growth of specific bacterial species from specimens that normally contain large numbers of bacteria (for example, stool, genital tract secretions, and

sputum). Isolation of a bacterium is usually performed on solid medium. **Liquid medium is used to grow larger quantities of a culture of bacteria that have already been isolated as a pure culture.**

❖ **Enriched media:** Media fortified with blood, yeast extracts, or brain or heart infusions are useful in growing fastidious organisms. **Blood agar** contains protein sources, sodium chloride, and 5 % blood and supports the growth of most gram-positive and gram-negative bacteria isolated from human sources. ***Haemophilus influenzae*** and ***Neisseria gonorrhoeae*** are highly fastidious organisms. They require **chocolate agar**, which contains red blood cells (RBCs) that have been lysed.

This releases intracellular nutrients, such as **hemoglobin**, **hemin (“X” factor)**, and **nicotinamide adenine dinucleotide (“V” factor)**, required by these organisms.

Enriched media are useful for culturing normally sterile body fluids, such as blood and CSF, in which the finding of any organisms provides reasonable evidence for infection by that organism. Failure to culture an organism may indicate that:

- 1- The culture medium is inadequate
- 2- The incubation conditions do not support bacterial growth

❖ **Selective media:** The most commonly used selective medium is **MacConkey agar**, which supports the growth of most gram-negative rods, especially the Enterobacteriaceae, but inhibits growth of gram-positive organisms and some fastidious gram-negative bacteria, like *Haemophilus* and *Neisseria* species.

Growth on blood agar and chocolate agar but not MacConkey agar suggests a gram-positive isolate or a fastidious gram-negative species. On the other hand, most gram-negative rods often form distinctive colonies on MacConkey agar. This agar is also used to detect organisms able to metabolize lactose.

Clinical samples are routinely plated on blood agar, chocolate agar, and MacConkey agar. **Hektoen enteric agar** is also a selective medium that differentiates lactose / sucrose fermenters and non-fermenters as well as **H₂S producers** and non-producers. It is often used to culture **Salmonella** and **Shigella** species.

Thayer-Martin agar is another selective medium composed of chocolate agar supplemented with several antibiotics that suppress the growth of nonpathogenic *Neisseria* and other normal and abnormal flora. This medium is normally used to isolate *Gonococci*.

When submitting samples for culture, the physician must alert the laboratory to likely pathogens whenever possible, especially when unusual organisms are suspected. This allows inclusion of selective media that might not be used routinely.

Bacterial Identification

The most widely used identification scheme involves determining the morphologic and metabolic properties of the unknown bacterium and comparing these with properties of known microorganisms. Alternate identification schemes using nucleic acid-based methods are used also. It is essential to start identification tests with pure bacterial isolates grown from a single colony.

**Single enzyme test for bacterial identification**

Different bacteria produce varying spectra of enzymes; e.g., some enzymes are necessary for the bacterium's individual metabolism, and some facilitate the bacterium's ability to compete with other bacteria or establish an infection.

Tests that measure single bacterial enzymes are simple, rapid, and generally easy to interpret. They can be performed on organisms already grown in culture and often provide presumptive identification.

- 1) **Catalase test:** The enzyme catalase catalyzes the degradation of hydrogen peroxide to water and molecular oxygen ($\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2$). Catalase positive organisms rapidly produce bubbles when exposed to a solution containing hydrogen peroxide. The catalase test is key in differentiating between many gram-positive organisms; e.g., *Staphylococci* are catalase positive, whereas *Streptococci* and *Enterococci* are catalase negative. The production of catalase is an important virulence factor because H_2O_2 is antimicrobial, and its degradation decreases the ability of neutrophils to kill invading bacteria.
- 2) **Oxidase test:** The enzyme *cytochrome c oxidase* is part of electron transport and nitrate metabolism in some bacteria. The enzyme can accept electrons from artificial substrates (such as a *phenylenediamine derivative*), producing a dark, and oxidized product. This test assists in differentiating between groups of gram-negative bacteria. *Pseudomonas aeruginosa*; e.g., is oxidase positive.
- 3) **Urease:** The enzyme urease hydrolyzes urea to ammonia and carbon dioxide ($\text{NH}_2\text{CONH}_2 + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2$). The ammonia produced can be detected with pH indicators that change color in response to the increased alkalinity. The test helps to identify certain species of *Enterobacteriaceae*, *Corynebacterium urealyticum*, and *Helicobacter pylori*.
- 4) **Coagulase test:** Coagulase is an enzyme that causes a clot to form when bacteria are incubated with plasma. The test is used to differentiate *Staphylococcus aureus* (coagulase positive) from coagulase-negative *Staphylococci*.

**Automated systems for bacterial identification**

Microbiology laboratories are increasingly using automated methods to identify bacterial pathogens; as in the **Vitek System**, small plastic reagent cards containing micro liter quantities of various biochemical test media in 30 wells provide a biochemical profile that allows for organism identification. An inoculum derived from cultured samples is automatically transferred into the card, and a photometer intermittently measures color changes in the card that result from the metabolic activity of the organism.

The data are analyzed, stored, and printed in a computerized database. There are many commercial variants of these automated systems and several can be used for simultaneous identification and antimicrobial susceptibility determination.

**Tests based on the presence of metabolic pathways (API; analytical Profile Index)**

These tests measure the presence of metabolic pathways in a bacterial isolate, rather than a single enzyme. Commonly used assays include those for oxidation and fermentation of different

carbohydrates, the ability to degrade amino acids, and use of specific substrates. A widely used manual system for rapid identification of members of the family *Enterobacteriaceae* and other gram-negative bacteria makes use of twenty micro tubes containing substrates for various biochemical pathways. The test substrates in the micro tubes are inoculated with the bacterial isolate to be identified, and, after 5 hours incubation, the metabolic profile of the organism is constructed from color changes in the micro tubes. These color changes indicate the presence or absence of the bacteria's ability to metabolize a particular substrate. The results are compared with a data bank containing test results from known bacteria. The probability of a match between the test organism and known pathogens is then calculated.



Immunological bacterial identification

In the diagnosis of infectious diseases, immunologic methods take advantage of the specificity of **antigen-antibody binding**, as known antigens and antibodies are used as diagnostic tools in identifying microorganisms.

Serologic detection of a patient's immune response to infection, or antigenic or nucleic acid evidence of a pathogen in a patient's body fluids, is frequently useful. Immunologic methods are **useful when the infecting microorganism is difficult or impossible to isolate or when a previous infection needs to be documented**.

I- Detection of microbial antigen with known antiserum

These methods of identification are often rapid and show favorable sensitivity and specificity; unlike microbial culturing techniques, these immunologic methods do not permit further characterization of the microorganism, such as determining its antibiotic sensitivity or characteristic metabolic patterns:

1. Quellung reaction: Some bacteria having capsules can be identified directly in clinical specimens by a reaction that occurs when the organisms are treated with serum containing specific antibodies. The Quellung reaction **makes the capsule more refractile and thus more visible, but the capsule does not actually swell**. This method can be used for all serotypes of *S. pneumoniae*, *H. influenzae type b*, and *Neisseria meningitidis* groups A and C.

2. Slide agglutination test: Some microorganisms, such as *Salmonella* and *Shigella* species, can be identified by **agglutination (clumping)** of a suspension of bacterial cells on a microscopic slide. Agglutination occurs when a specific antibody directed against the microbial antigen is added to the suspension, causing cross-linking of the bacteria.

II-Identification of serum antibodies

Detection in a patient's serum of antibodies that are directed against microbial antigens provides evidence for a current or past infection with a specific pathogen; and it is characterized by:

- 1) Antibody may not be detectable early in an infection
- 2) The presence of antibodies in a patient's serum cannot differentiate between a present and a prior infection
- 3) A significant rise in antibody titer over a 10 to 14-day period does distinguish between a present or prior infection

Techniques such as **complement fixation** and **agglutination** can be used to quantitate antimicrobial antibodies.

1. Complement fixation: It is the older method but still useful method for detecting serum antibody directed against a specific pathogen employs the ability of antibody to bind complement. A patient's serum is first incubated with antigen specific for the suspected infectious agent, followed by the addition of complement. If the patient's serum does contain immunoglobulin (**IgG or IgM**) that target the specific antigen (**indicating past or current infection**), then the added complement will be sequestered in an **antigen–antibody–complement complex** ("**complement fixation**").

Then the sensitized (**antibody-coated**) **indicator sheep RBCs** are added to the solution. If complement has been fixed (because the patient's serum contained antibodies against the added antigen), then little complement will be available to bind to the antibody–RBC complexes, and the cells will not lyse.

If complement has not been depleted by initial antigen–antibody complexes (because the patient's serum does not contain antibodies to the specific antigen), the complement will bind to the antibody–RBC complexes, causing the cells to lyse. **As hemolyzed RBCs release hemoglobin, the reaction can be monitored with a spectrophotometer.**

2. Direct agglutination: Direct bacterial agglutination testing is sometimes ordered when a suspected pathogen is difficult or dangerous to culture in the laboratory. This test measures the ability of a patient's serum antibody to directly agglutinate specific killed (yet intact) microorganisms. This test is used to evaluate patients suspected of being infected by **Brucella abortus** or **Francisella tularensis**.

3. Direct hemagglutination: Antibodies directed against RBCs can arise during the course of various infections; such antibodies are typically found during **infectious mononucleosis caused by Epstein-Barr virus**. When uncoated (native) animal or human RBCs are used in agglutination reactions with serum from a patient infected with such an organism, antibodies to RBC antigens can be detected. The patient's antibodies cause the RBCs to clump. This test is, therefore, a direct hemagglutination reaction. In the case of some diseases, including pneumonia caused by **Mycoplasma pneumoniae**, IgM auto antibodies may develop that agglutinate human RBCs at 4° C but not at 37° C (**termed the "cold agglutinins" test**))

Other tests used to identify serum antigens or antibodies

1. Latex agglutination test:

Latex and other particles can be readily coated with either antibody (for antigen detection) or antigen (for antibody detection). Addition of antigen to antibody-coated latex beads causes agglutination that can be visually observed; such methods are used to rapidly test CSF for antigens associated with common forms of **bacterial or fungal meningitis**. When antigen is coated onto the latex bead, antibody from a patient's serum can be detected. Latex agglutination tests are widely used for the identification of **β -hemolytic Streptococci group A**.

2. Enzyme-linked immunosorbent assay:

Elisa is a diagnostic technique in which antibody specific for an antigen of interest is bound to the walls of a plastic micro titer well. Patient serum is then incubated in the wells, and any antigen in the serum is bound by the antibody on the well walls. The wells are then washed, and a second antibody is added. This one is also specific for the antigen but recognizes epitopes different from those bound by the first antibody. After incubation, the wells are again washed, removing any unattached antibody.

Attached to the second antibody is an enzyme, which, when presented with its substrate, produces a colored product, the intensity of the color produced being proportional to the amount of bound antigen. Elisa can also be used to detect or quantitate antibody in a patient's serum; the wells are coated with antigen specific for the antibody in question. The patient's serum is allowed to react with the bound antigen, the wells are washed, and a secondary antibody (that recognizes the initial antibody) conjugated to a color product-producing enzyme is added to the well. After a final washing, substrate for the bound enzyme is added to the well, and the intensity of the colored product can be measured.

3. Fluorescent-antibody tests: Organisms in clinical samples can be detected directly by specific antibodies coupled to a fluorescent compound such as **fluorescein**. In the direct Immunofluorescence antibody technique, a sample of concentrated body fluid (like CSF or serum), tissue scraping (like skin), or cells in tissue culture is incubated with a **fluorescein-labeled antibody** directed against a specific pathogen.

The labeled antibody bound to the microorganism **absorbs ultraviolet light** and emits visible fluorescence that can be detected using a **fluorescence microscope**. A variation of the technique, the indirect Immunofluorescence antibody technique, involves the use of two antibodies. The first is unlabeled antibody (**the target antibody**), which binds a specific microbial antigen in a sample; and this clinical sample is subsequently stained with a fluorescent antibody that recognizes the target antibody. Because a number of labeled antibodies can bind to each target antibody, the fluorescence from the stained microorganism is intensified.

Nucleic acid based tests

The most widely used methods for detecting microbial DNA fall into three categories:

- 1) *Direct hybridization (non-amplified assay)*
- 2) *Amplification methods using the polymerase chain reaction (PCR)*
- 3) *DNA microarrays*

Although not likely to completely replace culture techniques in the near future, nucleic acid-based tests for the diagnosis of infectious diseases are gaining wider acceptance.

Direct detection of pathogens without target amplification

This highly specific method of pathogen detection involves identification of the DNA of the pathogen in a patient sample or, more commonly, organisms isolated in culture. The basic strategy is to detect a relatively short sequence of nucleotide bases of DNA sequence) that is unique to the pathogen. This is done by hybridization with a probe, a single-stranded piece of DNA (usually labeled with a fluorescent molecule) containing a complementary sequence of bases. In bacteria, DNA sequences coding for **16S ribosomal RNA sequences (rRNA)** are commonly used targets because each microorganism contains

multiple copies of its specific 16S rRNA gene, thereby increasing the sensitivity of the assay. When the probe is bound to the target, the label will give off a signal after the free probe is washed away. A limitation of standard direct probe hybridization is the requirement for a 10^4 or greater number of copies of target nucleic acid for detection.

Nucleic acid amplification for diagnosis

Nucleic acid amplification overcomes the principal limitation of direct detection with nucleic acid probes by selectively amplifying specific DNA targets present in low concentrations. The bacterial 16S rRNA gene has emerged as the most useful marker for microbial detection and identification. Ribosomal DNA genes contain highly conserved areas (***that are used as targets for primers***) separated by internal transcribed sequences containing variable, species-specific regions. These sequences are like fingerprints. Comparing certain locations on a 16s rRNA gene with a database of known organisms allows the identification of organisms. For virus detection, primers are constructed to target highly conserved DNA or RNA sequences unique to the pathogen. Amplification and detection of the viral genomes are highly sensitive and are especially valuable ***when the viral load is too low to be detected by culture or when results are needed rapidly.***

Conventional polymerase chain reaction

DNA polymerase repetitively amplifies targeted portions of DNA (ideally sequences that are highly conserved and unique to the pathogen). Each cycle of amplification doubles the amount of DNA in the sample, leading to an ***exponential increase in DNA*** with repeated cycles of amplification. The amplified DNA sequence can then be analyzed by gel electrophoresis, Southern blotting, or direct sequence determination.

Real-time polymerase chain reaction (RT-PCR)

This variant of PCR combines nucleic acid amplification and fluorescent detection of the amplified product in the same closed automated system. Real-time PCR limits the risk of contamination and provides a rapid (30-40 minutes) diagnosis. Real-time PCR ***is a quantitative method*** and allows the determination of the concentration of pathogens in various samples.

Advantages of polymerase chain reaction:

- 1- Methods employing nucleic acid amplification techniques have a major advantage over direct detection with nucleic acid probes because amplification methods allow specific DNA or RNA target sequences of the pathogen to be amplified millions of times without having to culture the microorganism itself for extended periods
- 2- PCR also permits identification of non-cultivable or slow-growing organisms, such as *Mycobacteria*, anaerobic bacteria, and viruses
- 3- Nucleic acid amplification methods are sensitive, specific for the target organism, and are unaffected by the prior administration of antibiotics
- 4- Nucleic acid amplification techniques are generally quick, easy, and accurate
- 5- They are useful in the detection of organisms that require complex media or cell cultures or prolonged incubation times

PCR amplification is limited by the occurrence of spurious false-positives due to cross-contamination with other microorganisms' nucleic acid. PCR tests are often costly and require skilled personnel

DNA microarrays

Although microarrays are now routinely used to measure gene expression, the technique is an emerging technology in the diagnostic microbiology laboratory. Microarrays have the unprecedented potential to simultaneously detect and identify many pathogens from the same specimen. For example, an oligonucleotide microarray targeting the 16S rRNA gene has been developed for the detection of a panel of forty predominant human intestinal bacterial pathogens in human fecal samples. DNA microarray consists of microscopic spots of immobilized DNA oligonucleotide, each containing specific DNA sequences, **known as probes**. The probes are constructed to be complementary to specific gene sequences of interest in suspected pathogens. DNA of the microorganism obtained from a clinical specimen, **known as the target**, is extracted and amplified using PCR and fluorescent labeling techniques. The target DNA is exposed to the probe microarray. If the labeled DNA from the microorganism and the immobilized probe has a complementary base sequence, they will hybridize, thereby increasing fluorescence intensity. After washing off of nonspecific bonding sequences only strongly paired strands will remain hybridized and fluoresce. The intensity of fluorescence at each spot is a measure of the amount of that particular microbial DNA in the sample. Correlating fluorescence with the identity of the probe allows for the detection and quantitation of specific pathogens.

Sterilization and Disinfection

Sterilization is the process of killing or removing all viable organisms achieved by: physical and chemical

Disinfection is the process of removing or killing most but not all, viable organisms

Methods:

1. **Chemical** the substance is called a disinfectant it kills pathogens but may not kill viruses or spores
2. **Physical process** boiling or low pressure steam it reduces only the bioburden

Antiseptics: A particular group of disinfectants which used to reduce the number of viable organisms in the skin; that act differentially on organism and host tissue

1. **Germicide** is a chemical agent capable of killing microbes
2. **Sporicide** is a germicide capable of killing bacterial spores

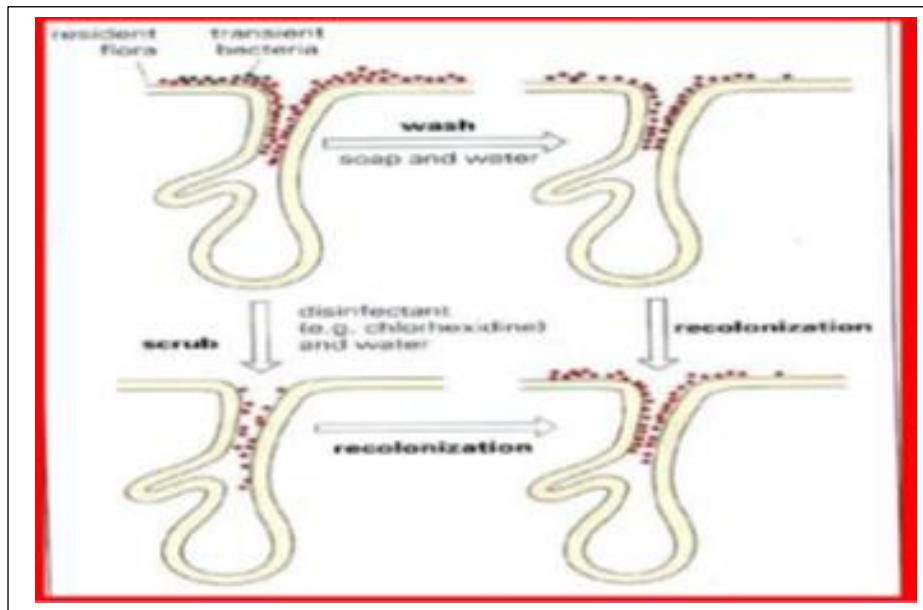


Figure shows the Antiseptics effect on normal skin flora

Pasteurization: it reduces the total number of viable microbes in bulk fluids such as milk and fruit juices without destroying flavor and palatability.

Decision whether to use Sterilization or Disinfection it depended upon:

- ❖ Cost
- ❖ Damage involved
- ❖ Low bioburden is a prerequisite for cost-effective sterilization.

Uses of sterilization and disinfection

1. Prevention of hospital infection:

- a. sterile equipments, instruments and dressings
- b. isolation facilities
- c. safe disposal of infected materials

2. Microbiologists: production of sterile media and the laboratory activities

3. Central to almost all areas of medical practice like surgery (from hand washing to needles and prosthesis)

Process: The rate of killing of microorganisms depends upon the concentration of the killing agent and time of exposure.

$$N = 1/CT$$

N – Number of survivors

C – Concentration of agent

T – Time of exposure to the agent

Mechanisms of action of antimicrobial agents:

1. Damage cell membrane
2. Denature proteins
3. Modify functional groups of proteins and nucleic acids

Activity of a particular disinfectant may result from one or combination of pathways.

Factors affecting efficacy:

1. Physical environment
2. Presence of moisture
3. Temperature and pH
4. Concentration of the agent
5. Hardness of water
6. Bioburden and the object
7. Mature and state of microbes in bioburden
8. Ability of microbes to inactivate the chemical agent

Techniques for Sterilization

I. Heat the preferred choice because of:

- a. ease of use
- b. controllability
- c. low cost
- d. efficiency

Types:

1. Dry heat

- Incineration
- use of Bunsen burner
- Hot air oven (one hour) 160-180 °C / 1 hour.

2. Moist heat under pressure

a- Autoclave the most effective for sterilization (121 °C / 15 minutes) in which the pressure aids in penetration; in sterilization of surgical instruments, dressing and heat resistant pharmaceuticals or culture media

b- Boiling water for a few minutes can be used as a rapid emergency measure to disinfect instruments; it kills vegetative bacteria but not all spores

c- Pasteurization: done at 62.8 – 65.6°C for 30 minutes, which used for fluids as it reduce the number of bacteria and eliminate pathogen present in small numbers

Flash pasteurization (71.7 °C for 15 seconds). After the process, the fluid should be kept at a temperature below 10°C to minimize, subsequent bacterial growth.

II. Irradiation: it include gamma and X-rays

Gamma irradiation which used for sterilizing large batches of small volume items such as:

1. Needles, syringes, catheters, gloves
2. Vaccines
3. To prevent food spoilage
4. Capital cost is high but process is 100% efficient
5. Killing mechanism involves production of free radicals that break the bonds in DNA
6. Sporocidal at higher doses (4.5 megarads)

III- Filtration

- 1-Used to produce particles and pyrogen-free fluid.
- 2-Composed of nitrocellulose
- 3-Work by electrostatic attraction and physical pore size
- 4-Purify drinking water
- 5-To recover very small number of organism from large volumes of fluid
- 6-Can be used for quantitating bacteria in fluids

Disinfection by chemical agents

1- Alkylating agents which include gases as

a- ethylene oxide (toxic and explosive)

b-Formaldehyde (extremely unpleasant odor; and irritant to mucous membrane decontaminate rooms and exhaust protective cabinets)

2- Liquid

I- Glutaraldehyde - disinfect heat-sensitive articles (endoscopes surfaces)

II- Oxidizing agents

1-Hydrogen peroxide (H₂O₂)

❖ 3 – 6% kills most bacteria

❖ 10-25% kills all including spores

❖ (Disinfects plastic implants, contact lenses and surgical prosthesis)

III-Halogens it includes:

a- Iodine compounds

1-Most effective, virtually effective among all organisms including spore- formers and *Mycobacterium*

2-Effective in acid pH because more free iodine is liberated

3-Acts more rapidly than other halogens and quaternary NH₄ (ammonium) compounds

4- Effectivity reduced by serum, feces, body fluids.

Povidone iodine used to disinfect metal surfaces and tissues.

b-Chlorine compounds

It available in 3 forms:

✓ Cl₂

✓ HOCl (hypochlorous acid)

✓ OCl (hypochlorite)

It has a good germicide activity, though spores are resistant to it.

IV. Phenolic compounds

a. rarely used as disinfectant nowadays

b. historical interest it was used as comparative standard for assessing the activity of germicidal compounds

V- Quaternary Ammonium compounds which are organic groups linked to nitrogen (Benzalkonium chloride and Cetyl pyridinium chloride. Bacteriostatic at low concentrations, bactericidal at high. Not effective against *Pseudomonas*, *Mycobacterium* and *Trichophyton*)

VI-Alcohols

- Germicidal activity increases with increasing chain length (5-8 carbons)
- Most commonly used – ethanol and isopropanol
- No activity against spores
- Activity is more active in the presence of water hence 70% alcohol is more active than 95% alcohol.
- Common disinfectants used for skin surfaces
- Extremely effective when followed by treatment with iodophor

VII- Heavy metals

✓ Soluble salts of Hg (mercury), arsenic, silver and other heavy metals

✓ By forming mercaptides with sulfhydryl groups of cysteine residue

✓ It examples as mercurials – merthiolate, mercurochrome

On the other hand the silver compounds – irritant and caustic effect; silver nitrate (propylaxis); sulfadiazine cream, colloidal silver compounds used in ophthalmology.

- **Antimicrobial agents** Chemotherapy: The use of drugs to treat a disease
- Antimicrobial drugs: Interfere with the growth of microbes within a host
- Antibiotic: It is of biological origin and Produced by a microbe, in order to inhibits other microbes
- Chemotherapeutic agent: synthetic chemicals
- Selective toxicity: Drug kills pathogens without damaging the host
- Therapeutic index: ratio between toxic dose and therapeutic dose or ratio of LD₅₀ (lethal) to ED₅₀ (effective).

- Antimicrobial action – either Bacteriostatic (which inhibit microbes without destruction) or bactericidal (destruct microbes and lyses them)
- *Some factors effect antibiotics action in our body like:*

Tissue distribution, metabolism, and excretion, passage through blood brain barriers (BBB); Unstable in acid; half-life duration or it shelf life.

Selection of antibiotics

Factors to take into consideration:

- The identity of the infecting organism.
- Drug sensitivity of the infecting organism
- Host factors (i.e. site of the infection, status of host defenses).
- **Empiric therapy prior to completion of lab tests: it may be necessary to begin treatment in patients with serious infections BEFORE the lab results.**
- **Take samples for culture PRIOR TO INITIATION of treatment**

HOST FACTORS

- ✓ Host defenses (immune system and phagocytic cells).
- ✓ Site of infection .To be effective an antibiotic must be present in the site of infection in a concentration greater than MIC

(Endocarditis, meningitis, abscesses)

- ✓ Age (infants and elderly highly vulnerable to drug toxicity).
- ✓ Pregnancy and lactation
- ✓ Previous allergic reactions
- ✓ Genetic factors (i.e. hemolysis in patients with G-6PD deficiency if given sulfonamides).

Antibiotic combinations:

The result may be additive, potentiative or antagonistic.

Additive response: one in which the antimicrobial effect of the combination is equal to the sum of the effects of the two drugs alone.

Potentiative interaction: one in which the effect of the combination is GREATER than the sum of the effects of the individual agents.

Antagonistic response: in certain cases the combination of two antibiotics may be less effective than one of the agents by itself (i.e. combination of a bacteriostatic with a bactericidal drug)

Disadvantages of antibiotic combinations

- 1) Increased risk of toxic and allergic reactions
- 2) Possible antagonism of antimicrobial effects
- 3) Increased risk of suprainfection

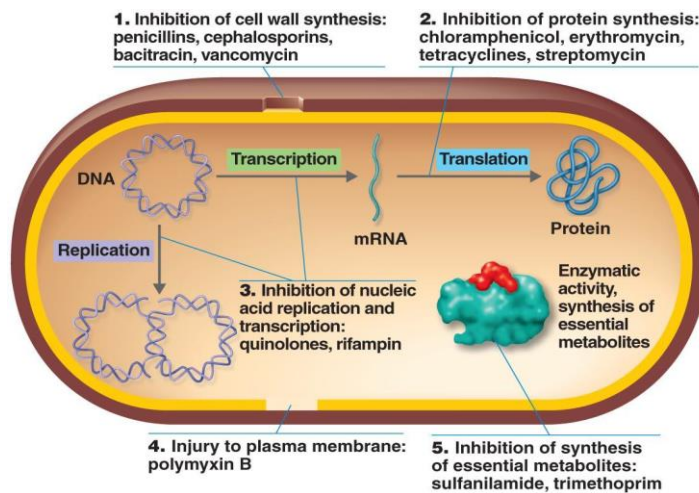


Figure shows the four main Actions of Antimicrobial Drugs

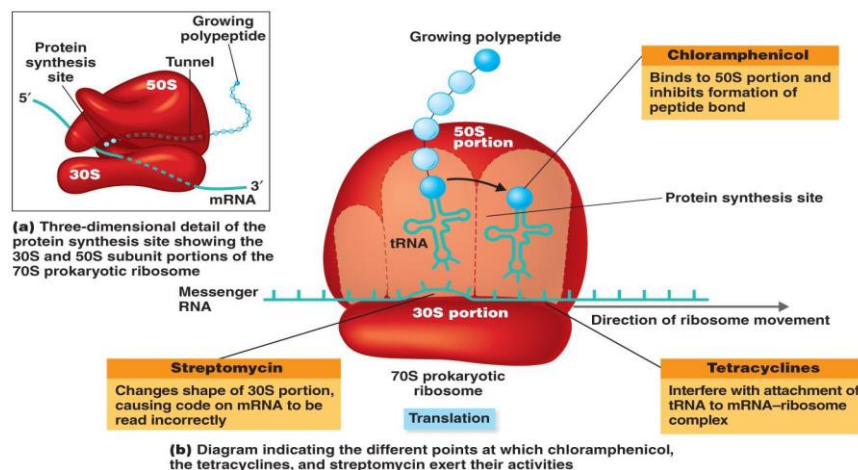


Figure shows the Inhibition of Protein Synthesis by Antibiotics

Mechanism of Action Drugs

☒ Inhibition of Cell Wall Synthesis

- ✚ Inhibit cross-linking of peptidoglycan by inactivating transpeptidases (PBPs)

Penicillins, Cephalosporins, Aztreonam, Imipenem

- ✚ Bind to terminal D-ala-D-ala & prevent incorporation into growing peptidoglycan

Vancomycin, Teicoplanin

- ✚ Inhibition of transglycosylation

Oritavancin, Teicoplanin, lipophilic vancomycin analogs, ramoplanin

- ✚ Inhibit dephosphorylation of phospholipid carrier in peptidoglycan structure

Bacitracin

- Prevents incorporation of D-alanine into peptidoglycan

Cycloserine

- Inhibition of Protein Synthesis**

- Bind to 50S ribosomal subunit

Macrolides, Chloramphenicol, Clindamycin

- Bind to 30S ribosomal subunit

Aminoglycosides, Tetracyclines

- Inhibition of Nucleic acid synthesis**

- Inhibition of DNA gyrase & topoisomerase

Quinolones

- Inhibition of nucleic acid biosynthesis

Flucytosine, Griseofulvin

- Inhibition of mRNA synthesis

Rifampin, Rifabutin, Rifapentine

- Alteration of Cell Membrane Function**

- Inhibition of ergosterol biosynthesis

Imidazole antifungals

- Bind to membrane sterols

Polymyxins, Amphotericin B, Nystatin

- Alteration of Cell Metabolism**

- Inhibition of tetrahydrofolic acid production (cofactor for nucleotide synthesis)

Sulfonamides, Trimethoprim, Trimetrexate Pyrimethamine

- Inhibition of mycolic acid biosynthesis

Isoniazid

- Interference with ubiquinone biosynthesis & cell respiration

Atovaquone

- Bind to macromolecules (Metronidazole, Nitrofurantoin)

Antibacterial Antibiotics Inhibitors of Cell Wall Synthesis

Since the gram-positive cell wall contains only two major components it is much less complicated than the gram-negative cell wall.

Teichoic acids are polymers that are interwoven in the peptidoglycan layer and extend as hair-like projections beyond the surface of the gram-positive cell. They also are major surface antigens in those organisms that possess them.

The **peptidoglycan layer**, or murein layer, of gram-positive bacteria is much thicker than that of gram-negative bacteria. It is responsible for maintaining the shape of the organism and often is referred to as the cell wall.

Penicillin is natural and semi synthetic penicillins contain β -lactam ring and natural penicillins produced by *Penicillium* are effective against Gram + cocci and spirochetes while semi synthetic penicillins: made in laboratory by adding different side chains onto β -lactam ring will lead to production of penicillin which are resistant and broader spectrum of activity

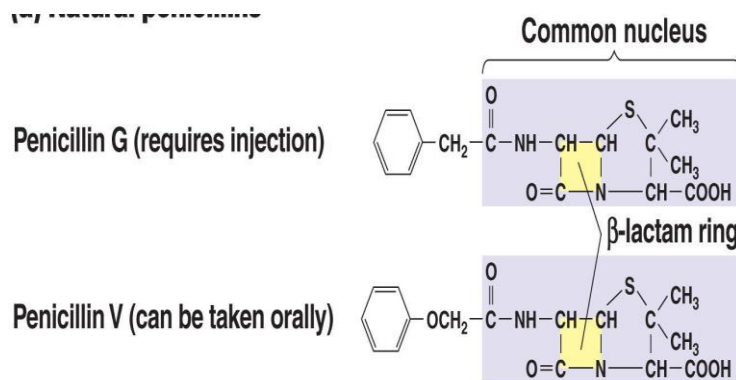


Figure shows The Penicillins nucleus morphology

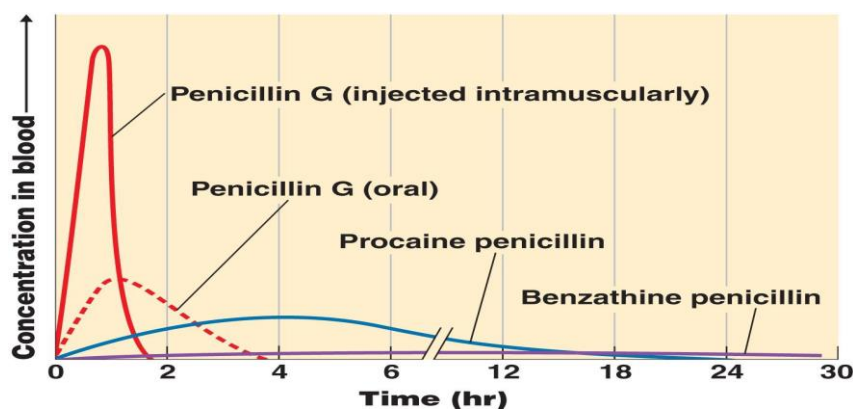


Figure shows The Penicillins Activity Cycle in the body

Penicillinase (β -lactamase): bacterial enzyme that destroys natural penicillins

Penicillinase resistant penicillins: methicillin replaced by oxacillin and nafcillin due to MRSA

Extended-spectrum penicillin: Ampicillin, amoxicillin; new: carboxypenicillins and ureidopenicillins (also good against *P. aeruginosa*)

Cephalosporin

Produced by fungi of genus *Cephalosporium*, its structure and mode of action resembles penicillin, there are 4 generations of cephalosporin

1. First-generation: Narrow spectrum, gram-positive
2. Second-generation: Extended spectrum includes gram-negative
3. Third-generation: Includes pseudomonads; mostly injected some oral.
4. Fourth-generation: Most extended spectrum

Cephalosporins are:

1. More stable to bacterial lactamase than penicillin
2. Broader spectrum and used against penicillin-resistant strains

Vancomycin

- ❖ It is a glycopeptide from *Streptomyces*
- ❖ Inhibition of cell wall synthesis
- ❖ Used to kill MRSA
- ❖ Emerging Vancomycin resistance: VRE and VRSA

Tetracyclines

A number of antibiotics, including tetracyclines, aminoglycosides, and macrolides, exert antimicrobial effects by targeting the bacterial ribosome, which has components that differ structurally from those of the mammalian cytoplasmic ribosomes. Binding of tetracyclines to the 30S subunit of the bacterial ribosome is believed to block access of the amino acyl-tRNA to the mRNA-ribosome complex at the acceptor site, thereby inhibiting bacterial protein synthesis.

Tetracyclines are broad-spectrum antibiotics (that is, many bacteria are sensitive to these drugs). Tetracyclines are generally bacteriostatic.

Aminoglycosides

Aminoglycosides inhibit bacterial protein synthesis. Susceptible organisms have an oxygen-dependent system that transports the antibiotic across the cell membrane. All aminoglycosides are bactericidal. They are effective only against aerobic organisms because anaerobes lack the oxygen-requiring transport system. Gentamicin is used to treat a variety of infectious diseases including those caused by many of the Enterobacteriaceae and, in combination with penicillin, endocarditis caused by viridans-group *Streptococci*.

Macrolides

Macrolides are a group of antibiotics with a macrocyclic lactone structure. Erythromycin was the first of these to find clinical application, both as the drug of first choice and as an alternative to penicillin in individuals who are allergic to β -lactam antibiotics. Newer macrolides, such as clarithromycin and azithromycin, offer extended activity against some organisms and less severe adverse reactions.

The macrolides bind irreversibly to a site on the 50S subunit of the bacterial ribosome, thereby inhibiting the translocation steps of protein synthesis. Generally considered to be bacteriostatic, they may be bactericidal at higher doses.

Fluoroquinolones

Fluoroquinolones uniquely inhibit the replication of bacterial DNA by interfering with the action of DNA gyrase (topoisomerase II) during bacterial growth. Binding Quinolones to both the enzyme and DNA to form a ternary complex inhibits the rejoining step, and, thus, can cause cell death by inducing cleavage of the DNA.

Because DNA gyrase is a distinct target for antimicrobial therapy, cross-resistance with other more commonly used antimicrobial drugs is rare but is increasing with multidrug-resistant organisms.

All of the Fluoroquinolones are bactericidal.

Carbapenems

Carbapenems are synthetic β -lactam antibiotics that differ in structure from the penicillins. Imipenem, meropenem, doripenem, and ertapenem are the drugs of this group currently available. Imipenem is compounded with cilastatin to protect it from metabolism by renal dehydropeptidase. Imipenem resists hydrolysis by most β -lactamases.

This drug plays a role in empiric therapy because it is active against β -lactamase-producing gram-positive and gram-negative organisms, anaerobes, and *P. aeruginosa*.

Meropenem and doripenem have antibacterial activity similar to that of Imipenem. However, ertapenem is not an alternative for *P. aeruginosa* coverage because most strains exhibit resistance.

Ertapenem also lacks coverage against *Enterococcus* species and *Acinetobacter* species.

Trimethoprim-sulfamethoxazole

A combination called co-trimoxazole shows greater antimicrobial activity than equivalent quantities of either drug used alone. The synergistic antimicrobial activity of Co-trimoxazole results from its inhibition of two sequential steps in the synthesis of tetrahydrofolic acid: sulfamethoxazole inhibits incorporation of PABA into folic acid, and trimethoprim prevents reduction of dihydrofolate to tetrahydrofolate. It is

effective in treating urinary tract infections and respiratory tract infections as well as in *Pneumocystis jiroveci* pneumonia and ampicillin- and chloramphenicol-resistant systemic *Salmonella* infections.

It has activity versus methicillin-resistant *S. aureus* and can be particularly useful for community acquired skin and soft tissue infections caused by this organism.

Drug Resistance

Intrinsic and acquired drug resistance modes:

In some species antimicrobial resistance is an **intrinsic or innate** property. For example, *E. coli* is intrinsically resistant to Vancomycin because Vancomycin is too large to pass through porin channels in their outer membrane.

Gram-positive bacteria, on the other hand, do not possess an outer membrane and thus are not intrinsically resistant to Vancomycin. Bacteria also can **acquire resistance** to antimicrobial agents by genetic events such as mutation, conjugation, transformation, transduction and transposition.

Mutation: Chromosomal resistance develops as a result of spontaneous mutation in a locus that controls susceptibility to a given antimicrobial agent. Spontaneous mutation occurs at a relatively low frequency but, when the bacteria are exposed to the antibiotic, only the mutant cell survives. It then multiplies and gives rise to a resistant population. Spontaneous mutations may also occur in plasmids. For example, mutations in plasmids containing genes for beta-lactamase enzymes can result in altered beta-lactamases often with extended activity.

Conjugation: Bacteria often contain extrachromosomal genetic elements called plasmids, many of which carry genes for antimicrobial resistance. When two bacterial cells are in close proximity, a bridge-like structure known as a pilus forms between them. This allows a copy of the plasmid as it is replicated, to be transferred to another cell. The result is a bacterium that expresses the antimicrobial resistance encoded in the plasmid.

Transformation: Bacteria may encounter naked fragments of DNA that carry antimicrobial resistance genes. These fragments are taken into the cell by a process called transformation. The DNA fragment is incorporated into the host cell chromosome by recombination and the resulting cell is resistant.

Transduction: When bacterial viruses (bacteriophage) are multiplying in the cytoplasm of a bacterium, fragments of DNA from plasmids or chromosomes may by chance be packaged in a viral coat and enter another host cell. When the fragments contain genes for resistance to an antimicrobial agent they can confer resistance in the new host cell.

Transposition: Specialized genetic sequences known as transposons are “mobile” sequences that have the capability of moving from one area of the bacterial chromosome to another or between the chromosome and plasmid or bacteriophage DNA. Since transposon DNA can carry genes for antimicrobial resistance they have contributed to the development of plasmids encoding genes for multiple antibiotic resistances. Some transposons are capable of moving from one bacterium to another without becoming incorporated into a chromosome, a plasmid or a bacteriophage.

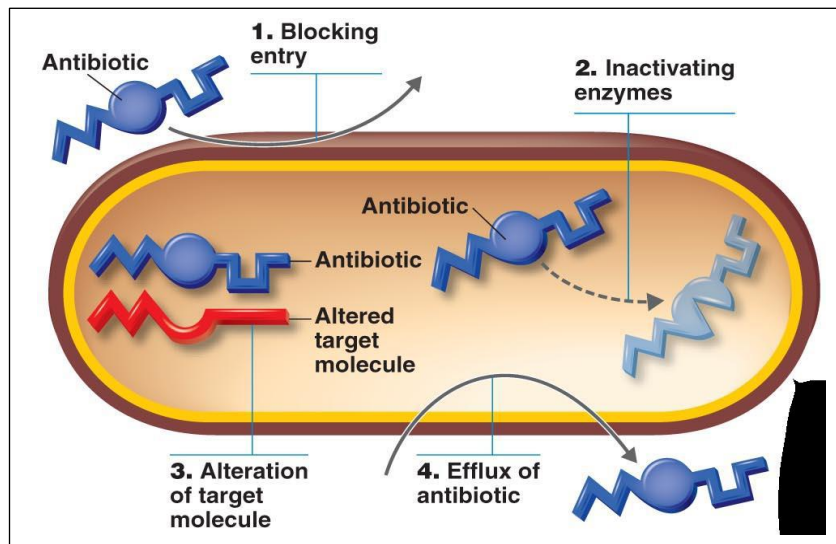
NOTES:

- ✓ Penicillin G resistance of *S. aureus* from 3% to > 90%
- ✓ Multidrug-resistant *S. aureus* = MRSA
- ✓ Vancomycin-resistance
- ✓ *Evolution of drug resistance:*
- ✓ Vertical evolution due to spontaneous mutation
- ✓ Horizontal evolution due to gene transfer

A variety of mutations can lead to antibiotic resistance

1. Enzymatic destruction of drug
2. Prevention of penetration of drug
3. Alteration of drug's target site
4. Rapid ejection of the drug

Resistance genes are often on plasmids or transposons that can be transferred between bacteria.



Misuse of antibiotics selects for resistance mutants; Misuse includes

- ☒ Using outdated or weakened antibiotics

- ☒ Using antibiotics for the common cold and other inappropriate conditions
- ☒ Using antibiotics in animal feed
- ☒ Failing complete the prescribed regimen
- ☒ Using someone else's leftover prescription

Classification of the Antibiotics

I- β -Lactam antibiotics which includes:

First: Penicillin which includes:-

1- Benzylpenicillins like

Penicillin G (benzylpenicillin sodium, procaine benzylpenicillin, benzathine penicillin)

2- Phenoxy-penicillins (oral penicillins) like Penicillin V , Propicillin

3- Penicillinase resistant penicillins (anti-staphylococcal penicillins) like Oxacillin, Dicloxacillin, Flucloxacillin

4- Amino benzyl penicillins like Ampicillin, Amoxicillin

5- Ureidopenicillins (broad-spectrum penicillins) like Mezlocillin, Piperacillin

6- β -Lactam inhibitors like Ampicillin with or without sulbactam, Amoxicillin with or without clavulanate, Piperacillin with or without tazobactam

Second: Cephalosporins which includes:-

1- (First generation) Cephalosporins like

✓ Cefazolin

✓ Cefalexin (oral)

✓ Cefadroxil (oral)

2- (Second generation) Cephalosporins like

✓ Cefuroxime

✓ Cefotiam

✓ Cefuroxime axetil

✓ Cefaclor (oral)

✓ Loracarbef

3- (third and fourth generation)

✓ Cefotaxime

✓ Ceftriaxone

✓ Ceftazidime

✓ Cefepime

✓ Cefixime (oral)

✓ Cefpodoxime

- ✓ Proxetil (oral)
- ✓ Ceftibuten (oral)

Third: other like

- ❖ Monobactams like Aztreonam
- ❖ Carbapenems like Imipenem, Meropenem, Ertapenem, and Doripenem
- ❖ β -Lactamase inhibitors like Clavulanic acid, Sulbactam, Tazobactam

II- Other drugs un related to β -lactam ring drugs like:

- 1- Aminoglycosides like Streptomycin, Gentamicin, Tobramycin, Netilmicin, and Amikacin
- 2- Tetracyclines like Tetracycline, long acting Doxycycline, oxytetracycline, and Minocycline
- 3- Quinolones or Fluoroquinolones like

Group I: Norfloxacin

Group II: Enoxacin, Ofloxacin, Ciprofloxacin

Group III: Levofloxacin

Group IV: Moxifloxacin

The usage of Quinolones groups in clinical situations:

I: Indications essentially limited to UTI

II: Widely indicated

III: Improved activity against Gram-positive and atypical pathogens

IV: Further enhanced activity against Gram-positive and atypical pathogens, also against anaerobic bacteria

- 4- Lincosamides like Clindamycin
- 5- Azol derivatives like Miconazole, Ketoconazole, Fluconazole, Itraconazole, Voriconazole, and Posaconazole
- 6- Nitroimidazoles like Metronidazole

Glycopeptides antibiotics like Vancomycin, Teicoplanin, and Telavancin

Macrolides like Erythromycin, Spiramycin, Roxithromycin, Clarithromycin, and Azithromycin

- 7- Polyenes like Amphotericin B, Nystatin
- 8- Glycylcyclines like Tigecycline
- 9- Echinocandins like Caspofungin, Anidulafungin, Micafungin
- 10- Streptogramins like Quinupristin / dalfopristin
- 11- Ketolides like Telithromycin
- 12- Oxazolidinones like Linezolid
- 13- Lipopeptides like Daptomycin
- 14- Epoxides like Fosfomycin
- 15- Polymyxins like Colistin (polymyxin E), Polymyxin B
- 16- Ansamycins like Rifampicin

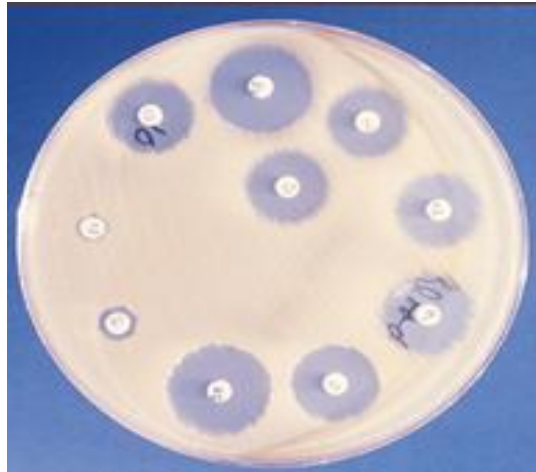


Figure shows antibiotics discs response against isolates in Mueller-Hinton agar plate

Antibiotic Assays to Guide Chemotherapy

Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs.

Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem.

Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a bacterial etiology, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis.

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections.

As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts.

Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic

panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

Principle

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal, Walker and others at the turn of the century, the discovery of antibiotics made these tests (or their modification) too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field.

The Oxford Group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms.

Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory.

Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

Factors Influencing Antimicrobial Susceptibility Testing like pH The pH of each batch of Mueller-Hinton agar should be checked when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, Quinolones, and macrolides). While other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected.

Moisture

If excess surface moisture is present, the plates should be placed in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the Petri dish covers when the plates are inoculated.

Testing strains that fail to grow satisfactorily

Only aerobic or facultative bacteria that grow well on unsupplemented Mueller-Hinton agar should be tested on that medium.

Certain fastidious bacteria such as *Haemophilus* spp., *N. gonorrhoeae*, *S. pneumoniae*, and viridans and β -haemolytic streptococci do not grow sufficiently on unsupplemented Mueller-Hinton agar. These organisms require supplements or different media to grow.

Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system; they include:

-  Diffusion Kirby-Bauer method
-  Dilution Minimum Inhibitory Concentration include Broth and Agar Dilution
-  Diffusion and Dilution E-Test method

Gram-positive bacilli

Aerobic non-spore forming bacilli

- ✚ Usual *Corynebacterium*
- ✚ Unusual *Arcanobacterium*, *Rothia*
- ✚ Acid-fast *Rhodococcus*, *Nocardia*, *Gordonia*

Aerotolerant anaerobes non-spore forming bacilli

Actinomyces, *Propionibacterium*

Corynebacterium

- ✚ Usual *C diphtheriae*
- ✚ Less *C amycolatum*, *C minutissimum*, *C jeikeium*, *C pseudodiphtheriticum*, *C striatum*, *C urealyticum*, *C xerosis*

Corynebacterium species and *Propionibacterium* species are normal flora in skin and mucus membrane, so they frequently contaminated our specimens; although the *Corynebacterium diphtheriae* is a pathogenic microbe that produces a powerful exotoxin which causes diphtheria disease.

Corynebacterium bacteria tend to be clubbed and irregular shaped, the coryneform or diphtheroid bacteria is a convenient name for this group. *Corynebacterium* has high concentration of guanosine and cytosine, its 0.5-1 µm in diameter and 3-4 µm in length; it posses irregular unipolar swelling (club shaped) and it composed of granules deeply stained by aniline dye known as metachromatic granules.



Figure shows Corynebacterium tends to be found in irregular distribution or parallel or on acute angles (Chinese letters)

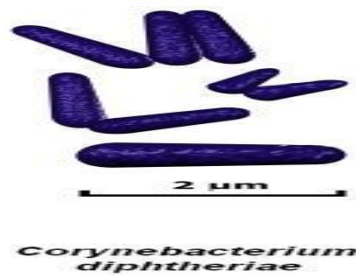


Figure shows Corynebacterium diphtheriae measurement

Its colonies on BA are small granular gray in color with irregular edges and have a small hemolysis zone around. If the plate containing potassium tellurite, so the colonies become black brown with a black brown halo as it reduces intracellular.



Figure shows Corynebacterium diphtheriae growth onto potassium tellurite agar plate

Corynebacterium has 4 biotypes or serovars; **gravis**, **mitis**, **intermedius**, **belfanti**, they are classified depending on colony morphology, biochemical reactions, and disease severity. *C. diphtheriae* grows on most culture media, on Loeffler serum medium it grows readily on it more than other respiratory microbes.

C. diphtheriae is found in 2 strains either toxigenic by *lysogenic conversion* or non-toxigenic strains and if infected by bacteriophages, so the offspring will be toxigenic also; so bacterial toxins are under phage gene control while bacterial invasiveness is under bacterial gene control.

C. diphtheriae causes respiratory and cutaneous infections (skin and soft tissues), so it spreads from person to other by droplets or direct contact; as it inhabits the area it will start toxin production. Bacterial exotoxin elaboration depends on media nitrogen or carbon or pH or amino acids or osmotic pressure or iron contents.

C. diphtheriae exotoxin is a heat-labile polypeptide and it is composed of 2 fragments; **fragment B** acts as a receptor of the toxin to special epithelial cell receptors and facilitates the **fragment A** entrance to the human cell which acts upon inhibition of polypeptide chain elongation through inactivation of elongation factor-2 (EF-2), so

arrest cell protein production and this step is responsible for tissue necrosis, this action is similar to the action of *P aeruginosa* exotoxin.

As the bacterial exotoxin is released it enters the cell and destructs them with embedding of fibrin, RBCs, WBCs exudate yielding *grayish pseudomembrane onto pharynx, tonsils, larynx*, any attempt to remove it will lead to bleeding, and the bacteria inside the membrane will continuo exotoxin production which will be absorbed to the blood to reach liver, heart, kidneys, adrenals, nerves and produce tissue necrosis among them i.e. the disease is not due to the bacterial cell itself but it due to the effect of its exotoxin.

Regarding the cutaneous infection there is the same membrane on wound failed to be healed, but here the exotoxin absorption is slight so the systemic picture is negligible, as this small quantity will elicit the production of antitoxin antibodies that neutralize it.

In diphtheria the clinical presentation is due to suffocation caused by membrane obstruction to the airways, the patient is feverish, irregular heartbeats, blurred vision or speech difficulties or swallowing, and weakness in arms or legs.

In general serovar gravis produce the sever presentation more than the serovar mitis.

C diphtheriae laboratory diagnosis

It is significant in order to improve the clinical impression plus for epidemiological purposes, it is very important not to delay the proper treatment.

Amie's swab from nose, throat and it collected from beneath any membrane seen, then smear is made and fix it to be stained by Gram or methylene blue; then inoculate the specimen onto BA to rule out *Streptococci* inoculate onto loeffler slant and Cystine - tellurite plate (known as modified Tinsdale agar), incubate at 37 °C for 36-48 hours, if got any suspected colonies then send our specimen to reference laboratory to *investigate microbial toxigenicity through different ways*:

- a- **Elek's test** as a filter paper containing antitoxin (10 IU) placed onto agar then inoculates the bacteria 7-9 mm far from the disk, after 48 hours if the bacterium is toxigenic so the antitoxin will react with the toxin to gain precipitin line in the mid line.

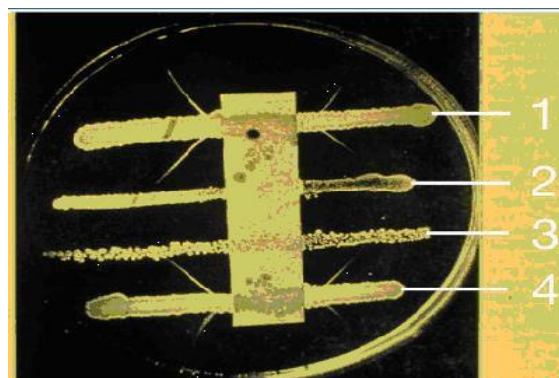


Figure shows the Elek's test reaction

- b- **PCR** to detect bacterium toxin genes (*tox*) if it positive and the culture is negative so here the diphtheria is possible and if both are positive so it is a confirmatory situation. PCR could be applied upon direct swab specimen or upon the resulted culture.
- c- **Elisa** to detect toxin from specimen directly
- d- **Immunochromatography strips** to detect the exotoxin directly after specimen manipulation and it highly sensitive.
- e- In the past they were detect bacterial isolates toxins through injection of guinea pigs with emulsified isolates, if it immunized so they will be survive.

Diphtheria treatment relay upon rapid elimination of the bacterium by antimicrobial agents (penicillin or erythromycin) to prevent toxins production; bacterial antitoxins produced by horses, rabbits, sheep, goats through repeated purified and concentrated toxoid injections, the antitoxin is injected to the patient in 20,000-100,000 units i.m. or i.v.

Before immunization diphtheria was disease of small children and as most of cases occurred sub clinically so a continuous antigenic stimulation is found and the population became immuned to the infection; active immunization in childhood with toxoid yielded antitoxin level enough till adulthood and adults must given booster doses of toxoid.

Diphtheria patient must be isolated in order to decrease contact with healthy personnel because without therapy the patient will continuo shedding the bacilli for weeks or months.

A filtrate broth of toxigenic culture is treated with 0.3% formalin and incubated at 37 °C till its toxigenicity is disappeared, then it called (*fluid toxoid*) which later purified and prepared in flocculation unit (Lf); after that add aluminum hydroxide or aluminum phosphate to be remain longer as a depot in the injection site beside it is a good antigenic stimulus.

Diphtheria toxoid is combined with tetanus toxoid plus pertussis vaccine (DPT)

Gram positive cocci**Genus Staphylococci**

It is gram positive spherical cells (cocci) 1 µm in diameter, arranged in grapelike clusters; producing pigments vary from white-deep yellow, found as single or pairs or tetrads, or chain in liquid broth, the young stained strongly with gram and by aging it will become colorless, it is non-motile and non-spore-forming. Some are members of human skin plus mucus membrane and other cause infections.

Micrococci it resembling *Staphylococci* found in the environment free, appeared in 4 or 8 cocci together and their colonies pigments yellow-red-orange; its rarely associated with infections.

Staphylococci got 40 species but 3 are significant in human infections; *S.aureus* (Coagulase positive), *S.epidermidis*, and *S.saprophyticus* (cause UTI in young females only).

The Coagulase negative *Staphylococci* (CNS) usually are normal flora in human and cause infections if introduced by intravascular catheterization or joints prostheses, especially in young or elderly immunocompromised patients. Less infections caused by CNS is reported (*S.ludunensis*, *S.warneri*, *S.hominis*).

Staphylococci grow in aerobic and microaerophilic conditions grow at 37 °C and produce its pigments at room temperature (20-25 °C), its shape onto solid media round smooth glistening raised and *S.aureus* colonies color vary from gray-golden yellow and its size about 3-4 mm with hemolysis area 1 cm around it; while *S.epidermidis* colonies gray-white in color and without hemolysis. *Staphylococci* are catalase positive to be differentiated from *Streptococci*.

Staphylococci drugs resistance

- 1- β-lactamase producers which under plasmids control, transmitted by transduction or conjugation (e.g., ampicillin, amoxicillin, ticarcillin, piperacillin)
- 2- Resist to nafcillin, methicillin, and oxacillin is not due to β-lactamase but due to locus on chromosome called staphylococci cassette chromosome (SCC *mec gene*) which reduce drug affinity to PBPs; there are 4 classes of *mec* genes (I, II, III) responsible for hospital acquired methicillin resist *Staphylococci* (MRSA), and class IV responsible for community acquired resistance of (MRSA).
- 3- *Staphylococci aureus* considered susceptible to vancomycin if its MIC (minimum inhibitory concentration) is ≤ 2 µg/ml, intermediate if MIC 4-8 µg/ml which known as (VISA) and resist if MIC ≥ 16 µg/ml (VRSA). *Staphylococci* resistance is due to bacteria cell wall changes and not due vancomycin resistance gene (*van gene*) which occur in *Enterococci*.
- 4- Tolerance; it is usually due to failure of the drug to induce the cell wall autolytic enzymes.
Staphylococci certain colonies sometimes differ from other population in shape, color, hemolysis, enzymes production, and pathogenicity, in nafcillin resistance as 10⁷ will be resist at 37 °C but in 30 °C incubation only 10³ will be resist.

Staphylococci antigen

Its cell wall *peptidoglycan* is important in production of endogenous pyrogen (interleukin-1) plus opsonic antibodies by monocytes beside it act as chemotactant for the polymorphonuclear leucocytes (PMNs), also it has endotoxin like activity to enhance the complements.

In addition the *teichonic acid* in the cell wall (composed of glycerol or ribitol phosphate polymers) which connects to the peptidoglycan can enhance antibodies detected by gel diffusion in patients with Staph endocarditis.

Another antigen is the cell wall *protein A* act as antigen and adhesin of bacteria to the target cell wall and it attach to Fc portion in the IgG molecules to enhance the agglutination process to specific antigen (*coagglutination*).

Some strains gain a *capsule* that inhibits bacteria phagocytosis by PMNs cells.

Coagulase (aggregation or clumping factor) also found in the cell wall, that connect enzymatically to fibrinogen

Staphylococci extracellular enzymes & toxins

The main pathogenesis is by invasion + multiplication + enzymes & toxins production

The bacterial enzymes are under plasmids or chromosomal control and they are:

- 1- *Catalase*: that convert H_2O_2 to H_2O and O_2 in *Staph* but not in *Strep*
- 2- *Coagulase & clumping factor*: it coagulate oxalated or citrated plasma; as it bind to prothrombin to be activated and initiate fibrin polymerization, there after the clumping factor is responsible for bacteria adherence to the fibrinogen.
- 3- *Hyaluronidase, staphylokinase that produce fibrinolysis, proteinase, lipase.*

The toxins are:

- 1- *α -toxins (hemolysin)* act upon leucocytes cell wall
- 2- *β -toxins* act upon RBCs act upon leucocytes wall
- 3- *γ -toxins (hemolysin)* act upon leucocytes wall
- 4- *δ -toxins* act upon human epithelial cells
- 5- *Exfoliative toxins (A+B)* they are epidermolytic toxins, cause skin desquamation (scalded skin), type A is under chromosomal control and it heat stable while type B is under plasmid control and it heat labile.
- 6- *Toxic shock syndrome toxin (TSST-1)* it bind to major histocompatibility complex class II (MHC-II) molecules and this will lead to T-lymphocytes stimulation.
- 7- *Enterotoxins (emetic effect)* and it types are (A-E, G-J, K-R, and U, V); it preformed as *Staph* grow in carbohydrate or protein diets and the personnel ingest the already produced toxins not the microbe itself.

Pathogenesis

S epidermidis usually found normally onto skin, respiratory, GIT. 20-50% of people normally harboring *S aureus* nasally (this is important in nursery), also found into fomites, clothing and lines.

Staphylococci infections

Local infection or abscess with painful inflammatory reaction that goes to central suppuration and heals as soon the pus is drained. The fibrin and immune cells around the lesion try to prevent infection spread, so must prevent its manipulation or trauma rupture.

Staphylococci occur also directly through wound contamination (surgical or traumatic) as meningitis after skull fracture or osteomyelitis after open fracture surgery. If the bacteria transmitted by blood stream we get bacteremia, pulmonary, osteomyelitis, meningitis, arthritis, etc.

In food poisoning the incubation period is short (1-8 hours); with vomiting and diarrhea & *NO FEVER*

Staphylococci laboratory diagnosis

The specimens are pus swab, blood, tracheal or abscess aspirates, urine, CSF

1-Gram stained smears

2-Culture onto 5% blood agar plate (BAP), Mannitol salt plate as *S aureus* ferment it to gain yellow colonies, in suspected mixed growth use CAN (Columbia agar plate) which contain colistin + nalidixic acid in order to suppress the G- bacteria; in addition we can use chromogenic culture media especially for nasal carriers detection.

3-Catalase test to detect cytochrome oxidase enzyme as using 3% hydrogen peroxide solution

4-Hemolysis in BAP

5-Coagulase test which is carried out in 2 ways (*slide*); we use particles covered with fibrinogen and IgG antibodies that bind coagulase to gain clumping within 20 seconds if the IgG is present in low concentration we turn onto *tube method* as we mix equal volumes from bacterial broth and citrated plasma then incubate it for 1-4 hours at 37 °C to gain the clot in positive reaction (*Coagulase positive Staph*) (*CPS*); *S intermedius* usually give positive test although it is not regarded as human pathogen.

((Most of the CPS is pathogenic to human))

1-Susceptibility testing done by broth microdilution or disk diffusion and as known 90% of *S aureus* is producing β – lactamase.

Resistance to nafcillin, oxacillin, and methicillin occur in 65% of *S aureus* and in 75% of *S epidermidis* isolates. As commercially methicillin disk production is ceased, so can be replaced with oxacillin disk; if the bacteria resist it that mean it resist methicillin i.e., it is *MRSA*.

2-Detection of *mec* gene through PCR, most laboratories use Mueller-Hinton agar (MHA) contain 4% NaCl + 6 μ g/ml oxacillin in order to inoculate *S aureus* on it, if the bacteria grow that's mean it resist nafcillin and gain *mec* gene.

3-Rapid staph detection through quantitative PCR (qPCR) also called real-time (RT-PCR) as in the ordinary culture we need few days to gain the bacteria while by PCR few copies of bacterial DNA enough to detect its presence within few

hours; in it we duplicate DNA using *thermus aquaticus* (Taq polymerase) and to start duplication need free nucleotides (primers).

4-*Serology* by agglutination to the isolates of little values nowadays.

As most persons harboring *Staph* on skin or nose and throat and they are eliminated only from the skin in eczema cases but later they will return as usual, the bacteria is transmitted in-between persons from fingers or clothes and fomites, so local antiseptics are of value in infection control.

Genus Streptococcus

It is gram positive cocci (spheres or ovoid) found in pairs or chains which divided in a perpendicular manner so sometimes this will give like a diplococci appearance. The bacteria elaborates wide range of substances and enzymes, its classification depend on colony morphology and blood agar plate hemolysis type; cell wall group-specific substances (*Lancefield classification*) and capsular antigens especially to *S.pneumoniae* (*Pneumococcus*; over 90 serotypes are present) and for *S.agalactiae* (group B), biochemical reactions (through sugar fermentation or for detection of enzymes or susceptibility and resistance for certain chemicals), molecular study of species.

Many *Streptococci* able to hemolysed RBCs invitro, if it's a complete it called (β -hemolysis), partial destruction with green pigment formation called (α -hemolysis), or they non-hemolytic which called (γ -hemolysis).

Group-specific substance is carbohydrate in the cell wall; it classified to A-H and K-U, and it determined by its amino-sugars e.g., for group A it is rhamnose-N-acetylglucosamine; for group B it is rhamnose-glucosamine polysaccharide; for group C it is rhamnose-N-acetylgalactosamine; for group D it is glycerol teichoic acid; for group E it is glucopyranosyl-N-acetylgalactosamine.

This group specific antigen is extract by colony centrifugation with:

- 1- Hot HCl treatment
- 2- Enzymatic extraction with trypsin or pepsin
- 3- Through bacterial broth autoclaving

Most streptococcal human infections are caused by groups A, B, C, F, and G.

Group A β -hemolytic Streptococcus pyogenes

It cause local and systemic infections plus post-streptococcal immunological disorders, usually it produce large hemolysis zone (1 cm) in diameter and colony size equal to 0.5 mm, it is susceptible to bacitracin, PYR test (+ve) (hydrolyze 1-Pyrrolidonyl-2-naphthalamide); its main habitat at pharynx and skin, and its main diseases are pharyngitis, impetigo, rheumatic fever, glomerulonephritis, toxic shock.

Most of group A produce a capsule composed of hyaluronic acid, it inhibit phagocytosis and it play role in bacterial virulence as it bind to hyaluronic acid binding protein (CD44) which found onto human epithelial cells, so disrupt the epithelium to allow the microbes to be inside; other capsule belonging *S pneumoniae* or *S agalactiae* is different.

Also its cell wall contain proteins (M, T, R antigens), M protein compose mainly the capsule projections (pili) which covered with lipoteichoic acid and it important for bacteria attachments.

Its growth onto solid media is enhanced by CO₂; they are facultative anaerobes grow well at 37 °C; *most of streptococci are grow under aerobic and anaerobic conditions except Peptostreptococcus which is strict anaerobic.*

S pyogenes also show colony variation as some matt (mostly virulent strains) due to excess M protein and other are glossy (mostly a virulent strains)

S pyogenes antigens

- 1- **M protein:** it appear as hair like projections through the cell wall, especially in virulent strains also it resist phagocytosis by polymorphonuclear leucocytes (PMNs); immunity to it infection related to absence of antibodies against it, and as it has more than 150 types of M protein so the person could gain repeated streptococcal infections. M protein is found in 2 functional structures, (class I, II), M protein has a role in rheumatic fever as it enhance antibodies towards cardiac sacrolemma
- 2- **T substance** its role in pathogenesis still unclear
- 3- **Nucleoproteins**

Streptococcal pyogenes toxins and enzymes

- 1- **Streptokinase** (fibrinolysin) it convert human plasminogen to plasmin which digest the fibrin clot, clinically streptokinase is given intravenously in cases of coronary or pulmonary or venous thrombosis
- 2- **Streptodornase** (deoxyribonuclease) it depolymerase DNA
- 3- **Hyaluronidase** it split hyaluronic acid which is important component of the connective tissue ground substance, it assist M.O. to spread in infected area (spreading factor)
- 4- **Pyrogenic (erythrogenic exotoxin)** they are 3 types (A, B, and C); it associated with toxic shock syndrome and scarlet fever; as it activate the T-lymphocytes through contact to the MHC class II which found onto T-cell surface so the cell will release cytokines that mediate the shock; this mechanism it seems to be the same as with staph enterotoxins.
- 5- **Diphosphopyridine nucleotidase** it related to killing of leucocytes
- 6- **Proteinase and amylase** produced by certain strains
- 7- **Hemolysins** it elaborate 2 hemolysin (streptolysin O) which rapidly inactivated by O₂ so it responsible for hemolysis in the deep area of BAP; it is responsible for antibodies elaborate after strep infections known as antistreptolysin O (ASO) that its normal range from 160-200 units and any elevation is due to recent infection or reactivation in hyperimmune personnel, the other streptolysin is (S) which is O₂ stable cause hemolysis at the BAP surface, elaboration occur as serum is found hence get this name.

Streptococcal pyogenes infections

I-Infections due to bacterial invasion

Mostly they introduced through skin abrasion by burns or wound or surgical incision e.g. in:

- 1- *Erysipelas*
- 2- *Cellulitis*
- 3- *Necrotizing fasciitis* (streptococcal gangrene) sometimes they called the microbe “flesh eating bacteria”
- 4- *Puerperal fever* (endometritis) a sit enter the uterus after delivery
- 5- *Bacteremia / sepsis* it usually follow cellulitis and rarely due to pharyngitis

II-Local infection plus bacterial by-products

- 1- *Sore throat or pharyngitis* as the bacteria attach to pharynx epithelial cells receptors (glycoprotein fibronectin) through its lipoteichoic acid which cover pili and hyaluronic acid in capsulated strains, in children the infection occur as subacute nasopharyngitis with serous discharge and mild fever plus tendency to extend to the mastoid, middle ear, and enlarged cervical lymph nodes.

In older children and adults get acute intense nasopharyngitis, tonsillitis with intense redness and purulent exudate over the mucus membrane, high fever plus tender lymphadenitis. Similar picture occur due to gonococcal infection or infectious mononucleosis or diphtheria or adenovirus infections.

- 2- *Streptococcal pyoderma* a skin local infection especially in children known as (impetigo) which similar to *S aureus* impetigo, composed of vesicles which rupture to crusted area, it highly communicable and prone to be occurred in eczematous or wounded or burned skin. Groups of M proteins 49+57 and 59-61 are prone to yield skin infections rather than glomerulonephritis.

III-Invasive infections (TSS, scarlet fever)

It characterized with bacteremia and respiratory failure plus multi-organs involvement, its rash will appear on the trunk and spread to extremities.

IV-post streptococcal diseases (rheumatic fever and glomerulonephritis)

After a latent period of *Strep* infection (1-4 weeks) and this period represent to the time needed to be hyper immunized to the bacterial antigens; nephritis more prone after skin infections and rheumatic fever more prone after respiratory infections.

The nephritogenic strains with M proteins 2, 42, 49, 56, 57, 60; nephritis initiated with Ag-Ab complex deposit onto the glomerular basement membrane, which lead to RBCs and albumin in urine (edema) and elevated blood pressure, low serum complements, its sequel the majority will recovered and the minority get renal failure.

In rheumatic fever it a serious sequel as damage the heart muscle and valves; as some *Strep* strain gain cell wall antigens cross react with cardiac muscles antigens, it major clinical picture as fever, malaise, non-suppurative polyarthrititis, signs of inflammation in all heart parts i.e. (endocardium which lead to thick deformed valves, myocardium, which lead to perivascular granuloma “Aschoff bodies” and pericardium).

This carditis has tendency to be reactivated with recurrent *Strep* infections, whereas in nephritis this will not happen; **so the chemoprophylaxis is valuable.**

Streptococcus A laboratory diagnosis

- 1- Specimen throat swab which is less valuable as always *viridians strep* is founded or pus or blood
- 2- Smears shows cocci or pairs rather than chain, if the gram stained film is positive and the culture is negative so anaerobes must be suspected
- 3- Culture on BA with the assist of bacitracin diagnostic disk that inhibit its growth and anaerobic culture if suspected, incubate at 5% CO₂ to enhance hemolysin elaboration, Strep in blood culture either grow within hours or need days to be positive as it grow slowly especially with *Enterococcus* or *viridians streptococci*
- 4- Antigen rapid detection with commercial kits, it based on enzymatic or chemical extraction of these Ag, then use elisa or agglutination kit to identify it.
- 5- Serological test:
 - a- ASOT in pharyngitis case
 - b- Anti DNase
 - c- Antihyaluronidase
 - d- Antistreptokinase
 - e- Anti specific M protein

Although human carry *S pyogenes* asymptotically in nasopharynx or perineal, its detection in culture should be considered as significant finding, they spread the microbes by skin contact or respiratory droplets, many other *Strep* as *viridians* or *enterococci* normally inhabitant the respiratory or intestinal or urinary flora and only produce diseases if they reach sites which not belong to them especially after dental or surgical intervention; so chemoprophylaxis is significant prior to it.

S agalactiae

It group B in Lancefield grouping, it classified to types depending on its capsular polysaccharides. It is β -hemolytic and produce 1-2 mm in diameter hemolysis area around the colony, give +ve CAMP test (Christie Atkins Munch Peterson) as using BA which inoculate with suspected *S agalactiae* and in the agar center inoculate a streak of *S aureus*, so the hemolysis will be exaggerated to form arrowhead character also it hydrolyze sodium hippurate.

S agalactiae is part of normal flora in 5-25% of women in the genital tract plus lower GIT (asymptomatic carriers), and not all bacteria will cause babies infection as it depend upon membrane rupture before 37 gestational week, or rupture more than 18 hours prior delivery.

S agalactiae cause invasive infections in adults like bacteremia, UTIs, bone and joints infections, skin and soft tissue, pneumonia, genitourinary.

Group C and G Streptococci

It habitat the nasopharynx and cause pharyngitis or sinusitis, bacteremia or endocarditis; it react like *S pyogenes* as produce β -hemolysin or α - type or none; and got the same M protein.

Group D Enterococcus (Streptococci faecalis and bovis)

S bovis group is the most valuable member in this group which cause human infections, it hemolysis is α - type or none, it habitat colon and biliary tree, and growth in bile (deoxycholate) presence, hydrolyze esculin, *S faecalis* grow in 6.5% NaCl and *S bovis* does not grow. They cause abdominal abscesses, UTIs, endocarditis, biliary infections and *bovis* commonly seen in colonic cancer.

Streptococcus anginosus group (anginosus, intermedius, milleri, and constellatus)

Consist of groups F (A, C, G) and untypeable groups, its hemolysis β or α -type or none, habitat the throat, colon, female genital tract; resist bacitracin, it cause Pyrogenic infections including brain abscesses. They are PYR –ve and Vogas-Proskauer +ve.

Group N Streptococcus

Rarely isolated from human and they are normally contributed in milk coagulation (souring).

Groups E, F, H, and K-U

Rarely isolated usually they are an animal's pathogens

Viridans Streptococci

It include *S mitis*, *S intermedius*, *S sanguis*, *S slivarius*, they hemolysis α –type or none, its growth not inhibited by optochin and their colonies not soluble in bile (deoxycholate), it is the most usual flora in the upper respiratory tract and they are important for the healthy state of the mucus membrane.

It reach blood stream through teeth manipulation to cause endocarditis, *S mutans* produce large polysaccharides like dextrans or levans from sucrose which contribute to the formation of dental caries.

Enterococci or pneumococci or viridans Streptococci as they reach the blood stream (bacteremia) it will settle on normal or already deformed heart valves.

Enterobacteriaceae

Genera and species to be considered

Opportunistic Pathogens:

Citrobacter freundii
Citrobacter (diversus) koseri
Citrobacter braakii
Cronobacter sakazakii (previously *Enterobacter sakazakii*)
Edwardsiella tarda
Enterobacter aerogenes
Enterobacter cloacae
Enterobacter gergoviae
Enterobacter amnigenus
Enterobacter (cancerogenous) taylorae
Escherichia coli (including extraintestinal)
Ewingella americana
Hafnia alvei
Klebsiella pneumoniae
Klebsiella oxytoca
Morganella morganii subsp. *morganii*
Morganella psychrotolerans
Pantoea agglomerans (previously *Enterobacter agglomerans*)
Proteus mirabilis
Proteus vulgaris
Proteus penneri
Providencia alcalifaciens
Providencia heimbachae
Providencia rettgeri
Providencia stuartii
Serratia marcescens
Serratia liquefaciens group
Serratia odorifera

Pathogenic Organisms:

Primary Intestinal Pathogens
E. coli (diarrheagenic)
Plesiomonas shigelloides
Salmonella, all serotypes
Shigella dysenteriae (group A)
Shigella flexneri (group B)

Shigella boydii (group C)

Shigella sonnei (group D)

Pathogenic *Yersinia* spp.

Yersinia pestis

Yersinia enterocolitica subsp. *enterocolitica*

Yersinia frederiksenii

Because of the large number and diversity of genera included in the Enterobacteriaceae, it is helpful to consider the bacteria of this family as belonging to one of two major groups. The first group comprises species that either commonly colonize the human gastrointestinal tract or are most notably associated with human infections. Although many Enterobacteriaceae that cause human infections are part of our normal gastrointestinal flora, there are exceptions, such as *Yersinia pestis*. The second group consists of genera capable of colonizing humans but rarely associated with human infection and commonly recognized as environmental inhabitants or colonizers of other animals. For this reason, the discovery of these species in clinical specimens should alert laboratorians to possible identification errors; careful confirmation of both the laboratory results and the clinical significance of such isolates is warranted.

General Characters

Molecular analysis has not proven effective for definitively characterizing all the organisms and genera included within the Enterobacteriaceae family. Therefore, species names and reclassification of organisms continually evolve. In general, the Enterobacteriaceae consist of a diverse group of gram negative bacilli or coccobacilli; they are non-spore forming, facultative anaerobes capable of fermenting glucose; they are oxidase negative (except for *Plesiomonas* sp.); and, with rare exception (*Photorhabdus* and *Xenorhabdus* spp.), they reduce nitrates to nitrites. Furthermore, except for *Shigella dysenteriae* type 1, all commonly isolated Enterobacteriaceae are catalase positive.

Epidemiology

Enterobacteriaceae inhabit a wide variety of niches, including the human gastrointestinal tract, the gastrointestinal tract of other animals, and various environmental sites. Some are agents of zoonoses, causing infections in animal populations (Table 1). Just as the reservoirs for these organisms vary, so do their modes of transmission to humans. For species capable of colonizing humans, infection may result when a patient's own bacterial strains (i.e., endogenous strains) establish infection in a normally sterile body site. These organisms can also be passed from one patient to another. Such infections often depend on the debilitated state of a hospitalized patient and are acquired during the patient's hospitalization (nosocomial). However, this is not always the case. For example, although *E. coli* is the most common cause of nosocomial infections, it is also the leading cause of community-acquired urinary tract infections. Other species, such as *Salmonella* spp., *Shigella* spp., and *Yersinia enterocolitica*, inhabit the bowel during infection and are acquired by ingestion of contaminated food or water. This is also the mode of transmission for the various types of *E. coli* known to cause gastrointestinal infections. In contrast, *Yersinia pestis* is unique among the Enterobacteriaceae that infect humans. This is the only species transmitted from animals by an insect vector (i.e., flea bite).

Table (1-1) Epidemiology of Clinically Relevant Enterobacteriaceae

Organism	Habitat (Reservoir)	Mode of Transmission
<i>Escherichia coli</i>	Normal bowel flora of humans and other animals; may also inhabit female genital tract	Varies with the type of infection. For nongastrointestinal infections, organisms may be endogenous or spread person to person, especially in the hospital setting. For gastrointestinal infections, the transmission mode varies with the strain of <i>E. coli</i> (see Table 20-2); it may involve fecal-oral spread between humans in contaminated food or water or consumption of undercooked beef or unpasteurized milk from colonized cattle
<i>Shigella</i> spp	Only found in humans at times of infection; not part of normal bowel flora	Person-to-person spread by fecal-oral route, especially in overcrowded areas, group settings (e.g., daycare) and areas with poor sanitary conditions
<i>Salmonella</i> serotype Typhi <i>Salmonella</i> serotypes Paratyphi A, B, C	Only found in humans but not part of normal bowel flora	Person-to-person spread by fecal-oral route by ingestion of food or water contaminated with human excreta
Other <i>Salmonella</i> spp	Widely disseminated in nature and associated with various animals	Ingestion of contaminated food products processed from animals, frequently of poultry or dairy origin. Direct person-to-person transmission by fecal-oral route can occur in health care settings when hand-washing guidelines are not followed
<i>Edwardsiella tarda</i>	Gastrointestinal tract of cold-blooded animals, such as reptiles	Uncertain; probably by ingestion of contaminated water or close contact with carrier animal
<i>Yersinia pestis</i>	Carried by urban and domestic rats and wild rodents, such as the	From rodents to humans by the bite of flea vectors or by ingestion of contaminated animal tissues; during human epidemics of pneumonic (i.e., respiratory)

	ground squirrel, rock squirrel, and prairie dog	disease, the organism can be spread directly from human to human by inhalation of contaminated airborne droplets; rarely transmitted by handling or inhalation of infected animal tissues or fluids
<i>Yersinia enterocolitica</i>	Dogs, cats, rodents, rabbits, pigs, sheep, and cattle; not part of normal human microbiota	Consumption of incompletely cooked food products (especially pork), dairy products such as milk, and, less commonly, by ingestion of contaminated water or by contact with infected animals
<i>Yersinia pseudotuberculosis</i>	Rodents, rabbits, deer, and birds; not part of normal human microbiota	Ingestion of organism during contact with infected animal or from contaminated food or water
<i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Klebsiella</i> spp., <i>Morganella</i> spp., <i>Proteus</i> spp., <i>Providencia</i> spp., and <i>Serratia</i> spp	Normal human gastrointestinal Microbiota	Endogenous or person-to-person spread, especially in hospitalized patients

Pathogenesis and spectrum of diseases:

The clinically relevant members of the Enterobacteriaceae can be considered as two groups: the opportunistic pathogens and the intestinal pathogens.

Typhi and Shigella spp. are among the latter group and are causative agents of typhoid fever and dysentery, respectively. *Yersinia pestis* is not an intestinal pathogen, but it is the causative agent of plague. The identification of these

organisms in clinical material is serious and always significant. These organisms, in addition to others, produce various potent virulence factors and can cause life threatening infections (Table 2). The opportunistic pathogens most commonly include *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Serratia* spp., and a variety of other organisms. Although considered opportunistic pathogens, these organisms produce significant virulence factors, such as endotoxins capable of mediating fatal infections.

However, because they generally do not initiate disease in healthy, uncompromised human hosts, they are considered opportunistic.

Although *E. coli* is a normal bowel inhabitant, its pathogenic classification is somewhere between that of the overt pathogens and the opportunistic organisms. Diuretic strains of this species, such as enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC), express potent toxins and cause serious gastrointestinal infections. Additionally, in the case of enterohemorrhagic *E. coli* (EHEC) also referred to as verocytotoxin producing *E. coli* (VTEC) or Shiga-like toxin producing *E. coli* (STEC), the organism may produce life-threatening systemic illness. Furthermore, as the leading cause of Enterobacteriaceae nosocomial infection, *E. coli* is likely to have greater virulence capabilities than the other species categorized as “opportunistic” Enterobacteriaceae.

Table (1-2)Pathogenesis and Spectrum of Disease for Clinically Relevant Enterobacteriaceae

Organism	Virulence Factors	Spectrum of Disease and Infections
<i>Escherichia coli</i> (as a cause of extraintestinal infections)	Several, including endotoxin, capsule production pili that mediate attachment to host cells	Urinary tract infections, bacteremia, neonatal meningitis, and nosocomial infections of other various body sites. Most common cause of gram-negative nosocomial infections.
Enterotoxigenic <i>E. coli</i> (ETEC)	Pili that permit gastrointestinal colonization. Heat-labile (LT) and heat-stable (ST) enterotoxins that mediate secretion of water and electrolytes into the bowel lumen	Traveler’s and childhood diarrhea, characterized by profuse, watery stools. Transmitted by contaminated food and water.
Enteroinvasive <i>E. coli</i> (EIEC)	Virulence factors uncertain, but organism invades enterocytes lining the large intestine in a manner nearly identical to <i>Shigella</i>	Dysentery (i.e., necrosis, ulceration, and inflammation of the large bowel); usually seen in young children living in areas of poor sanitation.
Enteropathogenic <i>E. coli</i> (EPEC)	Bundle-forming pilus, intimin, and other factors that mediate organism attachment to mucosal cells of the small bowel, resulting in changes in cell surface (i.e., loss of	Diarrhea in infants in developing, low-income nations; can cause a chronic diarrhea.

	microvilli	
Enterohemorrhagic <i>E. coli</i> (EHEC, VTEC, or STEC)	Toxin similar to Shiga toxin produced by <i>Shigella dysenteriae</i> . Most frequently associated with certain serotypes, such as <i>E. coli</i> O157:H7	Inflammation and bleeding of the mucosa of the large intestine (i.e., hemorrhagic colitis); can also lead to hemolytic-uremic syndrome, resulting from toxin-mediated damage to kidneys. Transmitted by ingestion of undercooked ground beef or raw milk.
Enteraggregative <i>E. coli</i> (EAEC)	Probably involves binding by pili, ST-like, and hemolysin-like toxins; actual pathogenic mechanism is unknown	Watery diarrhea that in some cases can be prolonged. Mode of transmission is not well understood
<i>Shigella</i> spp.	Several factors involved to mediate adherence and invasion of mucosal cells, escape from phagocytic vesicles, intercellular spread, and inflammation. Shiga toxin role in disease is uncertain, but it does have various effects on host cells.	Dysentery defined as acute inflammatory colitis and bloody diarrhea characterized by cramps, tenesmus, and bloody, mucoid stools. Infections with <i>S. sonnei</i> may produce only watery diarrhea
<i>Salmonella</i> serotypes	Several factors help protect organisms from stomach acids, promote attachment and phagocytosis by intestinal mucosal cells, allow survival in and destruction of phagocytes, and facilitate dissemination to other tissues.	Three general categories of infection are seen: - Gastroenteritis and diarrhea caused by a wide variety of serotypes that produce infections limited to the mucosa and submucosa of the gastrointestinal tract. <i>S.</i> serotype Typhimurium and <i>S.</i> serotype Enteritidis are the serotypes most commonly associated with <i>Salmonella</i> gastroenteritis in the United States. - Bacteremia and extraintestinal infections occur by spread from

		the gastrointestinal tract. These infections usually involve <i>S. Choleraesuis</i> or <i>S. dublin</i>, although any serotype may cause these infections. • Enteric fever (typhoid fever, or typhoid) is characterized by prolonged fever and multisystem involvement, including blood, lymph nodes, liver, and spleen. This life-threatening infection is most frequently caused by <i>S. serotype Typhi</i>; more rarely, <i>S. serotypes Paratyphi A, B</i> or <i>C</i>.
<i>Yersinia pestis</i>	Multiple factors play a role in the pathogenesis of this highly virulent organism. These include the ability to adapt for intracellular survival and production of an antiphagocytic capsule, exotoxins, endotoxins, coagulase, and fibrinolysin	Two major forms of infection are bubonic plague and pneumonic plague. Bubonic plague is characterized by high fever and painful inflammatory swelling of axilla and groin lymph nodes (i.e., the characteristic buboes); infection rapidly progresses to fulminant bacteremia that is frequently fatal if untreated. Pneumonic plague involves the lungs and is characterized by malaise and pulmonary signs; the respiratory infection can occur as a consequence of bacteremic spread associated with bubonic plague or can be acquired by the airborne route during close contact with other pneumonic plague victims; this form of plague is also rapidly fatal.
<i>Yersinia enterocolitica</i> subsp. <i>enterocolitica</i>	Various factors encoded on a virulence plasmid allow the organism to attach to and invade the intestinal	Enterocolitis characterized by fever, diarrhea, and abdominal pain; also can cause acute mesenteric lymphadenitis, which may present clinically as appendicitis (i.e., pseudoappendicular

	mucosa and spread to lymphatic tissue.	syndrome). Bacteremia can occur with this organism but is uncommon
<i>Yersinia Pseudotuberculosis</i>	Similar to those of <i>Y. enterocolitica</i>	Causes infections similar to those described for <i>Y. enterocolitica</i> but is much less common
<i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Klebsiella</i> spp., <i>Morganella</i> spp., <i>Proteus</i> spp., <i>Providencia</i> spp., and <i>Serratia</i> spp.	Several factors, including endotoxins, capsules, adhesion proteins, and resistance to multiple antimicrobial agents	Wide variety of nosocomial infections of the respiratory tract, urinary tract, blood, and several other normally sterile sites; most frequently infect hospitalized and seriously debilitated patients

Specific organisms:

Opportunistic human pathogens

***Citrobacter* spp.** (*C. freundii*, *C. koseri*, *C. braakii*) *Citrobacter* organisms are inhabitants of the intestinal tract. The most common clinical manifestation in patients as a result of infection occurs in the urinary tract. However, additional infections, including septicemias, meningitis, brain abscesses, and neurologic complications, have been person to person. (Table 3) provides an outline of the biochemical differentiation of the most common clinically isolated *Citrobacter* species. *C. freundii* may harbor inducible *AmpC* genes that encode resistance to ampicillin and first-generation cephalosporins.

Table(3) Biochemical Differentiation of *Citrobacter* Species

Species	Indole	ODC	Malonate	ACID FERMENTATION Adonitol	Dulcitol	Melibiose	Sucrose
<i>C. braakii</i>	V	pos	Neg	neg	v	v	neg
<i>C. freundii</i>	V	neg	Neg	neg	neg	pos	v
<i>C. koseri</i>	Pos	pos	Pos	pos	v	neg	v

From Versalovic J: *Manual of clinical microbiology*, ed 10, 2011, Washington, DC, ASM Press. neg, Negative < 15%; ODC, ornithine decarboxylase; pos, positive ≥ 85%; V, variable 15% to 84%.

Cronobacter sakazakii

Cronobacter sakazakii, formerly *Enterobacter sakazakii*, is a pathogen associated with bacteremia, meningitis, and necrotizing colitis in neonates. The organism produces a yellow pigment that is enhanced by incubation at 25°C.

C. sakazakii may be differentiated from *Enterobacter* spp. As Voges-Proskauer, arginine dihydrolase, ornithine decarboxylase positive. In addition, the organism displays the following fermentation reactions: D-sorbitol negative, raffinose positive, L-rhamnose positive, melibiose positive, D-arabitol negative, and sucrose positive. *C. sakazakii* is intrinsically resistant to ampicillin and first- and second-generation cephalosporins as a result of an inducible *AmpC* chromosomal β -lactamase. Mutations to the *AmpC* gene may result in overproduction of β -lactamase, conferring resistance to third-generation cephalosporins.

Edwardsiella tarda

Edwardsiella tarda is infrequently encountered in the clinical laboratory as a cause of gastroenteritis. The organism is typically associated with water harboring fish or turtles. Immunocompromised individuals are particularly susceptible and may develop serious wound infections and myonecrosis. Systemic infections occur in patients with underlying liver disease or conditions resulting in iron overload. *Enterobacter* spp.

(*E. aerogenes*, *E. cloacae*, *E. gergoviae*, *E. amnigenus*, *E. taylorae*)

Enterobacter spp. are motile lactose fermenters that produce mucoid colonies. *Enterobacter* spp. are reported as one of the genera listed in the top 10 most frequently isolated health care-associated infections by the National Healthcare Safety Network. The infections are typically associated with contaminated medical devices, such as

respirators and other medical instrumentation. The organism has a capsule that provides resistance to phagocytosis. *Enterobacter* spp. may harbor plasmids that encode multiple antibiotic resistance genes, requiring antibiotic susceptibility testing to identify appropriate therapeutic options.

***Escherichia coli* (UPEC, MNEC, ETEC, EIEC, EAEC, EPEC and EHEC)**

Molecular analysis of *E. coli* has resulted in the classification of several pathotypes as well as commensal strains. The genus consists of facultative anaerobic, glucosefermenting, gram-negative, oxidase-negative rods capable

of growth on MacConkey agar. The genus contains motile (peritrichous flagella) and nonmotile bacteria. Most *E. coli* strains are lactose fermenting, but this function may be delayed or absent in other *Escherichia* spp.

Isolates of extraintestinal *E. coli* strains have been grouped into two categories: uropathogenic *E. coli* (UPEC) and meningitis/sepsis-associated *E. coli* (MNEC).

UPEC strains are the major cause of *E. coli*-associated urinary tract infections. These strains contain a variety of pathogenicity islands that code for specific adhesions and toxins capable of causing disease, including cystitis and acute pyelonephritis. MNEC causes neonatal meningitis that results in high morbidity and mortality. Eighty percent of MNEC strains test positive for the K1 antigen.

The organisms are spread to the meninges from a blood infection and gain access to the central nervous system via membrane-bound vacuoles in microvascular endothelial cells.

As mentioned, intestinal *E. coli* may be classified as enterohemorrhagic (or serotoxigenic [STEC], or verotoxigenic [VTEC]), enterotoxigenic, enteropathogenic, enteroinvasive, or enteroaggregative. EHEC is recognized

as the cause of hemorrhagic diarrhea, colitis, and hemolytic uremic syndrome (HUS). HUS, which is characterized by a hemolytic anemia and low platelet

count, often results in kidney failure and death. Unlike in dysentery, no white blood cells are found in the stool. Although more than 150 non-O157 serotypes have been associated with diarrhea or HUS, the two most common

are O157:H7 and O157:NM (nonmotile). The O antigen is a component of the lipopolysaccharide of the outer membrane, and the H antigen is the specific flagellin associated with the organism. ETEC produces a heatlabile enterotoxin (LT) and a heat-stable enterotoxin (ST) capable of causing mild watery diarrhea. ETEC is uncommon in the United States but is an important pathogen in young children in developing countries.

EIEC may produce a watery to bloody diarrhea as a result of direct invasion of the epithelial cells of the colon. Cases are rare in the United States. EPEC typically does not produce exotoxins. The pathogenesis of these strains is associated with attachment and effacement of the intestinal cell wall through specialized adherence factors. Symptoms of infection include prolonged, nonbloody diarrhea; vomiting; and fever, typically in infants or children.

EAEC has been isolated from a variety of clinical cases of diarrhea. The classification as aggregative results from the control of virulence genes associated with a global aggregative regulator gene, *AggR*, responsible for cellular adherence. EAEC-associated stool specimens typically are not bloody and do not contain white blood cells. Inflammation is accompanied by fever and abdominal pain.

Ewingella americana

Ewingella americana has been identified from blood and wound isolates. The organism is biochemically inactive, and currently no recommended identification scheme has been identified.

Hafnia alvei

Hafnia alvei (formerly *Enterobacter hafniae*) has been associated with gastrointestinal infections. The organism, resides in the gastrointestinal tract of humans and many animals. It is a motile non-lactose fermenter and is often

isolated with other pathogens. Most infections with *H.alvei* are identified in patients with severe underlying disease (e.g., malignancies) or after surgery or trauma.

However, a distinct correlation with clinical signs and symptoms has not been clearly developed, probably because of the lack of identified clinical cases. Treatment is based on antimicrobial susceptibility testing.

***Klebsiella* spp. (*K. pneumoniae*, *K. oxytoca*)**

Klebsiella spp. are inhabitants of the nasopharynx and gastrointestinal tract. Isolates have been identified in association with a variety of infections, including liver abscesses, pneumonia, septicemia, and urinary tract infections. Some strains of *K. oxytoca* carry a heatlabile cytotoxin, which has been isolated from patients who have developed a self-limiting antibiotic-associated community-acquired pyogenic liver abscess worldwide.

All strains of *K. pneumoniae* are resistant to ampicillin. In addition, they may demonstrate multiple antibiotic resistance patterns from the acquisition of multidrug-resistant plasmids, with enzymes such as carbapenemase.

***Morganella* spp. (*M. morganii*, *M. psychrotolerans*)**

Morganella spp. are found ubiquitously throughout the environment and are often associated with stool specimens collected from patients with symptoms of diarrhea.

They are normal inhabitants of the gastrointestinal tract. *M. morganii* is commonly isolated in the clinical laboratory; however, its clinical significance has not been clearly defined. *Morganella* spp. are deaminase positive and urease positive.

Pantoea agglomerans

Pantoea agglomerans appears as a yellow-pigmented colony and is lysine, arginine, and ornithine negative. In addition, the organism is indole positive and mannitol, raffinose, salicin, sucrose, maltose, and xylose negative. The organism is difficult to identify using commercial or traditional biochemical methods due to the high variability of expression in the key reactions. Sporadic infections can occur due to trauma from objects contaminated with soil or from contaminated fluids (i.e., IV fluids).

Plesiomonas shigelloides

Plesiomonas shigelloides is a fresh water inhabitant that is transmitted to humans by ingestion of contaminated water or by exposure of disrupted skin and mucosal surfaces. *P. shigelloides* can cause gastroenteritis, most frequently in children, but its role in intestinal infections is still unclear.

P. shigelloides is unusual in that it is among the few species of clinically relevant bacteria that decarboxylate lysine, ornithine, and arginine. It is important to distinguish *Aeromonas* spp. from *P. shigelloides*, since both are oxidase positive. This is accomplished by using the string test. The DNase test may also be used to differentiate these organisms. *Aeromonas* spp. are DNase positive and *Plesiomonas* organisms are DNase negative.

***Proteus* spp. (*P. mirabilis*, *P. vulgaris*, *P. penneri*) and *Providencia* spp. (*P. alcalifaciens*, *P. heimbachae*, *P. rettgeri*, *P. stuartii*, *P. rustigianii*)**

The genera *Proteus* and *Providencia* are normal inhabitants of the gastrointestinal tract. They are motile, non-lactose fermenters capable of deaminating phenylalanine.

Proteus spp. are easily identified by their classic “swarming” appearance on culture media. However, some strains lack the swarming phenotype. *Proteus* has a distinct odor that is often referred to as a “chocolate cake” or “burnt chocolate” smell. For safety reasons, smelling plates is strongly discouraged in the clinical laboratory. Because of its motility, the organism is often associated

with urinary tract infections; however, it also has been isolated from wounds and ears. The organism has also been associated with diarrhea and sepsis.

Providencia spp. are most commonly associated with urinary tract infections and the feces of children with diarrhea. These organisms may be associated with nosocomial outbreaks.

***Serratia* spp. (*S. marcescens*, *S. liquefaciens* group)**

Serratia spp. are known for colonization and the cause of pathogenic infections in health care settings. *Serratia* spp.

are motile, slow lactose fermenters, DNase, and orthonitrophenyl galactoside (ONPG) positive. *Serratia* spp. Are ranked the twelfth most commonly isolated organism from pediatric patients in North America, Latin America, and Europe. Transmission may be person to person but is often associated with medical devices such as urinary catheters, respirators intravenous fluids, and other

medical solutions. *Serratia* spp. have also been isolated from the respiratory tract and wounds. The organism is capable of survival under very harsh environmental conditions and is resistant to many disinfectants. The red pigment (prodigiosin) produced by *S. marcescens* typically is the key to identification among laboratorians, although pigment-producing strains tend to be of lower virulence. Other species have also been isolated from human infections. *Serratia* spp. are resistant to ampicillin and first-generation cephalosporins because of the presence of an inducible, chromosomal *AmpC* β -lactamase. In addition, many strains have plasmid-encoded antimicrobial resistance to other cephalosporins, penicillins, carbapenems, and aminoglycosides.

Primary intestinal pathogens

***Salmonella* (All Serotypes)**

Salmonella are facultative anaerobic, motile gram-negative rods commonly isolated from the intestines of humans and animals. Identification is primarily based on the ability of the organism to use citrate as the sole carbon source and lysine as a nitrogen source in combination with hydrogen sulfide (H₂S) production. The genus is comprised of two primary species, *S. enterica* (human pathogen) and *S. bongori* (animal pathogen). *S. enterica* is subdivided into six subspecies: subsp. *enterica*, subsp. *salamae*, subsp. *arizonae*, subsp. *diarizonae*, subsp. *houtenae*, and subsp. *indica*. *S. enterica* subsp. *enterica* can be further divided into serotypes with unique virulence properties.

Serotypes are differentiated based on the characterization of the heat-stable O antigen, included in the LPS, the heat-labile H antigen flagellar protein, and the heat-labile Vi antigen, capsular polysaccharide. A DNA sequence-based method has been developed for molecular identification of DNA motifs in the flagella and O antigens.

***Shigella* spp. (*S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*)**

Shigella spp. are nonmotile; lysine decarboxylase–negative; citrate-, malonate-, and H₂S-negative; non–lactose fermenting; gram-negative rods that grow well on MacConkey agar. The four subgroups of *Shigella* spp. are: *S. dysenteriae* (group A), *S. flexneri* (group B), *S. boydii* (group C), and *S. sonnei* (group D). Each subgroup has several serotypes. Serotyping is based on the somatic LPS O antigen. After presumptive identification of a suspected *Shigella* species based on traditional biochemical methods, serotyping should be completed, especially in the case of *S. dysenteriae*. Suspected strains of *Shigella* sp. that cannot be typed by serologic methods should be referred to a reference laboratory for further testing.

***Yersinia* spp. (*Y. pestis*, *Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia*, *Y. pseudotuberculosis*)**

Yersinia spp. are gram-negative; catalase-, oxidase-, and indole-positive, non–lactose fermenting; facultative anaerobes capable of growth at temperatures ranging from 4° to 43°C. The gram-negative rods exhibit an unusual bipolar staining. Based on the composition of the LPS in the outer membrane, colonies may present with either a rough form lacking the O-specific polysaccharide chain (*Y. pestis*) or a smooth form containing the lipid A-oligosaccharide core and the complete O-polysaccharide (*Y. pseudotuberculosis* and *Y. enterocolitica*). Complex typing systems exist to differentiate the various *Yersinia* spp., including standard biochemical methods coupled with biotyping, serotyping, bacteriophage typing, and antibiogram analysis. In addition, epidemiologic studies often include pulsed-field gel electrophoresis (PFGE) studies.

Rare human pathogens

A variety of additional Enterobacteriaceae may be isolated from human specimens, such as *Cedecea* spp., *Kluyvera* spp., *Leclercia adcarboxylata*, *Moellerella wisconsensis*, *Rahnella aquatilis*, *Tatumella ptyseos*, and *Yokenella regensburgei*. These organisms are typically opportunistic pathogens found in environmental sources.

Laboratory diagnosis:

Specimen collection and transport

Enterobacteriaceae are typically isolated from a variety of sources in combination with other more fastidious organisms. No special considerations are required for specimen collection and transport of the organisms.

Direct detection methods

All Enterobacteriaceae have similar microscopic morphology; therefore, Gram staining is not significant for the presumptive identification of Enterobacteriaceae.

Generally isolation of gram-negative organisms from a sterile site, including cerebrospinal fluid (CSF), blood, and other body fluids, is critical and may assist the physician in prescribing appropriate therapy.

Direct detection of Enterobacteriaceae in stool by Gram staining is insignificant because of the presence of a large number of normal gram-negative microbiota. The presence of increased white blood cells may indicate an enteric infection; however, the absence is not sufficient to rule out a toxin-mediated enteric disease.

Other than Gram staining of patient specimens, specific procedures are required for direct detection of most Enterobacteriaceae. Microscopically the cells of these organisms generally appear as coccobacilli, or straight rods with rounded ends. *Y. pestis* resembles a closed safety pin when it is stained with methylene blue or Wayson stain; this is a key characteristic for rapid diagnosis of plague.

Klebsiella granulomatis can be visualized in scrapings of lesions stained with Wright's or Giemsa stain. Cultivation in vitro is very difficult, so direct examination is important diagnostically. Groups of organisms are seen in mononuclear endothelial cells; this pathognomonic entity is known as a *Donovan body*, named after the physician who first visualized the organism in such a lesion.

The organism stains as a blue rod with prominent polar granules, giving rise to the safety-pin appearance, surrounded by a large, pink capsule. Subsurface infected cells must be present; surface epithelium is not an adequate specimen.

P. shigelloides tend to be pleomorphic gram-negative rods that occur singly, in pairs, in short chains, or even as long, filamentous forms.

Cultivation

Media of Choice

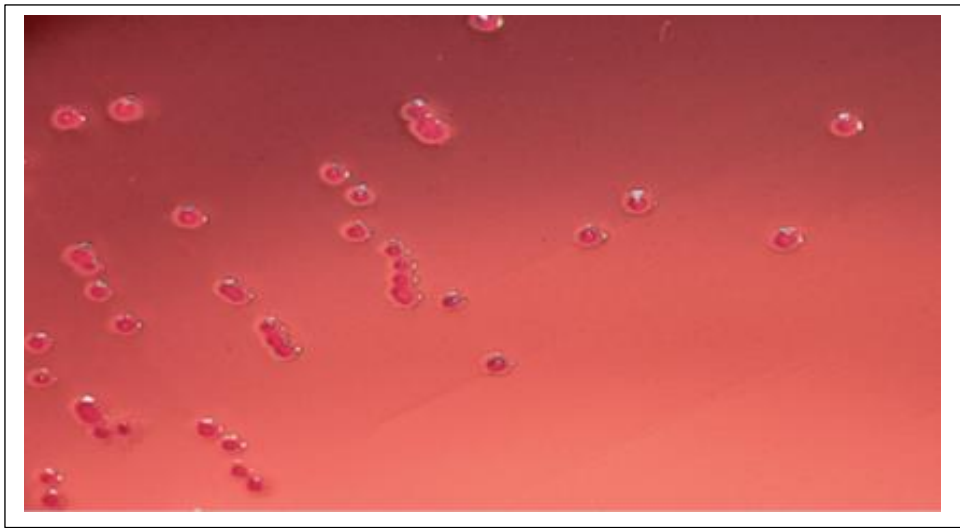
Most Enterobacteriaceae grow well on routine laboratory media, such as 5% sheep blood, chocolate, and MacConkey agars. In addition to these media, selective agars, such as Hektoen enteric (HE) agar, xylose-lysine-deoxycholate (XLD) agar, and *Salmonella-Shigella* (SS) agar, are commonly used to cultivate enteric pathogens from gastrointestinal. The broths used in blood culture systems, as well as thioglycollate and brain heart infusion broths, all support the growth of Enterobacteriaceae.

Cefsulodin-irgasan-novobiocin (CIN) agar is a selective medium specifically used for the isolation of *Y. enterocolitica* from gastrointestinal specimens. Similarly, MacConkey-sorbitol agar (MAC-SOR) is used to differentiate sorbitol-negative *E. coli* O157:H7 from other strains of *E. coli* that are capable of fermenting this sugar alcohol. *Klebsiella granulomatis* will not grow on routine agar media. Recently, the organism was cultured in human monocytes from biopsy specimens of genital ulcers of patients with donovanosis. Historically, the organism has also been cultivated on a special medium described by Dienst that contains growth factors found in egg yolk. In clinical practice, however, the diagnosis of granuloma inguinale is made solely on the basis of direct examination.

Incubation Conditions and Duration

Under normal circumstances, most Enterobacteriaceae produce detectable growth in commonly used broth and agar media within 24 hours of inoculation. For isolation, 5% sheep blood and chocolate agars may be incubated at 35°C in carbon dioxide or ambient air. However, Mac- Conkey agar and other selective agars (e.g., SS,

HE,XLD) should be incubated only in ambient air. Unlike most other Enterobacteriaceae, *Y. pestis* grows best at 25° to 30°C. Colonies of *Y. pestis* are pinpoint at 24 hours but resemble those of other Enterobacteriaceae after 48 hours. CIN agar, used for the isolation of *Y. enterocolitica*, should be incubated 48 hours at room temperature to allow for the development of typical “bull’s-eye” colonies (Figure 1).



Figure(1) Bull’s-eye colony of Yersinia enterocolitica on cefsulodin-irgasan-novobiocin (CIN) agar

Colonial Appearance

Table 4 presents the colonial appearance and other distinguishing characteristics (pigment and odor) of the most commonly isolated Enterobacteriaceae on MacConkey, HE, and XLD agars.

All Enterobacteriaceae produce similar growth on blood and chocolate agars; colonies are large, gray, and smooth. Colonies of *Klebsiella* or *Enterobacter* may be mucoid because of their polysaccharide capsule. *E. coli* is often beta-hemolytic on blood agar, but most other genera are nonhemolytic. As a result of motility, *Proteus mirabilis*, *P. penneri*, and *P. vulgaris* “swarm” on blood and chocolate agars. Swarming results in the production of a thin film of growth on the agar surface (Figure 3) as the motile organisms spread from the original site of inoculation. Colonies of *Y. pestis* on 5% sheep blood agar are pinpoint at 24 hours but exhibit a rough, cauliflower appearance at 48 hours. Broth cultures of *Y. pestis* exhibit a characteristic “stalactite pattern” in which clumps of cells adhere to one side of the tube.

Y. enterocolitica produces bull’s-eye colonies (dark red or burgundy centers surrounded by a translucent border; see Figure (1) on CIN agar at 48 hours. However, because most *Aeromonas* spp. produce similar colonies on CIN agar, it is important to perform an oxidase test to verify that the organisms are *Yersinia* spp. (oxidase negative).

The oxidase test should be performed on suspect colonies that have been subcultured to sheep blood agar. Pigments present in the CIN agar will interfere with correct interpretation of the oxidase test results.

Table (4) Colonial Appearance and Characteristics of the Most Commonly Isolated Enterobacteriaceae

Organism	Medium	Appearance

<i>Citrobacter</i> spp.	MAC	Late LF; therefore, NLF after 24 hr; LF after 48 hr; colonies are light pink after 48 hr
	MAC	Colorless
<i>Edwardsiella</i> spp.	MAC	NLF
	HE	Colorless
	XLD	Red, yellow, or colorless colonies, with or without black centers (H ₂ S)
<i>Enterobacter</i> spp.	MAC	LF; may be mucoid
	HE	Yellow
	XLD	Yellow
<i>Escherichia coli</i>	MAC	Most LF, some NLF (some isolates may demonstrate slow or late fermentation); and generally flat, dry, pink colonies with a surrounding darker pink area of precipitated bile salts†
	HE	Yellow
	XLD	Yellow
<i>Hafnia alvei</i>	MAC	NLF
	HE	Colorless
	XLD	Red or yellow
<i>Klebsiella</i> spp.	MAC	LF; mucoid
	HE	Yellow
	XLD	Yellow
<i>Morganella</i> spp.	MAC	NLF
	HE	Colorless
	XLD	Red or colorless
<i>Plesiomonas shigelloides</i>	BAP	Shiny, opaque, smooth, nonhemolytic
	MAC	Can be NLF or LF
<i>Proteus</i> spp.	MAC	NLF; may swarm, depending on the amount of agar in the medium; characteristic foul smell
	HE	Colorless
	XLD	Yellow or colorless, with or without black centers
<i>Providencia</i> spp.	MAC	NLF
	HE	Colorless
	XLD	Yellow or colorless
<i>Salmonella</i> spp.	MAC	NLF
	HE	Green, black center as a result of H ₂ S production
	XLD	Red with black center
<i>Serratia</i> spp.	MAC	Late LF; <i>S. marcescens</i> may be red pigmented, especially if plate is left at 25°C (Figure 20-2)

	HE	Colorless
	XLD	Yellow or colorless
<i>Shigella</i> spp.	MAC	NLF; <i>S. sonnei</i> produces flat colonies with jagged edges
	HE	Green
	XLD	Colorless
<i>Yersinia</i> spp.	MAC	NLF; may be colorless to peach
	HE	Salmon
	XLD	Yellow or colorless

HE, Hektoen enteric agar; *LF*, lactose fermenter, pink colony; *MAC*, MacConkey agar; *NLF*, non-lactose fermenter, colorless colony; *XLD*, xylose-lysinedeoxycholate agar.

*Most Enterobacteriaceae are indistinguishable on blood agar. Pink colonies on MacConkey agar with sorbitol are sorbitol fermenters; colorless colonies are non-sorbitol fermenters.

Approach to identification

In the early decades of the twentieth century, Enterobacteriaceae were identified using more than 50 biochemical tests in tubes; this method is still used today in reference and public health laboratories. Certain key tests such as indole, methyl red, Voges-Proskauer, and citrate, known by the acronym IMViC, were routinely performed to group the most commonly isolated pathogens.

Today, this type of conventional biochemical identification of enterics has become a historical footnote in most clinical and hospital laboratories in the United States.

In the latter part of the twentieth century, manufacturers began to produce panels of miniaturized tests for identification, first of enteric gram-negative rods and later of other groups of bacteria and yeast. Original panels were inoculated manually; these were followed by semiautomated and automated systems, the most sophisticated of which inoculate, incubate, read, and discard the panels. Practically any commercial identification system can be used to reliably identify the commonly isolated Enterobacteriaceae. Depending on the system, results are available within 4 hours or after overnight incubation. The extensive computer databases used by these systems include information on unusual biotypes.

The number of organisms used to define individual databases is important; in rare cases, isolated organisms or new microorganisms may be misidentified or not identified at all.

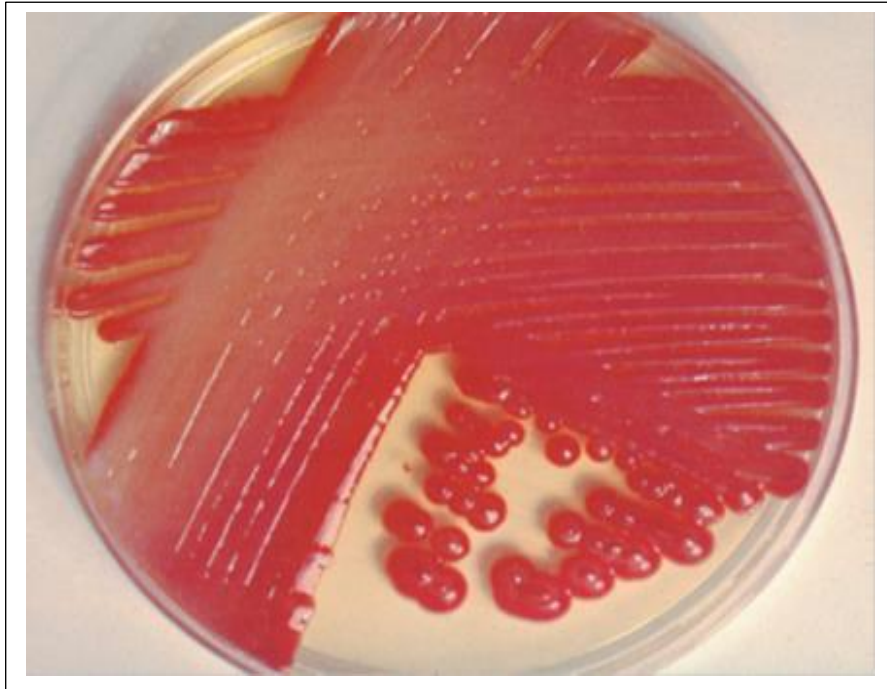
The definitive identification of enterics can be enhanced based on molecular methods, especially 16S ribosomal RNA (rRNA) sequencing and DNA-DNA

hybridization. Through the use of molecular methods, the genus *Plesiomonas*, composed of one species of oxidase-positive, gram-negative rods, now has been

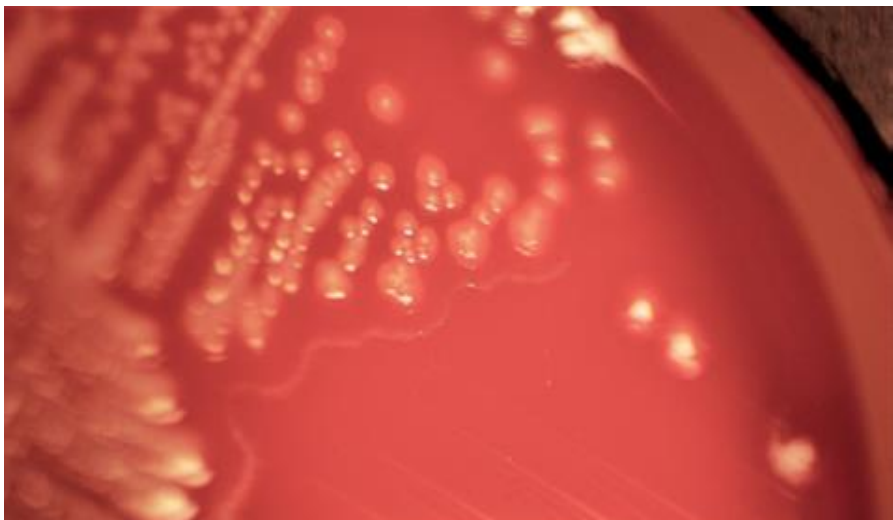
included in the family Enterobacteriaceae. *Plesiomonas* sp. clusters with the genus *Proteus* in the Enterobacteriaceae by 16S rRNA sequencing. However, like all other Enterobacteriaceae, *Proteus* organisms are Oxidase negative.

The clustering together of an oxidase-positive genus and an oxidase-negative genus is a revolutionary concept in microbial taxonomy.

In the interests of cost containment, many clinical laboratories use an abbreviated scheme to identify commonly isolated enterics. *E. coli*, for example, the most commonly isolated enteric organism, may be identified by a positive spot indole test. For presumptive identification of an organism as *E. coli*, the characteristic colonial appearance on MacConkey agar, as described in(Table 4), is documented along with positive spot indole test result. A spot indole test can also be used to quickly separate swarming *Proteae*, such as *P. mirabilis* and *P. penneri*, which are negative, from the indole-positive *P. vulgaris*.



Figure(2) Red-pigmented *Serratia marcescens* on MacConkey agar.



Figure(3) *Proteus mirabilis* swarming on blood agar (arrow points to swarming edge).

Specific Considerations for Identifying Enteric Pathogens

The common biochemical tests used to differentiate the species in the genus *Citrobacter* are illustrated in (Table 3.)

In most clinical laboratories, serotyping of Enterobacteriaceae is limited to the preliminary grouping of *Salmonella* spp., *Shigella* spp., and *E. coli* O157:H7. Typing should be performed from a non-sugar-containing medium, such as 5% sheep blood agar or LIA. Use of sugar-containing media, such as MacConkey or TSI agars, can cause the organisms to autoagglutinate.

Commercially available polyvalent antisera designated A, B, C1, C2, D, E, and Vi are commonly used to preliminarily group *Salmonella* spp. because 95% of isolates belong to groups A through E. The antisera A through E contain antibodies against somatic (“O”) antigens, and the Vi antiserum is prepared against the capsular (“K”) antigen of *S. serotype* Typhi. Typing is performed using a slide agglutination test. If an isolate agglutinates with the Vi antiserum and does not react with any of the “O” groups, then a saline suspension of the organism should be prepared and heated to 100°C for 10 minutes to inactivate

the Vi antigen. The organism should then be retested. *S. typhi* is positive with Vi and group D. Complete typing of *Salmonella* spp., including the use of antisera against the flagellar (“H”) antigens, is performed at reference laboratories. Preliminary serologic grouping of *Shigella* spp. is also performed using commercially available polyvalent somatic (“O”) antisera designated A, B, C, and D. As with *Salmonella* spp., *Shigella* spp. may produce a capsule and

therefore heating may be required before typing is successful. Subtyping of *Shigella* spp. beyond the groups A, B, and C (*Shigella* group D only has one serotype) is typically performed in reference laboratories.

P. shigelloides, a new member of the Enterobacteriaceae that can cause gastrointestinal infections might cross-react with *Shigella* grouping antisera, particularly group D, and lead to misidentification. This mistake can be avoided by performing an oxidase test. Sorbitol-negative *E. coli* can be serotyped using commercially available antisera to determine whether the somatic “O” antigen 157 and the flagellar “H” antigen 7 are present. Latex reagents and antisera are now also available for detecting some non-O157, sorbitol-fermenting, Shiga toxin-producing strains of *E. coli*.

Some national reference laboratories are simply performing tests for Shiga toxin rather than searching for O157 or non-O157 strains by culture. Unfortunately, isolates are not available then for strain typing for epidemiologic purposes. Laboratory tests to identify enteropathogenic, enterotoxigenic, enteroinvasive, and enteroaggregative *E. coli* that cause gastrointestinal infections usually involve animal, tissue culture, or molecular studies performed in reference laboratories.

The current recommendation for the diagnosis of Shiga toxin-producing *E. coli* includes testing all stools submitted from patients with acute community-acquired diarrhea to detect enteric pathogens (*Salmonella*, *Shigella*, and *Campylobacter* spp.) should be cultured for O157 STEC on selective and differential agar. In addition, these stools should be tested using either a Shiga toxin detection assay or a molecular assay to simultaneously determine whether the sample contains a non-O157 STEC. To save media, some laboratories may elect to perform the assay first, then attempt to grow organisms from broths with an assay-positive result

on selective media. In any case, any isolate or broth positive for O157STEC, non- O157STEC, or shiga toxin should be forwarded to the public health laboratory for confirmation and direct immunoassay testing. Any isolate positive for O157 STEC should be forwarded to the public health laboratory for additional epidemiologic analysis. Any specimens or enrichment broths that are positive for Shiga toxin or STEC but negative for O157 STEC should also be forward to the public health laboratory for further testing.

Most commercial systems can identify *Y. pestis* if a heavy inoculum is used. All isolates biochemically grouped as a *Yersinia* sp. should be reported to the public health laboratory. *Y. pestis* should always be reported and confirmed.

Serodiagnosis

Serodiagnostic techniques are used for only two members of the family Enterobacteriaceae; that is, *S. typhi* and *Y.pestis*. Agglutinating antibodies can be measured in the diagnosis of typhoid fever; a serologic test for *S. typhi* is part of the “febrile agglutinins” panel and is individually known as the Widal test. Because results obtained by using the Widal test are somewhat unreliable, this method is no longer widely used.

Serologic diagnosis of plague is possible using either a passive hemagglutination test or enzyme-linked immunosorbent assay; these tests are usually performed in reference laboratories.

Antimicrobial susceptibility testing and therapy:

For many of the gastrointestinal infections caused by Enterobacteriaceae, inclusion of antimicrobial agents as part of the therapeutic strategy is controversial or at least uncertain. The unpredictable nature of any clinical isolate’s antimicrobial susceptibility requires that testing be done as a guide to therapy. Several standard methods and commercial systems have been developed for this purpose.

The Clinical and Laboratory

Standards Institute and (CLSI) has created guidelines (CLSI document M-100 and M100-S23) for the minimum inhibitory concentration (MIC) and disk diffusion breakpoints for aztreonam, cefotaxime, cefpodoxime, ceftazidime, and ceftriaxone for *E. coli*, *Proteus*, and *Klebsiella* spp., as well as for cefpodoxime, ceftazidime, and cefotaxime for *P. mirabilis*. The sensitivity of the screening increases with the use of more than a single drug. ESBLs are inhibited by clavulanic acid; therefore, this property can be used as a confirmatory test in the identification process. In addition, with regard to cases in which moxalactam, cefonicid, cefamandole, or cefoperazone is being considered to treat infection caused by *E. coli*, *Klebsiella* spp., or *Proteus* spp., it is important to note that interpretive guidelines have not been evaluated, and ESBL testing should be performed. If isolates test ESBL positive, the results of the antibiotics listed should be reported as resistant.

CLSI has revised the interpretive criteria for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam. Using the new interpretive guidelines, routine ESBL testing is no longer

necessary, and it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins from susceptible to resistant. ESBL testing will remain useful for epidemiologic and infection control purposes.

Multidrug-resistant typhoid fever (MDRTF)

Multidrug-resistant typhoid fever is caused by *S. serotype Typhi* strains resistant to chloramphenicol, ampicillin, and cotrimoxazole. Isolates classified as MDRTF have been identified since the early 1990s in patients of all ages. The risk for the development of MDRTF is associated with the overuse, misuse and inappropriate use of antibiotic therapy.

Susceptibility tests should be performed using the typical first-line antibiotics, including chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, along with a fluoroquinolone and a nalidixic acid (to detect reduced susceptibility to fluoroquinolones), a third-generation cephalosporin, and any other antibiotic currently used for treatment.

Prevention

Vaccines are available for typhoid fever and bubonic plague; however, neither is routinely recommended in the United States. An oral, multiple-dose vaccine prepared against *S. serotype Typhi* strain or a parenteral single-dose vaccine containing Vi antigen is available for people traveling to an endemic area or for household contacts of a documented *S. serotype Typhi* carrier.

An inactivated multiple-dose, whole-cell bacterial vaccine is available for bubonic plague for people traveling to an endemic area. However, this vaccine does not provide protection against pneumonic plague. Individuals exposed to pneumonic plague should be given chemoprophylaxis with doxycycline (adults) or trimethoprim-sulfamethoxazole (children younger than 8 years of age)

Vibrio, Aeromonas, Chromobacterium, and Related Organisms

Genera and species to be considered:

<u>Current Name</u>	<u>Previous Name</u>
<i>Aeromonas caviae</i> complex	
<i>A. caviae</i>	
<i>A. media</i>	
<i>Aeromonas hydrophila</i> complex	
<i>A. hydrophila</i> subsp. <i>hydrophila</i>	
<i>A. hydrophila</i> subsp. <i>dhakensis</i>	
<i>A. bestiarum</i>	
<i>A. salmonicida</i>	
<i>Aeromonas veronii</i> complex	
<i>A. veronii</i> biovar <i>sobria</i>	
<i>A. veronii</i> biovar <i>veronii</i>	
<i>A. jandaei</i>	
<i>A. schubertii</i>	
<i>Chromobacterium violaceum</i>	
<i>Photobacterium damsela</i>	<i>Vibrio damsela</i>
<i>Grimontia hollisae</i>	CDC group EF-13; <i>Vibrio hollisae</i>
<i>Vibrio alginolyticus</i>	<i>Vibrio parahaemolyticus</i> biotype 2
<i>Vibrio cholerae</i>	
<i>Vibrio cincinnatiensis</i>	
<i>Vibrio fluvialis</i>	CDC group EF-6
<i>Vibrio furnissii</i>	
<i>Vibrio harveyi</i>	<i>Vibrio carchariae</i>
<i>Vibrio metschnikovii</i>	CDC enteric group 16
<i>Vibrio mimicus</i>	<i>Vibrio cholerae</i> (sucrose negative)
<i>Vibrio parahaemolyticus</i>	<i>Pasteurella parahaemolyticus</i>
<i>Vibrio vulnificus</i>	CDC group EF-3

General characteristics:

The organisms discussed in this lecture are considered together because they are all oxidase-positive glucosefermenting, gram negative bacilli capable of growth on MacConkey agar. Their individual morphologic and physiologic features are presented. Other halophilic organisms, such as *Halomonas venusta* and *Shewanella algae*, require salt but do not ferment glucose, as do the halophilic *Vibrio* spp.

Aeromonas spp. are gram-negative straight rods with rounded ends or coccobacillary facultative anaerobes that occur singly, in pairs, or in short chains. They are typically oxidase and catalase positive and produce acid from

oxidative and fermentative metabolism. *Chromobacterium violaceum* is a facultative anaerobic, motile, gram negative rod or cocci.

The family Vibrionaceae includes six genera, three of which are discussed in this lecture. The *Photobacterium* and *Grimontia* each include a single species. The genus *Vibrio* consists of 10 species of gram-negative, facultative anaerobic, curved or comma-shaped rods. Most species are motile and are catalase and oxidase positive except

Vibrio metschnikovii. All *Vibrio* spp. require sodium for growth and ferment glucose.

Epidemiology

Many aspects of the epidemiology of *Vibrio* spp., *Aeromonas* spp., and *C. violaceum* are similar (Table 1). The primary habitat for most of these organisms is water; generally, brackish or marine water for *Vibrio* spp., freshwater for *Aeromonas* spp., and soil or water for *C. violaceum*. *Aeromonas* spp. may also be found in brackish water

or marine water with a low salt content. None of these organisms are considered part of the normal human flora. Transmission to humans is by ingestion of contaminated water, fresh produce, meat, dairy products, or seafood or by exposure of disrupted skin and mucosal surfaces to contaminated water.

The epidemiology of the most notable human pathogen in this lecture, *Vibrio cholerae*, is far from being fully understood. This organism causes epidemics and pandemics (i.e., worldwide epidemics) of the diarrheal disease cholera. Since 1817 the world has witnessed seven cholera pandemics. During these outbreaks the organism is spread among people by the fecal-oral route, usually in environments with poor sanitation.

The niche that *V. cholerae* inhabits between epidemics is uncertain. The form of the organism shed from infected humans is somewhat fragile and cannot survive long in the environment. However, evidence suggests that the bacillus has survival, or dormant, stages that allow its long-term survival in brackish water or saltwater environments during interepidemic periods. These dormant stages are considered viable but nonculturable.

Asymptomatic carriers of *V. cholerae* have been documented, but they are not thought to be a significant reservoir for maintaining the organism between outbreaks.

Table (1) Epidemiology

Species	Habitat (Reservoir)	Mode of Transmission
<i>Vibrio cholerae</i>	Niche outside of human gastrointestinal tract between occurrence of epidemics and pandemics is uncertain; may survive in a dormant state in brackish or saltwater; human carriers also are known but are uncommon	Fecal-oral route, by ingestion of contaminated washing, swimming, cooking, or drinking water; also by ingestion of contaminated shellfish or other seafood
<i>V. alginolyticus</i>	Brackish or saltwater	Exposure to contaminated water

<i>V. cincinnatiensis</i>	Unknown	Unknown
<i>Photobacterium damsela</i>	Brackish or saltwater	Exposure of wound to contaminated water
<i>V. fluvialis</i>	Brackish or saltwater	Ingestion of contaminated water or seafood
<i>V. furnissii</i>	Brackish or saltwater	Ingestion of contaminated water or seafood
<i>Grimontia hollisae</i>	Brackish or saltwater	Ingestion of contaminated water or seafood
<i>V. metschnikovii</i>	Brackish, salt and freshwater	Unknown
<i>V. mimicus</i>	Brackish or saltwater	Ingestion of contaminated water or seafood
<i>V. parahaemolyticus</i>	Brackish or saltwater	Ingestion of contaminated water or seafood
<i>V. vulnificus</i>	Brackish or saltwater	Ingestion of contaminated water or seafood
<i>Aeromonas</i> spp.	Aquatic environments around the world, including freshwater, polluted or chlorinated water, brackish water and, occasionally, marine water; may transiently colonize gastrointestinal tract; often infect various warm- and cold-blooded animal species	Ingestion of contaminated food (e.g., dairy, meat, produce) or, water; exposure of disrupted skin or mucosal surfaces to contaminated water or soil; traumatic inoculation of fish fins and or fishing hooks
<i>Chromobacterium</i> <i>Violaceum</i>	Environmental, soil and water of tropical and subtropical regions. Not part of human flora	Exposure of disrupted skin to contaminated soil or water

Pathogenesis and spectrum of disease :

As a notorious pathogen, *V. cholerae* elaborates several toxins and factors that play important roles in the organism's virulence. Cholera toxin (CT) is primarily responsible for the key features of cholera (Table 2). Release of this toxin causes mucosal cells to hypersecrete water and electrolytes into the lumen of the gastrointestinal tract.

The result is profuse, watery diarrhea, leading to dramatic fluid loss. The fluid loss results in severe dehydration and hypotension that, without medical intervention, frequently lead to death. This toxin-mediated disease does not require the organism to penetrate the mucosal barrier. Therefore, blood and the inflammatory cells typical of dysenteric stools are notably absent in cholera. Instead, "rice water stools," composed of fluids and mucous flecks, are the hallmark of cholera toxin activity.

V. cholerae is divided into three major subgroups; *V. cholerae* O1, *V. cholerae* O129, and *V. cholerae* non-O1. The somatic antigens O1 and O139 associated with the *V. cholerae* cell envelope are positive markers for strains capable of epidemic and pandemic spread of the disease.

Strains carrying these markers almost always produce cholera toxin, whereas non-O1/non-O139 strains do not produce the toxin and hence do not produce cholera. Therefore, although these somatic antigens are not

virulence factors per se, they are important virulence and epidemiologic markers that provide important information about *V. cholerae* isolates. The non-O1/non-O139 strains are associated with nonepidemic diarrhea and extraintestinal infections.

V. cholerae produces several other toxins and factors, but the exact role of these in disease is still uncertain (see Table 2). To effectively release toxin, the organism first must infiltrate and distribute itself along the cells lining the mucosal surface of the gastrointestinal tract. Motility and chemotaxis mediate the distribution of organisms, and mucinase production allows penetration of the mucous layer. Toxin coregulated pili (TCP) provide the means by which bacilli attach to mucosal cells for release of cholera toxin.

Depending on the species, other vibrios are variably involved in three types of infection: gastroenteritis, wound infections, and bacteremia. Although some of these organisms have not been definitively associated with human infections, others, such as *Vibrio vulnificus*, are known to cause fatal septicemia, especially in patients suffering from an underlying liver disease.

Aeromonas spp. are similar to *Vibrio* spp. in terms of the types of infections they cause. Although these organisms can cause gastroenteritis, most frequently in children, their role in intestinal infections is not always clear.

Therefore, the significance of their isolation in stool specimens should be interpreted with caution. Severe watery diarrhea has been associated with *Aeromonas* strains that produce a heat-labile enterotoxin and a heat-stable enterotoxin. In addition to diarrhea, complications of infection with *Aeromonas* spp. include hemolytic-uremic syndrome and kidney disease.

Table (2) Pathogenesis and Spectrum of Diseases

Species	Virulence Factors	Spectrum of Disease and Infections
<i>Vibrio cholerae</i>	Cholera toxin; zonula occludens (Zot) toxin (enterotoxin); accessory cholera enterotoxin (Ace) toxin; O1 and O139 somatic antigens, hemolysin/cytotoxins, motility, chemotaxis, mucinase, and toxin coregulated pili (TCP) pili.	Cholera: profuse, watery diarrhea leading to dehydration, hypotension, and often death; occurs in epidemics and pandemics that span the globe. May also cause nonepidemic diarrhea and, occasionally, extra intestinal infections of wounds, respiratory tract, urinary tract, and central nervous system
<i>V. alginolyticus</i>	Specific virulence factors for the non- <i>V. cholerae</i> species are uncertain	Ear infections, wound infections; rare cause of septicemia; involvement in gastroenteritis is uncertain
<i>V. cincinnatiensis</i>		Rare cause of septicemia

<i>P. damsela</i>		Wound infections and rare cause of septicemia
<i>V. fluvialis</i>		Gastroenteritis
<i>V. furnissii</i>		Rarely associated with human infections
<i>Grimontia hollisa</i>		Gastroenteritis; rare cause of septicemia
<i>V. metschnikovii</i>		Rare cause of septicemia; involvement in gastroenteritis is uncertain
<i>V. mimicus</i>		Gastroenteritis; rare cause of ear infection
<i>V. vulnificus</i>		Wound infections and septicemia; involvement in gastroenteritis is uncertain
<i>Aeromonas</i> spp.	<i>Aeromonas</i> spp. produce various toxins and factors, but their specific role in virulence is uncertain	Gastroenteritis, wound infections, bacteremia, and miscellaneous other infections, including endocarditis, meningitis, pneumonia, conjunctivitis, and osteomyelitis
<i>Chromobacterium</i> <i>Violaceum</i>	Endotoxin, adhesins, invasins and cytolytic proteins have been described.	Rare but dangerous infection. Begins with cellulitis or lymphadenitis and can rapidly progress to systemic infection with abscess formation in various organs and septic shock

C. violaceum is not associated with gastrointestinal infections, but acquisition of this organism by contamination of wounds can lead to fulminant, life-threatening systemic infections.

Laboratory diagnosis

Specimen collection and transport

Because no special considerations are required for isolation of these genera. However, stool specimens suspected of containing *Vibrio* spp. should be collected and transported only in Cary-Blair medium.

Buffered glycerol saline is not acceptable, because glycerol is toxic for vibrios. Feces is preferable, but rectal swabs are acceptable during the acute phase of diarrheal illness.

Specimen processing

No special considerations are required for processing of the organisms .

Direct detection methods

V. cholerae toxin can be detected in stool using an enzyme linked immunosorbent assay (ELISA) or a commercially available latex agglutination test but these tests are not widely used in the United States. Microscopically, vibrios are gram-negative, straight or slightly curved rods (Figure 1).

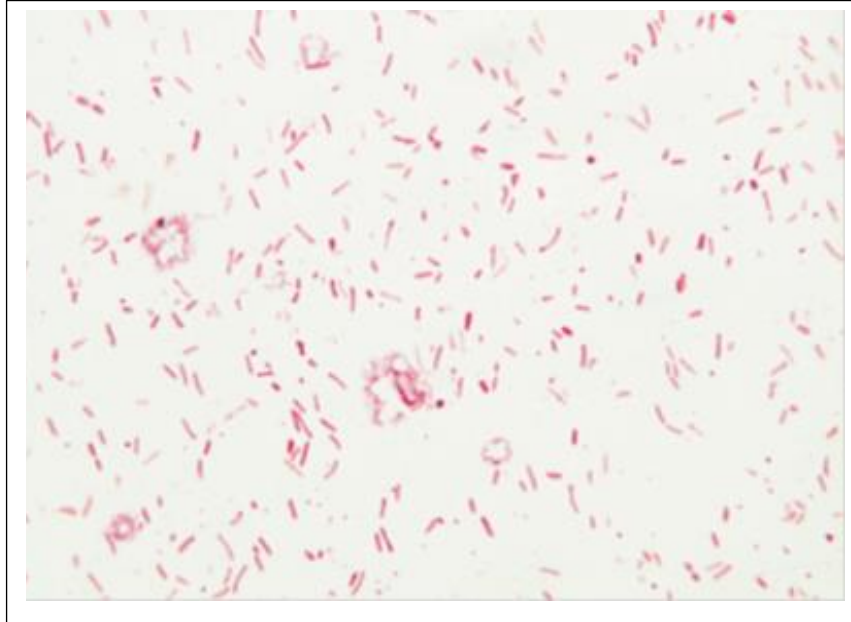


Figure 1 Gram stain of *Vibrio parahaemolyticus*

When stool specimen from patients with cholera are examined using darkfield microscopy, the bacilli exhibit characteristic rapid darting or shooting-star motility. However, direct microscopic examination of stools by any method is not commonly used for laboratory diagnosis of enteric bacterial infections.

Aeromonas spp. are gram-negative, straight rods with rounded ends or coccobacilli. No molecular or serologic methods are available for direct detection of *Aeromonas* spp. Cells of *C. violaceum* are slightly curved, medium to long, gram-negative rods with rounded ends. A polymerase chain reaction (PCR) amplification assay has been developed for identification of *C. violaceum*.

Cultivation:

Media of Choice

Stool cultures for *Vibrio* spp. are plated on the selective medium thiosulfate citrate bile salts sucrose (TCBS) agar.

TCBS contains 1% sodium chloride, bile salts that inhibit the growth of gram-positive organisms, and sucrose for the differentiation of the various *Vibrio* spp. Bromothymol blue and thymol blue pH indicators are added to the medium. The high pH of the medium (8.6) inhibits the growth of other intestinal flora. Although some *Vibrio* spp. grow very poorly on this medium, those that grow well produce either yellow or green colonies, depending on whether they are able to ferment sucrose (which produces yellow colonies).

Alkaline peptone water (pH 8.4) may be used as an enrichment broth for obtaining growth of vibrios from stool. After inoculation, the broth is incubated for 5 to 8 hours at 35°C and then subcultured to TCBS.

Chromogenic *Vibrio* agar, which was developed for the recovery of *Vibrio parahaemolyticus* from seafood, supports the growth of other *Vibrio* spp. Colonies on this agar range from white to pale blue and violet.

Aeromonas spp. are indistinguishable from *Yersinia enterocolitica* on modified cefsulodin-irgasan-novobiocin (CIN) agar (4 µg/mL of cefsulodin); therefore, it is important to perform an oxidase test to differentiate the two genera. *Aeromonas* agar is a relatively new alternative medium that uses D-xylose as a differential characteristic.

These organisms typically grow on a variety of differential and selective agars used for the identification of enteric pathogens. They are also beta-hemolytic on blood agar.

C. violaceum grows on most routine laboratory media. The colonies may be beta-hemolytic and have an almond like odor. Most strains produce violacein, an ethanol-soluble violet pigment.

All of the genera considered in this lecture grow well on 5% sheep blood, chocolate, and MacConkey agars.

They also grow well in the broth of blood culture systems and in thioglycollate or brain-heart infusion broths.

Incubation Conditions and Duration

These organisms produce detectable growth on 5% sheep blood and chocolate agars when incubated at 35°C in carbon dioxide or ambient air for a minimum of 24 hours. MacConkey and TCBS agars only should be incubated at 35°C in ambient air. The typical violet pigment of *C. violaceum* colonies (Figure 2) is optimally produced when cultures are incubated at room temperature (22°C).

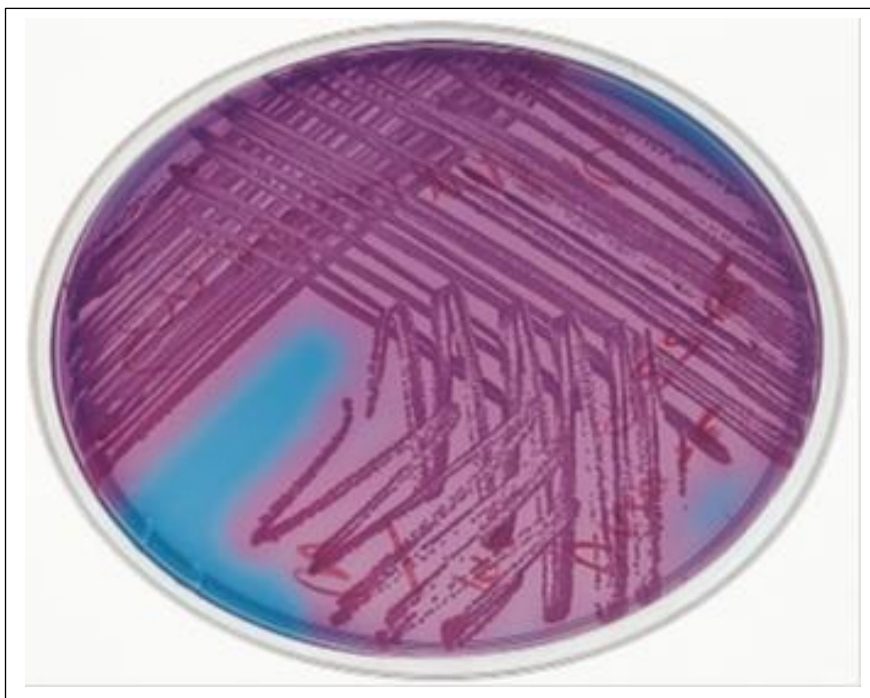


Figure 2 Colonies of Chromo bacterium violaceum on DNase agar. Note violet pigment

Table(3) Colonial Appearance and Characteristic

Organism	Medium	Appearance
Aeromonas spp.	BA Mac	Large, round, raised, opaque; most pathogenic strains are beta-hemolytic except <i>A. caviae</i> , which is usually nonhemolytic Both NLF and LF
Chromobacterium violaceum	BA Mac	Round, smooth, convex, some strains are beta-hemolytic; most colonies appear black or very dark purple; cultures smell of ammonium cyanide (almond-like) NLF
Vibrio spp. and Grimontia hollisae	BA Mac	Medium to large, smooth, opaque, iridescent with a greenish hue; <i>V. cholerae</i> , <i>V. fluvialis</i> , and <i>V. mimicus</i> can be beta-hemolytic NLF except <i>V. vulnificus</i> , which may be LF
<i>P. damsela</i>	BA Mac	Medium to large, smooth, opaque, iridescent with a greenish hue; may be beta-hemolytic NLF

BA, 5% sheep blood agar; *Mac*, MacConkey agar; LF, lactose fermenter, *NLF*, non-lactose fermenter.

Colonial Appearance

Table 3 describes the colonial appearance and other distinguishing characteristics (e.g., hemolysis and odor) of each genus on 5% sheep blood and MacConkey agars. The appearance of *Vibrio* spp. on TCBS is shown in Figure (3).

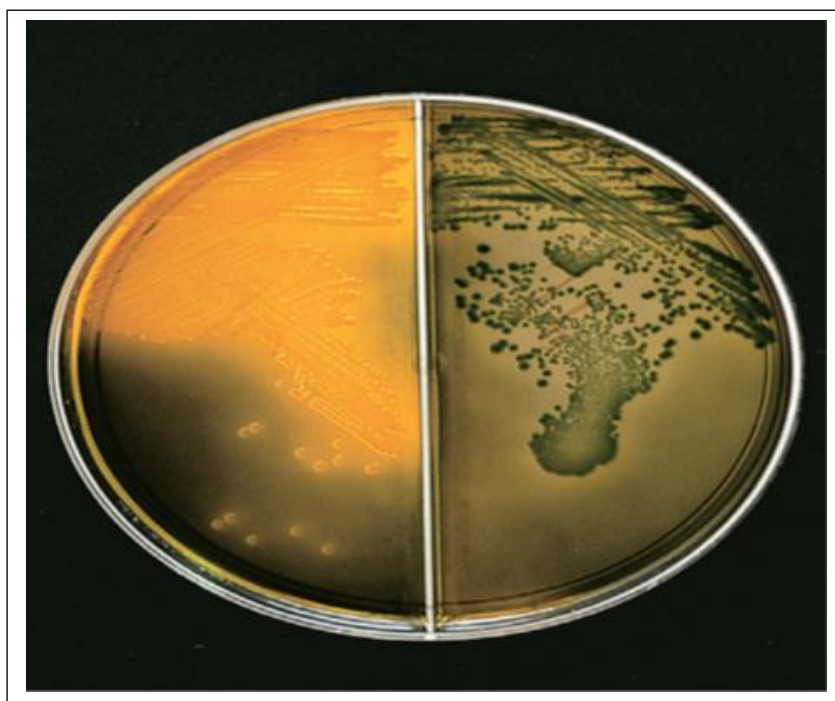


Figure 3 Colonies of *Vibrio cholerae* (A) and *V. parahaemolyticus* (B) on TCBS agar.

Approach to identification

The colonies of these genera resemble those of the family Enterobacteriaceae but can be distinguished notably by their positive oxidase test result (except for *V. metschnikovii*, which is oxidase negative). The oxidase test must be performed from 5% sheep blood or another medium without a fermentable sugar (e.g., lactose in MacConkey agar or sucrose in TCBS), because fermentation of a carbohydrate results in acidification of the medium, and a false-negative result may occur if the surrounding pH is below 5.1. Likewise, if the violet pigment of a suspected *C. violaceum* isolate interferes with performance of the oxidase test, the organism should be grown under anaerobic conditions (where it cannot produce pigment) and retested.

The reliability of commercial identification systems has not been widely validated for identification of these organisms, although most are listed in the databases of several systems. The API 20E system (bioMérieux, St.Louis, Missouri) is one of the best for vibrios. Because the inoculum is prepared in 0.85% saline, the amount of salt often is enough to allow growth of the halophilic (salt-loving) organism.

The ability of most commercial identification systems to accurately identify *Aeromonas* organisms to the species level is limited and uncertain, and with some kits, difficulty arises in separating *Aeromonas* spp. from *Vibrio* spp. Therefore, identification of potential pathogens should be confirmed using conventional biochemical tests or serotyping. show several characteristics that can be used to presumptively group *Vibrio* spp., *Aeromonas* spp., and *Chromobacterium violaceum*

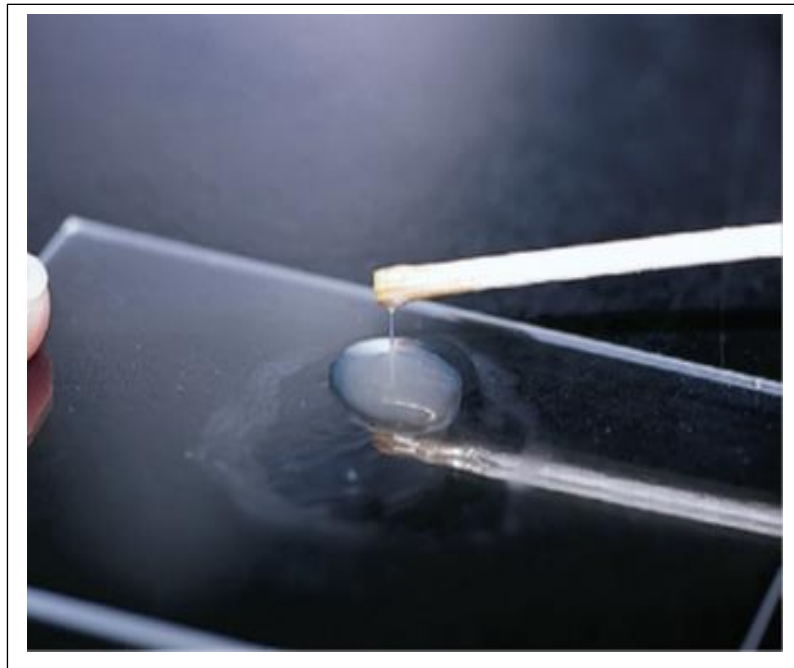


Figure 4 String test used to differentiate *Vibrio* spp. (positive) from *Aeromonas* spp. and *P. shigelloides* (negative).

Comments Regarding Specific Organisms

V. cholerae and *Vibrio mimicus* are the only *Vibrio* spp. That do not require salt for growth. Therefore, a key test for distinguishing the halophilic species from *V. cholerae*, *V. mimicus*, and *Aeromonas* spp. is growth in nutrient broth with 6% salt. Furthermore, the addition of 1% NaCl to conventional biochemical tests is recommended to allow growth of halophilic species.

The string test can be used to differentiate *Vibrio* spp. from *Aeromonas* spp. In this test, organisms are emulsified in 0.5% sodium deoxycholate, which lyses *Vibrio* cells, but not those of *Aeromonas* spp. Cell lysis releases DNA, which can be pulled up into a string with an inoculating loop Figure (4).

A *Vibrio* static test using 0/129 (2,4-diamino-6,7-diisopropylpteridine)–impregnated disks also has been used to separate vibrios (susceptible) from other Oxidase positive glucose fermenters (resistant) and to differentiate *V. cholerae* O1 and non-O1 (susceptible) from other *Vibrio* spp. (resistant). However, recent strains of *V. cholerae* O139 have demonstrated resistance, so the dependability of this test is questionable.

Serotyping should be performed immediately to further characterize *V. cholerae* isolates. Toxigenic strains of serogroup O1 and O139 can be involved in cholera epidemics. Strains that do not type in either antiserum are identified as non-O1. Although typing sera are commercially available, isolates of *V. cholerae* are usually sent to a reference laboratory for serotyping.

Identification of *V. cholerae* or *V. vulnificus* should be reported immediately because of the life-threatening nature of these organisms.

Aeromonas spp. and *C. violaceum* can be identified using the characteristics. *Aeromonas* spp. identified in clinical specimens should be identified as *A. hydrophilia*, *A. caviae* complex, or *A. veronii* complex.

Pigmented strains of *C. violaceum* are so distinctive that a presumptive identification can be made based on colonial appearance, oxidase, and Gram staining. Nonpigmented strains (approximately 9% of isolates) may be differentiated from *Pseudomonas*, *Burkholderia*, *Brevundimonas*, and *Ralstonia* organisms based on glucose fermentation and a positive test result for indole. Negative lysine and ornithine reactions are useful criteria for distinguishing *C. violaceum* from *Plesiomonas shigelloides*. Failure to ferment either maltose or mannitol also differentiates *C. violaceum* from *Aeromonas* spp.

Serodiagnosis

Agglutination, vibriocidal, or antitoxin tests are available for diagnosing cholera using acute and convalescent sera. However, these methods are most commonly used for epidemiologic purposes. Serodiagnostic techniques are not generally used for laboratory diagnosis of infections caused by the other organisms discussed in this lecture.

Antimicrobial susceptibility testing and therapy:

Two components of the management of patients with cholera are rehydration and antimicrobial therapy (Table4). Antimicrobials reduce the severity of the illness and shorten the duration of organism shedding. The drug of choice for cholera is tetracycline or doxycycline; however, resistance to these agents is known, and the use of other agents, such as chloramphenicol, ampicillin, or trimethoprim-sulfamethoxazole, may be necessary.

The Clinical and Laboratory Standards Institute (CLSI) has established methods for testing for *V. cholerae*, and the CLSI document should be consulted for this purpose.

The need for antimicrobial intervention for gastrointestinal infections caused by other *Vibrio* spp. and *Aeromonas* spp. is less clear. However, extraintestinal infections with these organisms and with *C. violaceum* can be lifethreatening, and directed therapy is required.

C. violaceum is often resistant to β -lactams and colistin. Antimicrobial agents with potential activity are listed, where appropriate, in Table 4. It is important to note these organisms' ability to show resistance to therapeutic agents; especially noteworthy is the ability of *Aeromonas* spp. to produce various beta-lactamases.

Table 4 Antimicrobial Therapy and Susceptibility Testing

Species	Therapeutic Options	Potential Resistance to Therapeutic Options
<i>Vibrio cholerae</i>	Adequate rehydration plus antibiotics. Recommended agents include tetracycline or doxycycline; alternatives include trimethoprim-sulfamethoxazole, erythromycin, chloramphenicol, and quinolones	Adequate rehydration plus antibiotics. Recommended agents include tetracycline or doxycycline; alternatives include trimethoprim-sulfamethoxazole, erythromycin, chloramphenicol, and quinolones
Other <i>Vibrio</i> spp.	No definitive guidelines. For gastroenteritis, therapy may not be needed; for wound infections and septicemia, potentially active agents include tetracycline, chloramphenicol, nalidixic acid, most cephalosporins, and quinolones	Similar to resistance reported for <i>V. cholerae</i>
<i>Aeromonas</i> spp.	No definitive guidelines. For gastroenteritis, therapy may not be needed; for soft tissue infections and septicemia, potentially active agents include ceftriaxone, cefotaxime, ceftazidime, imipenem, aztreonam, amoxicillin-clavulanate, quinolones, and trimethoprim-sulfamethoxazole	Capable of producing various beta-lactamases that mediate resistance to penicillins and certain cephalosporins
<i>Chromobacterium violaceum</i>	No definitive guidelines. Potentially active agents include cefotaxime,	Activity of penicillins is variable; activity of first- and

	ceftazidime, imipenem, and aminoglycosides	second-generation cephalosporins is poor

*Validated testing methods include standard methods recommended by the Clinical and Laboratory Standards Institute (CLSI) and commercial methods approved by the U.S. Food and Drug Administration (FDA).

Prevention:

No cholera vaccine is available in the United States. Two oral vaccines are available outside the United States although the World Health Organization no longer recommends immunization for travel to or from cholera-infected areas. Individuals who have recently shared food and drink with a patient with cholera (e.g., household contacts) should be given chemoprophylaxis with tetracycline, doxycycline, or trimethoprim-sulfamethoxazole. However, mass chemoprophylaxis during epidemics is not indicated.

Genus *Campylobacter*

Campylobacter jejuni and *C. coli* can colonize the intestinal tract of most mammals and birds and are the most frequently isolated *Campylobacter* species in humans with gastro-enteritis. Transmission from animals to humans is mainly through consumption and handling of animal food products but also direct contact with colonized animals may contribute to human Campylobacteriosis.

The fecal contamination of meat (especially poultry meat) during processing is considered to be a major source of human food-borne disease.

In humans, extra intestinal infections, including bacteremia, Guillain–Barré syndrome, reactive arthritis, and abortion could occur as sequelae of infection.

Detection of intestinal colonization is based on the isolation of the organism from feces, rectal swabs and / or caecal contents.

Alternatively, their high motility can be exploited using filtration techniques for isolation. Preliminary confirmation of isolates can be made by light microscopy. The organisms in the log growth phase are short and S-shaped in appearance, while coccoid forms predominate in older cultures.

Under phase-contrast microscopy the organisms have a characteristic rapid corkscrew-like motility. Phenotypic identification is based on reactions under different growth conditions. Biochemical and molecular tests can be used to confirm various *Campylobacter* species. Polymerase chain reaction assays also can be used for the direct detection of *C. jejuni* and *C. coli*.

In 1991 a revision of the taxonomy and nomenclature of the genus *Campylobacter* was proposed. According to *Bergey's Manual*, the genus *Campylobacter* comprises sixteen species and six subspecies. More recently, two additional species have been added.

Members of the genus are typically gram-negative, non-spore-forming, S-shaped or spiral shaped bacteria (0.2–0.8 µm wide and 0.5–5 µm long), with single polar flagella at one or both ends, conferring a characteristic corkscrew-like motility. These bacteria require micro aerobic conditions, but some strains also grow aerobically or anaerobically. They neither ferment nor oxidise carbohydrates. Some species, particularly *C. jejuni*, *C. coli* and *C. lari*, are thermophilic, growing optimally at 42°C. They can colonize mucosal surfaces, usually the intestinal tract, of most mammalian and avian species tested. The species *C. jejuni* includes two subspecies (*C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*) that can be discriminated on the basis of several phenotypic tests (nitrate reduction, selenite reduction, sodium fluoride, and safranin) and growth at 42°C (subsp. *doylei* does not grow at 42°C). Subspecies *jejuni* is much more frequently isolated than subspecies *doylei*.

Passive filtration

Passive filtration, a method developed by *Steele and McDermott* for the need for selective media; thus it is very useful for the isolation of antimicrobial-sensitive *Campylobacter* species.

The method does not use expensive selective media; it may be used in laboratories with fewer resources.

For passive filtration, feces are mixed with phosphorylate buffered saline (approximately 1/10 dilution) to produce a suspension. Approximately 100 µl of this suspension are then carefully layered on to a 0.45 or 0.65 µm filter, which has been previously placed on top of a non-selective blood agar plate. Care must be taken not to allow the inoculum to spill over the edge of the filter. The bacteria are allowed to migrate through the filter for 30–45 minutes at 37°C or room temperature. The filter is then removed, the fluid that has passed through

the filter is spread with a sterile glass or plastic spreader, and the plate is incubated micro aerobically at 42°C (Micro aerobic atmospheres of 5–10% oxygen, 5–10% carbon dioxide are required for optimal growth).

Specimen Types

Fresh stool specimens or rectal swabs from patients suspected to be infected with *Campylobacter* species, or meat.

Stool specimens, swabs, and food samples should not be older than 24 to 48 hours. Swabs must be inserted into appropriate transport media (*e.g.*, *Cary Blair medium*). If not processed immediately, store specimens in transport media at 4 to 8° C; avoid exposure to oxygen.

Samples Processing

Streak the specimen for dilution as soon as possible after it is received in the laboratory onto Butzler or Skirrow. Meat or other foods should first be minced or homogenized and then inoculated directly or after suspension in a small amount of peptone broth onto the medium.

If material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate inoculated plates, protected from light, at $35 \pm 2^\circ \text{C}$ or $42 \pm 2^\circ \text{C}$ in a reduced oxygen, increased CO₂ (micro aerobic) atmosphere. The incubation at 42° C results in better selectivity, but is inhibitory to *Campylobacter jejuni subsp. doylei* and a variety of other species.

The micro aerobic atmosphere can be achieved by using *Campy gaspak*. An incubation period of 2 to 3 days is usually sufficient, but 5 to 7 days of incubation were shown to increase the isolation rates.

Cultivation Media

Campylobacter agar known as Butzler plus *Campylobacter* agar known as Skirrow; are 2 selective media for the isolation of *Campylobacter* species from clinical specimens.

The genus *Campylobacter* includes important pathogens causing intestinal infections such as diarrhea. In rural areas and in less developed countries, *Campylobacters* are at least as frequent as *Salmonella* as intestinal pathogens.

The most frequently isolated species is *Campylobacter jejuni subsp. jejuni*, whereas *C. coli* and *C. lari* are rarer.

Butzler, in 1973, developed a selective medium containing five antimicrobials. Skirrow, in 1977, reported a selective culture medium containing three antimicrobial agents.

In Butzler, meat extract and peptone provide the nutrients, and sodium chloride maintains the osmotic stability. Novobiocin and colistin inhibit gram negative enteric bacteria; cephalosporin and bacitracin inhibit gram positive bacteria. Cycloheximide inhibits many fungi.

In Skirrow, heart infusion, casein peptone, and yeast extract provide nutrients, and sodium chloride maintains the osmotic stability. Vancomycin inhibits gram positives, and trimethoprim and polymyxin B inhibit many gram negative organisms. Lysed horse blood provides nutrients and heme for bacterial catalase.

Isolate Confirmation

A pure culture is required for confirmatory tests, but a preliminary confirmation can be obtained by direct microscopic examination of suspect colony material.

- 1- *Identification on solid medium:* on Skirrow or other blood-containing agars, characteristic *Campylobacter* colonies are slightly pink, round, convex, smooth and shiny, with a regular edge. On charcoal-based media, the characteristic colonies are grayish, flat and moistened, with a tendency to spread, and may have a metal sheen.
- 2- *Microscopic examination of morphology and motility:* material from a suspect colony is suspended in saline and evaluated, preferably by a phase-contrast microscope, for characteristic, spiral or curved slender rods with a corkscrew-like motility. Older cultures show less motile coccoid forms.
- 3- *Detection of oxidase:* take material from a suspect colony and place it on to a filter paper moistened with oxidase reagent. The appearance of a violet or deep blue color within 10 seconds is a positive reaction.
- 4- *Micro aerobic growth at 25°C:* Inoculate the pure culture on to a non-selective blood agar plate and incubate at 25°C in a micro aerobic atmosphere for 48 hours.
- 5- *Aerobic growth at 41.5°C,* inoculate the pure culture on to a non-selective blood agar plate and incubate at 41.5°C in an aerobic atmosphere for 48 hours.
- 6- *Latex agglutination tests* for confirmation of pure cultures of *C. jejuni* / *C. coli* (often also including *C. lari*) are commercially available.

Helicobacter pylorus

Helicobacter pylorus (*H. pylori*) is a spiral-shaped bacterium that is found in the gastric mucous layer or adherent to the epithelial lining of the stomach.

H. pylori cause more than 90% of duodenal ulcers and up to 80% of gastric ulcers. Spicy food, acid, stress, and lifestyle were considered the major causes of ulcers.

Majority of patients were given long-term medications, such as H2 blockers, and more recently, proton pump inhibitors, without a chance for permanent cure.

These medications relieve ulcer-related symptoms, heal gastric mucosal inflammation, and may heal the ulcer, but they do not treat the infection.

When acid suppression is removed, the majority of ulcers, particularly those caused by *H. pylori*, recur. So most ulcers which caused by *H. pylori*, appropriate antibiotic regimens can successfully eradicate the infection in most patients, with complete resolution of mucosal inflammation and a minimal chance for recurrence of ulcers; approximately 2/3 of the world's population is infected with *H. pylori*.

H. pylori are more prevalent among adults and lower socioeconomic groups.

Most persons who are infected with *H. pylori* never suffer any symptoms related to the infection; however, *H. pylori* cause chronic active, chronic persistent and atrophic gastritis in adults and children. Infection with *H. pylori* also causes duodenal and gastric ulcers.

Infected persons have a 2- to 6-fold increased risk of developing gastric cancer and mucosal associated-lymphoid-type lymphoma compared with their uninfected counterparts.

The role of *H. pylori* in non-ulcer dyspepsia remains unclear. Peptic ulcer disease at some point in their lifetime. Each year there are 500,000 to 850,000 new cases of peptic ulcer disease and more than one million ulcer-related hospitalizations.

The pain typically occurs when the stomach is empty, between meals and in the early morning hours, but it can also occur at other times.

It may last from minutes to hours and may be relieved by eating or by taking antacids.

Less common ulcer symptoms include nausea, vomiting, and loss of appetite. Bleeding can also occur; prolonged bleeding may cause anemia leading to weakness and fatigue.

Persons with active gastric or duodenal ulcers or documented history of ulcers should be tested for *H. pylori*, and if found to be infected, they should be treated.

Several methods may be used to diagnose *H. pylori* infection. Serological tests that measure specific *H. pylori* IgG antibodies can determine if a person has been infected. The sensitivity and specificity of these assays range from 80% to 95% depending upon the assay used.

Other diagnostic method is the breath test. In this test, the patient is given either ¹³C or ¹⁴C-labeled urea to drink. *H. pylori* metabolize the urea rapidly, and the labeled carbon is absorbed. This labeled carbon can then be measured as CO₂ in the patient's expired breath to determine whether *H. pylori* is present. The sensitivity and specificity of the breath test ranges from 94% to 98%.

During endoscopy, biopsy specimens of the stomach and duodenum are obtained and the diagnosis of *H. pylori* can be made by several methods:

1. The biopsy urease test - a colorimetric test based on the ability of *H. pylori* to produce urease; it provides rapid testing at the time of biopsy.
2. Histological identification of organisms - considered the gold standard of diagnostic tests.
3. Culture of biopsy specimens for *H. pylori*, which requires an experienced laboratory and is necessary when antimicrobial susceptibility testing is desired.

Therapy for *H. pylori* infection consists of 10 days to 2 weeks of one or two effective antibiotics, such as amoxicillin, tetracycline (not to be used for children <12 yrs.), metronidazole, or clarithromycin, plus either ranitidine bismuth citrate, bismuth subsalicylate, or a proton pump inhibitor.

Acid suppression by the H₂ blocker or proton pump inhibitor in conjunction with the antibiotics helps in relieve ulcer-related symptoms (i.e., abdominal pain, nausea), helps heal gastric mucosal inflammation, and may enhance efficacy of the antibiotics against *H. pylori* at the gastric mucosal surface.

Haemophilus

is a genus of Gram-negative, pleomorphic, coccobacilli bacteria belonging to the *Pasteurellaceae* family. While *Haemophilus* bacteria are typically small coccobacilli, they are categorized as *pleomorphic* bacteria because of the wide range of shapes they occasionally assume.

All *Haemophilus* are nonmotile, they are minute in size, *H. influenzae* measuring 0.3 micrometer across and up to 2 micrometers long.

The genus includes commensal organisms along with some significant pathogenic species such as *H. influenzae* a cause of sepsis and bacterial meningitis in young children and *H. ducreyi*, the causative agent of chancroid. All members are either aerobic or facultative anaerobic.

Members of the *Haemophilus* genus will not grow on blood agar plates as all species require at least one of the following blood factors for growth: hemin (factor X) and/or nicotinamide adenine dinucleotide (factor V).

Chocolate agar is an excellent *Haemophilus* growth medium as it allows for increased accessibility to these factors. Alternatively, *Haemophilus* is sometimes cultured using the "**Staph streak**" technique; both *Staphylococcus* and *Haemophilus* organisms are cultured together on a single blood agar plate. In this case, *Haemophilus* colonies will frequently grow in small "**satellite**" colonies around the larger *Staphylococcus* colonies because the metabolism of *Staphylococcus* produces the necessary blood factor by-products required for *Haemophilus* growth

Most strains of *H. influenzae* are opportunistic pathogens; that is, they usually live in their host without causing disease, but cause problems only when other factors (such as a viral infection, reduced immune function or chronically inflamed tissues, e.g. from allergies) create an opportunity. They infect the host by sticking to the host cell using Trimeric Auto transporter Adhesins (TAA).

Naturally acquired disease caused by *H. influenzae* seems to occur in humans only. In infants and young children, ***H. influenzae* type b (Hib)** causes bacteremia, pneumonia, epiglottitis and acute bacterial meningitis. On occasion, it causes cellulitis, osteomyelitis, and infectious arthritis.

Due to routine use of the **Hib conjugate vaccine** in the U.S.A. since 1990, the incidence of invasive Hib disease has decreased to 1.3/100,000 in children. However, Hib remains a major cause of lower respiratory tract infections in infants and children in developing countries where the vaccine is not widely used.

Unencapsulated *H. influenzae* strains are unaffected by the Hib vaccine and cause ear infections (otitis media), eye infections (conjunctivitis), and sinusitis in children, and are associated with pneumonia.

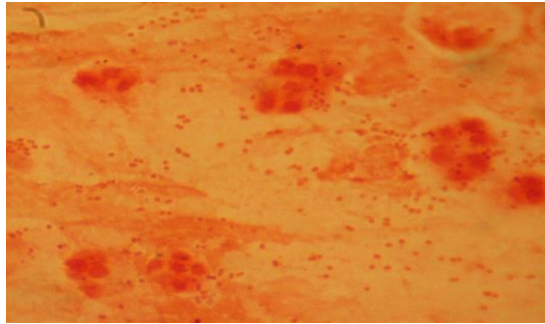


Figure shows Gram film *H. influenzae* in the sputum (coccobacilli)



Figure shows *Haemophilus influenzae* which requires X and V factors for growth. In this culture *Haemophilus* has only grown around the paper disc that has been impregnated with X and V factors. There is no bacterial growth around the discs that only contain either X or V factor

Laboratory Diagnosis

- ❖ Most *Haemophilus* species are normal inhabitants of upper respiratory tract of humans and other animals.
- ❖ The species of *Haemophilus* that most frequently cause human infections are *H. influenzae* (respiratory and invasive infections), *H. aegyptius* (acute conjunctivitis) and *H. parainfluenzae*, *H. haemolyticus*, *H. parahaemolyticus*, *H. aphrophilus*, *H. paraphrophilus* and *H. segnis* (abscesses and infective endocarditis)
- ❖ These are pleomorphic Gram negative coccobacilli.
- ❖ The laboratory diagnosis of *H. influenzae* is based on growth and colony morphology, and cell morphology on Gram staining.

- ❖ These are confirmed by the haemophilic character of the genus that reflects a requirement for either or both of the two factors called X and V.

Culture and identification for *Haemophilus*

Direct Examination

- ✓ Perform Gram stain
- ✓ Gram staining shows: Gram negative pleomorphic thin rods or coccobacilli.

Culture

- Inoculate samples onto chocolate agar media
- Incubate at 37°C in aerobic atmosphere containing 3-5 % CO₂ (i.e. candle jar) for 24-48 hours.

Colony morphology

- + On chocolate agar: large flat, colorless to gray or opaque colonies.
- + Colonies are 0.5 – 1mm circular, low convex, smooth, and pale grey and transparent.
- + With a characteristic “mouse nest” odor. No haemolysis or discoloration is seen.
- + Encapsulated strains appear more mucoid (watery) and non capsulated strains appear as compact grayish colonies.

Note: Growth is enhanced on chocolate agar and satellitism around *Staphylococcus aureus* is seen on blood agar.

Biochemical reactions for differentiation:

Confirmatory tests for X and V factor requirements as follow:

1. Inoculate a single suspected colony from chocolate agar onto **Mueller Hinton agar** plates.
2. Place commercially available X, V, and XV factor discs/strips on streaked plates.
3. Incubate plates at 37°C in 3-5 % CO₂ atmosphere for 18-24 hours.
4. Observe growth around the discs and *H. influenzae* will only grow around the combined XV disc.

Note that if only X and V factor discs (without XV) are applied, place them at least 2 cm apart and *H. influenzae* will grow between the two discs.

Serological identification (serotyping) of *Haemophilus influenzae*

Slide agglutination test.

- 1) Agglutinating antisera for serotypes “a” to “f” are available commercially. Such sera contain antibodies directed towards somatic antigens present in patient’s sera which result in agglutination.
- 2) Apply one drop of normal saline on a slide and make a homogenous suspension with a single suspected colony of *H. influenzae*.
- 3) Add one drop of specific antiserum and mix thoroughly.

Sexually transmitted diseases (STD)**Genus Neisseria**

The family Neisseriaceae includes the many genera, important one is genus *Neisseria*. The *Neisseriae* are gram-negative cocci that usually occur in pairs. *Neisseria gonorrhea* (gonococci) and *Neisseria meningitidis* (meningococci) are pathogenic for humans and typically are found associated with or inside polymorphonuclear cells.

Other *neisseriae* are normal inhabitants of the human respiratory tract, rarely if ever cause disease, and occur extracellularly.

- *N. catarrhalis* (*Moraxella* or *Branhamella catarrhalis*)
- *N. sicca*
- *N. subflava*
- *N. lactamica*
- *N. mucosa*

Common or general characteristics of Neisseria are:

Gram negative diplococci, kidney shape, with flat or concave opposing edge, oxidase positive, pathogenic to human only, Ferment carbohydrates and producing acid only, pyrogenic (produce pus), Non - hemolytic, non-motile, non-spore forming, intracellular while non-pathogenic are extracellular, needs 48h of culturing time, they are rapidly killed by drying, sunlight and many disinfectants, the non-pathogenic species can grow on simple media while the pathogenic species need enriched media e.g. blood and chocolate agar, Gonococci and meningococci are closely related with 70% DNA homology and are differentiated by few laboratory tests and specific characteristics.

Neisseria gonorrhea (gonococcus)

- ✚ Reservoir: human genital tract
- ✚ Transmission: Sexual contact, birth
- ✚ Sensitive to drying and cold

Gonorrhea; which differs in male than in female as in male it causes urethritis associated with yellowish mucopurulent discharge from anterior urethra and dysuria, the infection can extend to the epididymis leading to epididymitis and if not treated it will be complicated by fibrosis and urethral stricture.

In female the first site of infection is the cervix causing cervicitis, the infection is going to spread from cervix to the vagina causing vaginitis and presented by vaginal discharge which is mucopurulent in nature, if not treated it will spread upward to involve fallopian tube resulting in salpingitis plus PID (pelvic inflammatory disease) which leads to fibrosis with tube stricture leading to infertility due to tubal damage.

Rectal involvement will lead to proctitis; the patient presenting symptoms is purulent discharge from anus with tenesmus

Eye infection (infection of the conjunctival sac); occur in new born babies during their passage through infected birth canal causing a condition called (ophthalmia neonatorum) which is a very serious condition and if untreated it will lead to blindness. This case could be prevented through using antimicrobial agent as silver nitrate and erythromycin

Throat infection (Gonococcal pharyngitis)

Sometimes Gonococcal can cause systemic infection as it disseminated by the blood stream to the distant organs (skin, bone, joints) to gain arthritis or meningitis or endocarditis or vasculitis

Laboratory diagnosis

- ✓ Specimens: Pus and secretion are taken from the urethra, cervix, rectum, conjunctiva, throat or synovial fluid for culture and smear.
- ✓ Smears: Gram stained smears of urethral or endocervical exudates reveal many diplococci within pus cells. In male the finding of numerous neutrophils containing gram negative diplococci in a smear of urethral exudates permits a provisional diagnosis of gonococcal infection and indicates that the individual should be treated. In contrast, a positive culture is needed to diagnose gonococcal infection in female
- ✓ Culture: Immediately after collection of pus or mucous is streaked on enriched selective media such as Thayer – Martin which is a chocolate agar + Enrichment element +Antibiotics like colistin (against G – ve rods); vancomycin (against G + ve), Nystatin (against fungi)
- ✓ Modified Thayer - Martin: same as the above culture medium but plus trimethoprim (inhibit *proteus*)

It needs 5 – 7% CO₂ (candle jar, CO₂ generating kit); Humid atmosphere; 35 – 37 C°, 72 incubation hours

- ✓ Biochemical test:
 - I. Oxidase test (*N, N, N, N, tetramethyl-P-phenylenediamine*) positive for all *Neisseria* species, when get contact with oxidase enzyme they will gain purple color i.e. positive oxidase test.
 - II. Sugar fermentation test: (important in differentiation between *Neisseria* spp.)

Sucrose + Maltose + Glucose is (negative + positive + positive) regarding *N. meningitidis*, (negative + negative + positive) regarding *N. gonorrhea* and regarding *M. catarrhalis* all are negative

III- Nitrate reduction into nitrite it is positive, while in *Moraxella catarrhalis* it is negative

- ✓ Serology: It is not of great value in the diagnosis to detect antibodies against this bacterium; because of the antigen diversity of *Gonococci* and there is delay in the development of these antibodies, so it is more important to detect gonococcal antigens using elisa (EIA) or using radioimmunoassay (RIA).
- ✓ PCR (molecular method): Detection of gonococcal nucleic acid using DNA probe which can detect nucleic acid of the microorganism, this is the most sensitive and specific method.

Antigenic and virulence structures

Gonococci are antigenically heterogeneous both in vivo and in vitro. These antigenic changes help the microorganism to escape from the immune system and it includes:

- a- Pilli which is responsible for attachment to mucosal surfaces, also inhibit phagocytic uptake, and antigenic (immunogenic) variation as more than 1 million variants are found
- b- Porin (por) protein (protein I) that extends through the gonococcal cell membrane, each strain of gonococcus expresses only one type of por.
- c- Opacity (Opa) proteins (protein II) responsible for attachment to mucosal surfaces
- d- RMP (protein III) It is associated with (Opa) protein in the formation of pores
- e- Lipooligosaccharide (LOS) it has endotoxin effect and it is responsible for toxicity of gonococci
- f- IgA protease it split IgA making it in non-functioning form, so IgA protease is a virulent factor that enhances colonization of bacteria.

Treatment and prevention

More than 20% of current isolates of *N. gonorrhoea* are resistant to penicillin, A single intramuscular dose of ceftriaxone is the recommended therapy for uncomplicated gonococcal infections. Intramuscular spectinomycin is indicated in patients who are allergic to cephalosporines. Many patients with gonorrhea have coexisting Chlamydia infections; therefore Doxycycline is effective against Chlamydia.

Prevention of gonorrhea involves evaluation and management of sexual contacts, plus antibiotic prophylactically in an exposed individual even in the absence of symptoms. The use of barrier methods is also a preventive measure against gonorrhea as is the case for all sexually transmitted infections. No vaccine is available for gonorrhea.

Neisseria meningitidis (meningococcus)

N. meningitidis is one of the most frequent causes of meningitis. Infection with *N. meningitidis* can also take the form of a fulminant meningococcemia, with intravascular coagulation, circulatory collapse, and potentially fatal shock, but without meningitis.

In each case, symptoms can occur with extremely rapid onset and great intensity. Outbreaks of meningitis, most common in winter and early spring, are favored by close contact between individuals, such as occurs in schools, institutions, and military barracks. *N. meningitidis* tends to strike young, previously well individuals and can progress over a matter of hours to death.

It is gram-negative, kidney coffee bean-shaped diplococci with large antigenic capsule, grows on chocolate (not blood) agar in 5% CO₂ atmosphere and ferments maltose in contrast to gonococci

Its reservoir is human nasopharynx (5-10% carriers) and its transmission is through respiratory droplets; as it is an oropharyngeal colonizer, so it spreads to the meninges via the bloodstream and disease occurs in only small percentage of colonized individuals.

Antigenic and virulence structures

- a- Capsule: At least 13 serogroups of meningococci have been identified by immunologic specificity and capsular polysaccharides the most important serogroups associated with disease in human are A,B,C,Y, W135 ,they are associated with fulminant sepsis with or without meningitis, which is used for the preparation of vaccines
- b- Outer membrane protein (OMP): about 20 antigenic types, used for serotyping
- c- Pilli and outer membrane proteins important in ability to colonize and invasion
- d- IgA protease it cleaves IgA and allows oropharynx colonization
- e- Opa protein for attachment
- f- Lipooligopolysaccharide (Endotoxin) responsible for fever, septic shock in meningococemia.

Epidemiology

Transmission occurs through inhalation of respiratory droplets from a carrier or a patient in the early stages of the disease. In addition to contact with a carrier

Risk factors for disease include:

- 1- Recent viral or *Mycoplasma* upper respiratory tract infection
- 2- Active or passive smoking
- 3- Complement deficiency (C5-C8)

In susceptible persons, pathogenic strains may invade the bloodstream and cause systemic illness after an incubation period of 2 to 10 days. An incidence peak among adolescents and young adults led the Centers for Disease Control (CDC) to recently recommend vaccination of this at risk group. Humans are the only natural host

Diseases and pathogenicity

The source of the infection is either the patient or the carriers, the route of entry is through the nasopharynx by droplet, and meningococci will attach to the epithelial cell by pili.

From the nasopharynx the meningococci will spread through the blood stream to the target organ causing bacteremia, so the sequel is either meningitis or suffers from fulminating meningococemia.

Meningitis: mainly in adult ages 11 to 55 years, it is usually begun suddenly with headache, vomiting and stiff neck, this will progress to coma within few hours. Fulminating meningococemia is more severe with high fever and hemorrhagic rash; there may be disseminated intravascular coagulation and circulatory collapse (*waterhouse-friderichsen syndrome*).

Laboratory diagnosis

- a- Specimens: Blood, CSF, Nasopharyngeal swab are taken for carrier surveys
- b- Smears as gram stained smear of CSF show typical *Neisseria* within polymorphonuclear leukocytes
- c- Culture onto chocolate agar and Thayer - Martin media, incubated at 37 °C

- d- Serology as rapid detection test to measure antigens or antibodies to meningococcal polysaccharides through latex agglutination test, to identify *N. meningitidis* capsular antigens in CSF, or by Immunoelectrophoresis
- e- PCR which is the most sensitive and specific method

Treatment

Bacterial meningitis is a medical emergency. Accordingly, antibiotic treatment cannot await a definitive bacteriologic diagnosis.

Penicillin G is the drug of choice for treating meningococcal disease. In patient allergic to penicillin chloramphenicol and cefotaxime (or ceftriaxone) can be used.

Prevention

- a- Irradiation of the carrier states (major source)
- b- Isolation of the patient
- c- Chemoprophylaxis for contact people
- d- Vaccination with a conjugate meningococcal vaccine which was approved in the United States in 2005 for use in adolescents and adults ages 11 to 55 years, and has replaced the Unconjugated polysaccharide vaccine; this is a tetravalent vaccine that contains capsular polysaccharides from serogroups A, C, W-135, and Y conjugated to diphtheria toxoid

Moraxella catarrhalis

Previously they are called *Neisseria catarrhalis*, the name changed to *Branhamella* and now they are a separated genus "*Moraxella*". They are normally found in upper respiratory tract especially among school children as (50% of school children carry this microorganism), it may cause pneumonia, otitis media, sinusitis and other infections.

It is nonmotile, gram-negative coccobacilli that are generally found in pairs. *Moraxella* are aerobic, oxidase positive, non-fastidious organisms, can grow on nutrient agar, and does not ferment carbohydrates.

Legionella

Legionella was first recovered from the blood of a soldier more than 50 years ago, but its importance as a human pathogen was not recognized until 1976, when a mysterious epidemic of pneumonia struck members of the Pennsylvania American Legion.

The disease was named Legionnaire's disease by the press. Within 6 months a bacterium, subsequently named *Legionella pneumophila*, had been isolated and definitively established as the agent. A general term for disease produced by *Legionella* species is legionellosis.

The most common presentation of *Legionella pneumophila* is acute pneumonia (legionellosis); potentially any species of *Legionella* may cause the disease.

Extra pulmonary disease (e.g., pericarditis and endocarditis) is rare. Less often, disease presents as a nonpneumonic epidemic, influenza like illness called Pontiac fever.

Structure, Classification, and Antigenic Types

Legionella species are Gram-negative bacilli. There are currently 39 species and 60 distinct antigenic types of *Legionella*.

Legionella cells are thin, somewhat pleomorphic Gram-negative bacilli that measure 2 to 20 µm. Long, filamentous forms may develop, particularly after growth on the surface of agar. Ultra structurally, *Legionella* has the inner and outer membranes typical of Gram-negative bacteria. It possesses pili (fimbriae), and most species are motile by means of a single polar flagellum.

Pathogenesis

Legionella bacilli reside in surface and drinking water and are usually transmitted to humans in aerosols. The bacteria multiply intracellularly in alveolar macrophages.

Recruited neutrophils and monocytes, as well as bacterial enzymes, produce destructive alveolar inflammation. Direct inoculation of surgical wounds by contaminated tap water has been described.

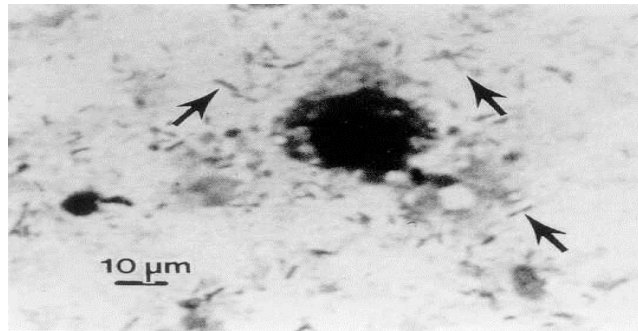


*Figure shows smear from the lung of a patient fatally infected with *L pneumophila* serogroup1, demonstrating many thin, Gram-negative bacilli (arrows). These bacteria stain less intensely with safranin than do enteric bacilli*

Host Defenses

Nonspecific physical and inflammatory pulmonary defenses are important, but cell-mediated immunity is critical. Immunologically activated monocytes and macrophages restrict intracellular bacterial growth. The role of humoral immunity is unclear.

Epidemiology

Legionella species are widespread in nature. Interactions with other environmental organisms may facilitate growth. Disease may be sporadic or epidemic and may occur in the community or in hospitals. People with compromised host defenses are at increased risk.

The **clinical manifestations** of *Legionella* infections are primarily respiratory. The most common presentation is acute pneumonia, which varies in severity from mild illness that does not require hospitalization (walking pneumonia) to fatal multilobar pneumonia.

Typically, patients have high, unremitting fever and cough **but do not produce much sputum**. Extra pulmonary symptoms, such as headache, confusion, muscle aches, and gastrointestinal disturbances, are common. Most patients respond promptly to appropriate antimicrobial therapy, but convalescence is often prolonged (lasting many weeks or even months).

Laboratory Diagnosis

There are no reliable distinguishing clinical features of *Legionella* pneumonia, so the diagnosis must come from the laboratory.

Some clinical features suggest legionnaire's disease; however, and should prompt the selection of appropriate laboratory tests. The diagnosis is confirmed in the laboratory by culture, demonstration of bacterial antigen in body fluids, or a serologic response.

The preferred diagnostic method is culturing, because it is both sensitive and specific; however, appropriate specimens are not always available.

The laboratory must be alerted to the possibility of legionellosis, because specially designed media must be used. The medium of choice is **buffered charcoal-yeast extract - α -ketoglutarate medium**.

This medium contains yeast extract, iron, L-cysteine, and α -ketoglutarate for bacterial growth; activated charcoal to inactivate toxic peroxides that develop in the media; and buffer with a pH 6.9, the optimum for growth of *Legionella* organisms.

Addition of albumin to the media may further facilitate growth of species other than *L. pneumophila*. For contaminated specimens such as sputum, antibiotics should be added. Morphologically distinctive bacterial colonies can usually be detected within 3 to 5 days and identified presumptively as *Legionella* species if the isolated bacteria depend on cysteine for growth. The identification can be confirmed by specific immunologic typing of the isolated bacteria or, in problematic cases, by molecular analysis.

Direct detection of bacterial antigen in clinical specimens is potentially much faster than culturing. Unfortunately, **direct immunofluorescence detection (DFA)** of *Legionella* antigen in respiratory specimens is neither sensitive nor specific enough to warrant general use. A commercially available **radioimmunoassay for bacterial antigen in urine** is satisfactory, but is available only for serogroup 1 of *L. pneumophila*.

Serologic diagnosis is moderately sensitive and reasonably specific. It should be considered as an adjunct to diagnosis by culture. Indirect immunofluorescence has been used most frequently.

It is important to use an assay that detects IgM and IgG. The advantages of serologic diagnosis are that it is performed on easily obtained blood specimens and can detect mild or even asymptomatic infection.

Bordetella

Bordetella pertussis and *Bordetella parapertussis* are the human pathogens of this genus. The former causes the disease pertussis (also known as whooping cough), and the latter causes a mild pertussis-like illness. Whooping cough is a highly contagious disease and a significant cause of morbidity and mortality worldwide (51 million cases and 600,000 deaths each year). Members of the genus *Bordetella* are aerobic. They are small, encapsulated coccobacilli that grow singly or in pairs. They can be serotyped on the basis of cell-surface molecules including adhesins and fimbriae.

Epidemiology

The major mode of transmission of *Bordetella* is via droplets spread by coughing, but the organism survives only briefly outside the human respiratory tract. The incidence of whooping cough among different age groups can vary substantially, depending on whether active immunization of young children is widespread in the community. In the absence of an immunization program, disease is most common among young children (ages 1 to 5 years). Adolescent and adult household members, whose pertussis immunity has disappeared, are an important reservoir of pertussis for young children.

Pathogenesis

B. pertussis binds to ciliated epithelium in the upper respiratory tract. There, the bacteria produce a variety of toxins and other virulence factors that interfere with ciliary activity, eventually causing death of these cells.

Clinical significance

The incubation period for pertussis generally ranges from 1 to 3 weeks. The disease can be divided into two phases: catarrhal and paroxysmal.

1. Catarrhal phase: This phase begins with relatively nonspecific symptoms, such as rhinorrhea, mild conjunctival infection (hyperemia, or bloodshot conjunctivae), malaise, and/or mild fever, and then progresses to include a dry, nonproductive cough. Patients in this phase of disease are highly contagious.

2. Paroxysmal phase: With worsening of the cough, the paroxysmal phase begins. The term “whooping cough” derives from the paroxysms of coughing followed by a “whoop” as the patient inspires rapidly. Large amounts of mucus may be produced. Paroxysms may cause cyanosis and/or end with vomiting. Pertussis typically causes leukocytosis that can be quite striking as the total white blood cell count sometimes exceeds 50,000 cells/ μ l (normal range = 4,500–11,000 white blood cells/ μ l), with a striking predominance of lymphocytes. Following the paroxysmal phase, convalescence requires at least an additional 3 to 4 weeks. During this period, secondary complications, such as infections (for example, otitis media and pneumonia) and central nervous system (CNS) dysfunction (for example, encephalopathy or seizures), may occur. Disease is generally most severe in infants.

Laboratory identification

Presumptive diagnosis may be made on clinical grounds once the paroxysmal phase of classic pertussis begins. Pertussis may be suspected in an individual who has onset of catarrhal symptoms within 1 to 3 weeks of exposure to a diagnosed case of pertussis.

Culture of *B. pertussis* on Bordet-Gengou or Regan-Lowe media (selective and enrichment media) from the nasopharynx of a symptomatic patient supports the diagnosis. The organism produces pin-point colonies in 3 to 6 days on selective agar medium (for example, one that contains blood and charcoal), which serves to absorb and/or neutralize inhibitory substances and is supplemented with antibiotics to inhibit growth of normal flora. More rapid diagnosis may be

accomplished using a direct fluorescent antibody test to detect *B. pertussis* in smears of nasopharyngeal specimens. Serologic tests for antibodies to *B. pertussis* are primarily useful for epidemiologic surveys.

Treatment

Erythromycin is the drug of choice for infections with *B. pertussis*, both as chemotherapy (where it reduces both the duration and severity of disease) and as chemoprophylaxis for household contacts. For erythromycin treatment failures, trimethoprim-sulfamethoxazole is an alternative choice. Patients are most contagious during the catarrhal stage and during the first 2 weeks after onset of coughing. Treatment of the infected individuals during this period limits the spread of infection among household contacts.

Prevention

Pertussis vaccine is available and has had a significant effect on lowering the incidence of whooping cough. It contains proteins purified from *B. pertussis* and is formulated in combination with diphtheria and tetanus toxoids. To protect infants who are at greatest risk of life-threatening *B. pertussis* disease, immunization is generally initiated when the infant is 2 months old. Until the middle of the first decade of the 21st century. However, because neither disease- nor vaccine-induced immunity is durable, there has been resurgence, with reported cases in 2010 the highest since the 1950's. A new vaccine, licensed for adolescents and adults, and vaccination of women even during the last trimester of pregnancy.

Anaerobic Bacteriology (Part-one)

Infections caused by anaerobic bacteria are common. The infections are often polymicrobial; that is, the anaerobic bacteria are found in mixed infections with other anaerobes, facultative anaerobes

Anaerobic bacteria are found throughout the human body; on the skin, on mucosal surfaces, and in high concentrations in the mouth and gastrointestinal tract, as part of the normal microbiota

Infection results when anaerobes and other bacteria of the normal microbiota contaminate normally sterile body sites.

Several important diseases are caused by anaerobic *Clostridium* species from the environment or from normal flora: botulism, tetanus, gas gangrene, food poisoning, and

Pseudomembranous colitis

Aerobic bacteria: Bacteria that require oxygen as a terminal electron acceptor and will not grow under anaerobic conditions (i.e., in the absence of O₂). Some *Bacillus* species and *Mycobacterium tuberculosis* are obligate aerobes (i.e., they must have oxygen to survive).

Anaerobic bacteria: Bacteria that do not use oxygen for growth and metabolism but obtain their energy from fermentation reactions. A functional definition of anaerobes is that they require reduced oxygen tension for growth and fail to grow on the surface of

solid medium in 10% CO₂ in ambient air. *Bacteroides* and *Clostridium* species are examples of anaerobes.

Facultative anaerobes: Bacteria that can grow either oxidatively, using oxygen as a terminal electron acceptor, or anaerobically, using fermentation reactions to obtain energy. Such bacteria are common pathogens. *Streptococcus* species and the Enterobacteriaceae (e.g., *Escherichia coli*) are among the many facultative anaerobes that cause disease. Often, bacteria that are facultative anaerobes are called “aerobes.”

Anaerobic bacteria do not grow in the presence of oxygen and are killed by oxygen or toxic oxygen radicals.

Aerobes and facultative anaerobes often have the metabolic systems listed below, but anaerobic bacteria frequently do not.

1. Cytochrome systems for the metabolism of O₂
2. Superoxide dismutase (SOD), which catalyzes the following reaction:
$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$
3. Catalase, which catalyzes the following reaction:
$$2H_2O_2 \rightarrow 2H_2O + O_2 \text{ (gas bubbles)}$$

Anaerobic bacteria do not have cytochrome systems for oxygen metabolism. Less fastidious anaerobes may have low levels of SOD and may or may not have catalase. Most bacteria of the *Bacteroides fragilis* group have small amounts of both catalase and SOD. There appear to be multiple mechanisms for oxygen toxicity. Presumably, when anaerobes have SOD or catalase (or both), they are able to negate the toxic effects of oxygen radicals and hydrogen peroxide and thus tolerate oxygen. **Obligate anaerobes** usually lack SOD and catalase and are susceptible to the lethal effects of oxygen; such strict obligate anaerobes are infrequently isolated from human infections, and most anaerobic infections of humans are caused by “moderately obligate anaerobes.”

Facultative anaerobes grow as well or better under anaerobic conditions than they do under aerobic conditions. Bacteria that are facultative anaerobes are often termed *aerobes*. When a facultative anaerobe such as *E. coli* is present at the site of an infection (e.g., abdominal abscess), it can rapidly consume all available

oxygen and change to anaerobic metabolism, producing an anaerobic environment, and thus allow the anaerobic bacteria that are present to grow and produce disease.

Gram-Negative Anaerobes

A. Gram-Negative Bacilli

1. *Bacteroides*: The *Bacteroides* species are very important anaerobes that cause human infection. They are a large group of bile-resistant, non-spore-forming, slender gram negative rods that may appear as coccobacilli. Many species previously included in the genus *Bacteroides* have been reclassified into the genus *Prevotella* or the genus *Porphyromonas*. Those species retained in the *Bacteroides* genus are members of the *B fragilis* group. *Bacteroides* species are normal inhabitants of the bowel and other sites. Normal stools contain 10^{11} *B fragilis* organisms per gram (compared with 10^8 /g for facultative anaerobes).

Other commonly isolated members of the *B fragilis* group include *Bacteroides ovatus*, *Bacteroides distasonis*, *Bacteroides vulgatus*, and *Bacteroides thetaiotaomicron*. *Bacteroides* species are most often implicated in intra-abdominal

infections, usually under circumstances of disruption of the intestinal wall as occurs in perforations related to surgery or trauma, acute appendicitis, and diverticulitis.

These infections are often polymicrobial. Both *B fragilis* and *B thetaiotaomicron*

are implicated in serious intrapelvic infections such as pelvic inflammatory disease and ovarian abscesses.

B fragilis group species are the most common species recovered in some series of anaerobic bacteremia, and these organisms are associated with a very high mortality rate. *B fragilis* is capable of elaborating numerous virulence factors, which contribute to its pathogenicity and mortality in the host.

2. *Prevotella*—*Prevotella* species are gram-negative bacilli and may appear as slender rods or coccobacilli. Most commonly isolated are *P melaninogenica*, *Prevotella bivia*, and *Prevotella disiens*. *P melaninogenica* and similar species are

found in infections associated with the upper respiratory tract. *P bivia* and *P disiens* occur in the female genital tract.

Prevotella species are found in brain and lung abscesses, in empyema, and in pelvic inflammatory disease and tubo-ovarian abscesses. In these infections, the *Prevotella* are often associated with other anaerobic organisms that are part of the normal microbiota; particularly *Peptostreptococcus*, anaerobic Gram-positive rods, and *Fusobacterium* species as well as Gram-positive and Gram-negative facultative anaerobes that are part of the normal microbiota.

3. *Porphyromonas*: The *Porphyromonas* species also are Gram-negative bacilli that are part of the normal oral microbiota and occur at other anatomic sites as well. *Porphyromonas* species can be cultured from gingival and periapical tooth infections and, more commonly, breast, axillary, perianal, and male genital infections.

4. *Fusobacteria*: There are approximately 13 *Fusobacterium* species, but most human infections are caused by *Fusobacterium necrophorum* and *Fusobacterium nucleatum*. Both species differ in morphology and habitat as well as the range of associated infections. *F necrophorum* is a very pleomorphic; long rod with round ends and tends to make bizarre forms. It is not a component of the healthy oral cavity. *F necrophorum* is quite virulent, causing severe infections of the head and neck.

Bacterial Vaginosis

Bacterial vaginosis is a common vaginal condition of women of reproductive age. It is associated with premature rupture of membranes and preterm labor and birth. Bacterial vaginosis has a complex microbiology; one organism, *Gardnerella vaginalis*, has been most specifically associated with the disease process.

G vaginalis is a serologically distinct organism isolated from the normal female genitourinary tract and also associated with vaginosis, so named because inflammatory cells are not present. In wet smears, this “nonspecific” vaginitis, or bacterial vaginosis, yields “clue cells,” which are vaginal epithelial cells covered with many Gram-variable bacilli (pleomorphic), and there is an absence of other common causes of vaginitis such as trichomonads or yeasts. Vaginal discharge often has a distinct “fishy” odor and **contains many anaerobes in addition to *G vaginalis***. The pH of the vaginal secretions is greater than 4.5 (normal pH is <4.5). The vaginosis attributed to this organism is suppressed by metronidazole, suggesting an association with anaerobes. Oral metronidazole is generally curative.

Gram-Positive Anaerobes***A. Gram-Positive Bacilli***

1. *Actinomyces*: The *Actinomyces* group includes several species that cause actinomycosis, of which *Actinomyces israelii* and *Actinomyces gerencseriae* are the ones most commonly encountered. Several new, recently described species that are not associated with actinomycosis have been associated with infections of the groin, urogenital area, breast, and axilla and postoperative infections of the mandible, eye, and head and neck.

On Gram stain, they vary considerably in length; they may be short and club shaped or long, thin, beaded filaments. They may be branched or unbranched. Because they often grow slowly, prolonged incubation of the culture may be necessary before laboratory confirmation of the clinical diagnosis of actinomycosis can be made. Some strains produce colonies on agar that resemble molar teeth.



Figure shows colony of Actinomyces

Species after 72 hours growth on brain–heart infusion agar, which usually yields colonies about 2 mm in diameter; they are often termed “Molar tooth” colonies

Some *Actinomyces* species are oxygen tolerant (aerotolerant) and grow in the presence of air; these strains may be confused with *Corynebacterium* species. Actinomycosis is a chronic suppurative and granulomatous infection that produces pyogenic lesions with interconnecting sinus tracts that contain granules composed of microcolonies of the bacteria embedded in tissue elements.

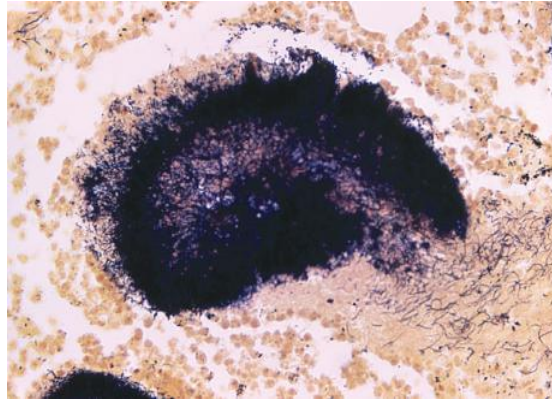


Figure shows granule of Actinomyces species in tissue with Brown and Breen stain. Filaments of the branching bacilli are visible at the periphery of the granule. Such granules are commonly called “sulfur granules” because of their unstained gross yellow color

Infection is initiated by trauma that introduces these endogenous bacteria into the mucosa. The organisms grow in an anaerobic niche, induce a mixed inflammatory response, and spread with the formation of sinuses, which contain the granules and may drain to the surface.

The infection causes swelling and may spread to neighboring organs, including the bones. Based on the site of involvement, the three common forms are cervicofacial, thoracic, and abdominal actinomycosis. Cervicofacial disease presents as a swollen, erythematous process in the jaw area (*known as “lumpy jaw”*). With progression, the mass becomes fluctuant, producing draining fistulas. The disease will extend to contiguous tissue, bone, and lymph nodes of the head and neck. The symptoms of thoracic actinomycosis resemble those of a subacute pulmonary infection and include a mild fever, cough, and purulent sputum. Eventually, lung tissue is destroyed, sinus tracts may erupt through to the chest wall, and invasion of the ribs may occur.

Abdominal actinomycosis often follows a ruptured appendix or an ulcer. In the peritoneal cavity, the pathology is the same, but any of several organs may be involved. Genital actinomycosis is a rare occurrence in women that results from colonization of an intrauterine device with subsequent invasion.

Diagnosis can be made by examining pus from draining sinuses, sputum, or specimens of tissue for the presence of sulfur granules. The granules are hard, lobulated, and composed of tissue and bacterial filaments, which are club shaped at the periphery. Specimens should be cultured anaerobically on appropriate media.

Treatment requires prolonged administration of penicillin (6-12 months). Clindamycin or erythromycin is effective in penicillin-allergic patients. Surgical excision and drainage may be required.

2. *Propionibacterium* species are members of the normal microbiota of the skin, oral cavity, large intestine, conjunctiva, and external ear canal. Their metabolic products include propionic acid, from which the genus name derives. On Gram stain, they are highly pleomorphic; showing curved, clubbed, or pointed ends; long forms with beaded uneven staining; and occasionally coccoid or spherical forms. ***Propionibacterium acnes***, often considered an opportunistic pathogen, causes the disease ***acne vulgaris*** and is associated with a variety of inflammatory conditions.

It causes acne by producing lipases that split free fatty acids off from skin lipids. These fatty acids can produce tissue inflammation that contributes to acne formation.

P. acnes is frequently a cause of postsurgical wound infections, particularly those that involve insertion of devices, such as prosthetic joint infections, particularly of the shoulder, central nervous system shunt infections, osteomyelitis, endocarditis, and endophthalmitis.

3. *Clostridia*

(In lecture of spore-forming gram-positive Bacilli: *Bacillus* and *Clostridium* Species)

B. Gram-Positive Cocci

The group of anaerobic gram-positive cocci has undergone significant taxonomic expansion. Many species within the genus *Peptostreptococcus* have been reassigned to new genera such as *Anaerococcus*, *Finegoldia*, and *Peptoniphilus*. The species contained within these genera, as well as *Peptococcus niger*, are important members of the normal microbiota of the skin, oral cavity, upper respiratory tract, gastrointestinal tract, and female genitourinary system. The members of this group are opportunistic pathogens and are most frequently found in mixed infections particularly from specimens that have not been carefully procured. However, these organisms have been associated with serious infections such as brain abscesses, pleuropulmonary infections, necrotizing fasciitis, and other deep skin and soft tissue infections, intra-abdominal infections, and infections of the female genital tract.

The polymicrobial anaerobic infections

Most anaerobic infections are associated with contamination of tissue by normal microbiota of the mucosa of the mouth, pharynx, gastrointestinal tract, or genital tract. Typically, multiple species (five or six species or more when standard culture conditions are used) are found, including both anaerobes and facultative anaerobes. Oropharyngeal, pleuropulmonary, abdominal, and female pelvic infections associated with contamination by normal mucosal microbiota have a relatively equal distribution of anaerobes and facultative anaerobes as causative agents; about 25% have anaerobes alone, about 25% have facultative anaerobes alone, and about 50% have both anaerobes and facultative anaerobes. Aerobic bacteria may also be present, but obligate aerobes are much less common than anaerobes and facultative anaerobes.

Diagnosis of anaerobic infections

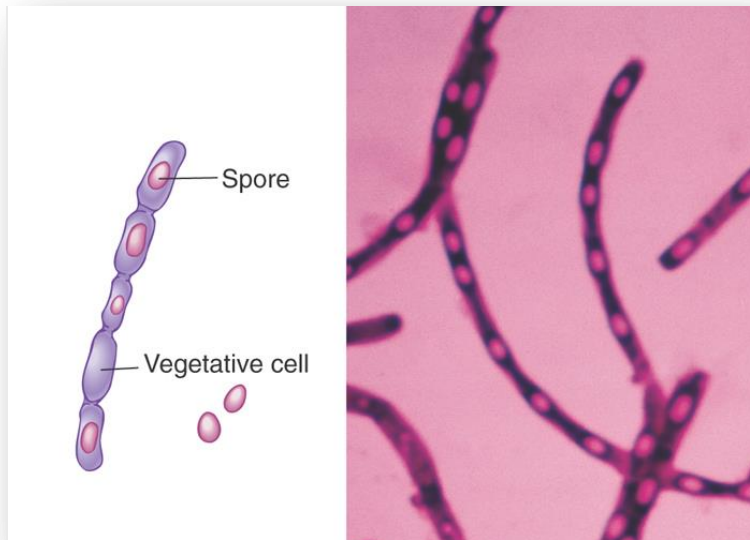
Clinical signs suggesting possible infection with anaerobes include the following:

1. ***Foul-smelling discharge*** (caused by short-chain fatty-acid products of anaerobic metabolism)
2. ***Infection in proximity to a mucosal surface*** (anaerobes are part of the normal microbiota)
3. ***Gas in tissues*** (production of CO₂ and H₂)
4. ***Negative aerobic culture results***

Diagnosis of anaerobic infection is made by anaerobic culture of properly obtained and transported specimens. Anaerobes grow most readily on complex media such as trypticase soy agar base, blood agar, brucella agar, brain–heart infusion agar, and each highly supplemented (e.g., with hemin, vitamin K1, blood). A selective complex medium containing kanamycin is used in parallel. Kanamycin (similar to all aminoglycosides) does not inhibit the growth of obligate anaerobes; thus, it permits them to proliferate without being overshadowed by rapidly growing facultative anaerobes. Cultures are incubated at 35-37°C in an anaerobic atmosphere containing CO₂. Colony morphology, pigmentation, and fluorescence are helpful in identifying anaerobes. Biochemical activities and production of short-chain fatty acids as measured by gas liquid chromatography are used for laboratory confirmation.

Spore-forming gram-positive Bacilli: *Bacillus* and *Clostridium* Species(Part –Two)

These bacilli are ubiquitous, and because they form spores, they can survive in the environment for many years. *Bacillus* species are aerobes and the *Clostridium* species are anaerobes.



Many species of *Bacillus* and related genera, most do not cause disease and are not well characterized. There are a few species, however, that cause important diseases in humans. Anthrax, a classical disease in the history of microbiology, is caused by *Bacillus anthracis*.

Anthrax remains an important disease of animals and occasionally of humans. Because of its potent toxins, *B anthracis* is a major potential agent of bioterrorism and biologic warfare.

Bacillus cereus and *Bacillus thuringiensis* cause food poisoning and occasionally eye or other localized infections.

The genus *Clostridium* is extremely heterogeneous and more than 200 species have been described.

Clostridia cause several important toxin mediated diseases, including tetanus (*Clostridium tetani*), botulism (*Clostridium botulinum*), and gas gangrene (*Clostridium perfringens*), and antibiotic-associated diarrhea and pseudomembranous colitis (*Clostridium difficile*)

Bacillus species

The genus *Bacillus* includes large aerobic, gram-positive rods occurring in chains. The members of this genus are closely related but differ both phenotypically and in terms of pathogenesis.

Pathogenic species possess virulence plasmids. Most members of this genus are saprophytic organisms prevalent in soil, water, and air, and on vegetation (e.g., *Bacillus subtilis*).

Some are insect pathogens, such as *B thuringiensis*. This organism is also capable of causing disease in humans. *B cereus* can grow in foods and cause food poisoning by producing either an enterotoxin (diarrhea) or an emetic toxin (vomiting). Both *B cereus* and *B thuringiensis* may occasionally produce disease in immunocompromised humans (e.g., meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastroenteritis). *B anthracis*, which causes anthrax, is the principal pathogen of the genus.

The typical cells, measuring 3–4 μm , have square ends and are arranged in long chains; spores are located in the center of the bacilli

Colonies of *B anthracis* are round and have a “cut glass” appearance in transmitted light. Hemolysis is uncommon with *B anthracis* but common with *B cereus* and the saprophytic bacilli. Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree.

Anthrax is primarily a disease of herbivores—goats, sheep, cattle, horses, and so on; other animals (e.g., rats) are relatively resistant to the infection.

Humans become infected incidentally by contact with infected animals or their products. In animals, the portal of entry is the mouth and the gastrointestinal tract. Spores from contaminated soil find easy access when ingested with spiny or irritating vegetation. In humans, the infection is usually acquired by the entry of spores through injured skin (cutaneous anthrax) or rarely the mucous membranes (gastrointestinal anthrax) or by inhalation of spores into the lung (inhalation anthrax). A fourth category of the disease, injection anthrax, has caused outbreaks among persons who inject heroin that has been contaminated with anthrax spores. The spores germinate in the tissue at the site of entry, and growth of the vegetative organisms results in formation of a gelatinous edema and congestion. Bacilli spread via lymphatics to the bloodstream, and they multiply freely in the blood and tissues shortly before and after the animal’s death.

Anthrax toxins are made up of three proteins, protective antigen (PA), edema factor (EF), and lethal factor (LF). PA is a protein that binds to specific cell receptors, and after proteolytic activation, it forms a membrane channel that mediates entry of EF and LF into the cell. EF is an adenylate cyclase; with PA, it forms a toxin known as edema toxin. Edema toxin is responsible for cell and tissue edema. LF plus PA form lethal toxin, which is a major virulence factor and cause of death in infected animals and humans.

When injected into laboratory animals (e.g., rats), the lethal toxin can quickly kill the animals by impairing both innate and adaptive immunity, allowing organism proliferation and cell death.

In inhalation anthrax (wool sorters’ disease), the spores from the dust of wool, hair, or hides are inhaled; phagocytosed in the lungs; and transported by the lymphatic drainage to the mediastinal lymph nodes, where germination occurs. This is followed by toxin production and the development of hemorrhagic mediastinitis and sepsis.



Figure shows Bacillus anthracis in broth culture

In humans, approximately 95% of cases are cutaneous anthrax, and 5% are inhalation. Gastrointestinal anthrax is very rare; it has been reported from Africa, Asia, and the United States when people have eaten meat from infected animals. The bioterrorism events in the fall of 2001 resulted in 22 cases of anthrax 11 inhalation and 11 cutaneous. Five of the patients with inhalation anthrax died.

Cutaneous anthrax generally occurs on exposed surfaces of the arms or hands followed in frequency by the face and neck. A pruritic papule develops 1–7 days after entry of the organisms or spores through a scratch. Initially, it resembles an insect bite. The papule rapidly changes into a vesicle or small ring of vesicles that coalesce, and a necrotic ulcer develops. The lesions typically are 1–3 cm in diameter and have a characteristic central black eschar. Marked edema occurs. Lymphangitis, lymphadenopathy, and systemic signs and symptoms of fever, malaise, and headache may occur.

After 7–10 days, the eschar is fully developed. Eventually, it dries, loosens, and separates; healing is by granulation and leaves a scar. It may take many weeks for the lesion to heal and the edema to subside. Antibiotic therapy does not appear to change the natural progression of the disease but prevents dissemination. In as many as 20% of patients, cutaneous anthrax can lead to sepsis, the consequences of systemic infection including meningitis and death.

The incubation period in inhalation anthrax may be as long as 6 weeks. The early clinical manifestations are associated with marked hemorrhagic necrosis and edema of the mediastinum. Substernal pain may be prominent, and there is pronounced mediastinal widening visible on chest radiographs. Hemorrhagic pleural effusions follow involvement of the pleura; cough is secondary to the effects on the trachea.

Sepsis occurs, and there may be hematogenous spread to the gastrointestinal tract, causing bowel ulceration, or to the meninges, causing hemorrhagic meningitis. The fatality rate in inhalation anthrax is high in the setting of known exposure; it is higher when the diagnosis is not initially suspected.

Animals acquire anthrax through ingestion of spores and spread of the organisms from the intestinal tract. This is rare in humans, and gastrointestinal anthrax is extremely uncommon. Abdominal pain, vomiting, and bloody diarrhea are clinical signs.

Injection anthrax is characterized by extensive, painless, subcutaneous edema and the notable absence of the eschar characteristic of cutaneous anthrax. Patients may progress to hemodynamic instability due to septicemia.

Laboratory Diagnosis

Specimens to be examined are fluid or pus from a local lesion, blood, pleural fluid, and cerebrospinal fluid in inhalational anthrax associated with sepsis and stool or other intestinal contents in the case of gastrointestinal anthrax. Stained smears from the local lesion or of blood from dead animals often show chains of large gram-positive rods. Anthrax can be identified in dried smears by immunofluorescence staining techniques.

When grown on blood agar plates, the organisms produce nonhemolytic gray to white, tenacious colonies with a rough texture and a ground-glass appearance. Comma-shaped outgrowths (Medusa head, “curled hair”) may project from the colony. Demonstration of capsule requires growth on bicarbonate-containing medium in 5–7% carbon dioxide. Gram stain shows large gram-positive rods. Carbohydrate fermentation is not useful. In semisolid medium, anthrax bacilli are always nonmotile, but related organisms (e.g., *B cereus*) exhibit motility by “swarming.”

Definitive identification requires lysis by a specific anthrax γ -bacteriophage, detection of the capsule by fluorescent antibody, or identification of toxin genes by polymerase chain reaction (PCR). These tests are available in most public health laboratories. A rapid enzyme-linked immunoassay (ELISA) that measures total antibody to PA, but the test result is not positive early in disease.

Bacillus cereus

Food poisoning caused by *B cereus* has two distinct forms;

1. **The emetic type**; which is associated with fried rice, milk, and pasta
2. **The diarrheal type**; which is associated with meat dishes and sauces

B cereus produces toxins that cause disease that is more of intoxication than a food-borne infection. The emetic form is manifested by nausea, vomiting, abdominal cramps, and occasionally diarrhea and is self-limiting, with recovery occurring within 24 hours. It begins 1-5 hours after ingestion of a plasmid-encoded preformed cyclic peptide (emetic toxin) in the contaminated food products.

B cereus is a soil organism that commonly contaminates rice. When large amounts of rice are cooked and allowed to cool slowly, the *B cereus* spores germinate, and the vegetative cells produce the toxin during log-phase growth or during sporulation. The diarrheal form has an incubation period of 1-24 hours and is manifested by profuse diarrhea with abdominal pain and cramps; fever and vomiting are uncommon. In this syndrome, ingested spores that develop into vegetative cells of *B cereus* secrete one of three possible enterotoxins which induce fluid accumulation in the small intestine.

The presence of *B cereus* in a patient's stool is not sufficient to make a diagnosis because the bacteria may be present in normal stool specimens; a concentration of 10^5 bacteria or more / gram of food is considered diagnostic.

Bacillus anthracis

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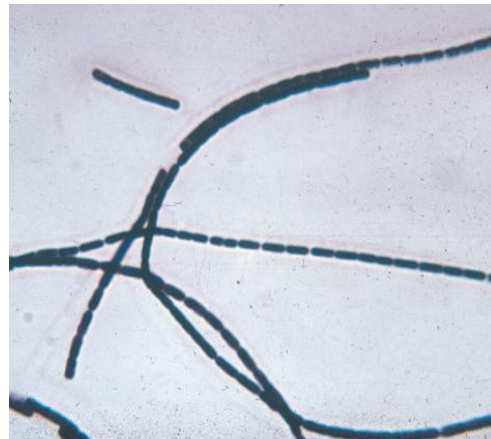


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Clostridia species

The *Clostridia* are large anaerobic, gram-positive, motile rods. Many decompose proteins or form toxins, and some do both.

Their natural habitat is the soil, marine sediments, sewage, or the intestinal tract of animals and humans, where they live as saprophytes. Among the pathogens are the organisms causing ***botulism, tetanus, gas gangrene, and pseudomembranous colitis.***

Clostridia are usually wider than the diameter of the rods in which they are formed; the spore is placed ***centrally, subterminally, or terminally.***

Most species of *Clostridia* are motile and possess ***peritrichous flagella***. They are anaerobes and grow under anaerobic conditions ((grow well on the blood-enriched media)).

Clostridia produce large raised colonies (e.g., ***C perfringens***); others produce smaller colonies (e.g., ***C tetani***). Some *Clostridia* form colonies that spread or swarm on the agar surface (***Clostridium septicum***). Many *Clostridia* produce a zone of β -hemolysis on blood agar. ***C perfringens characteristically produces a double zone of β -hemolysis around colonies.***

Clostridia can ferment a variety of sugars (***saccharolytic***) and many can digest proteins (***proteolytic***); some species do both. These metabolic characteristics are used to divide the *Clostridia* into groups. Milk is turned acid by some and digested by others and undergoes “***stormy fermentation***” (clot torn by gas).

Clostridium botulinum

C botulinum which causes the disease ***botulism*** is worldwide in distribution; it is found in soil and occasionally in animal feces. Types of *C botulinum* are distinguished by the antigenic type of toxin they produce. Spores of the organism are highly resistant to heat, withstanding 100°C for several hours. Heat resistance is diminished at acid pH or high salt concentration.

During the growth of *C botulinum* and during autolysis of the bacteria, toxin is liberated into the environment. Seven antigenic varieties of toxin (***serotypes A-G***) are known. ***Types A, B, E, and F are the principal causes of human illness.***

Botulinum toxins have three domains:

- 1- Two of the domains facilitate binding to and entry of toxin into the nerve cell
- 2-The third domain is the toxin which is protein that is cleaved into a heavy chain and a light chain that are linked by a disulfide bond.

Botulinum toxin is absorbed from the gut, enters the blood circulation, and binds to receptors of motor neurons of the peripheral nervous system and cranial nerves. The toxin does not cross the blood brain barrier or affect the central nervous system. ***It inhibits the release of acetylcholine at the neurons synapse, resulting in lack of muscle contraction and paralysis.***

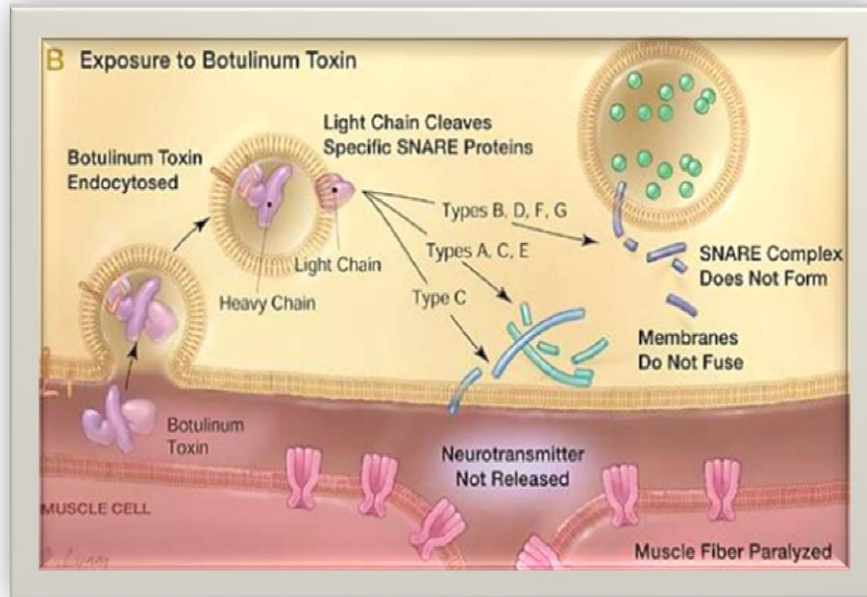


Figure shows *C botulinum* toxins action pattern

Pathogenesis

Most cases of botulism represent an intoxication resulting from the ingestion of food in which *C botulinum* has grown and produced toxin. The most common offenders are **spiced, smoked, vacuum packed or canned alkaline foods that are eaten without cooking**. In such foods, spores of *C botulinum* germinate; that is, under anaerobic conditions, vegetative forms grow and produce toxin. **In infant botulism, honey is the most frequent vehicle of infection.**

The infant ingests the spores of *C botulinum*, and the spores germinate within the intestinal tract. The vegetative cells produce toxin as they multiply; the neurotoxin then gets absorbed into the bloodstream.

The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions; the result is flaccid paralysis.

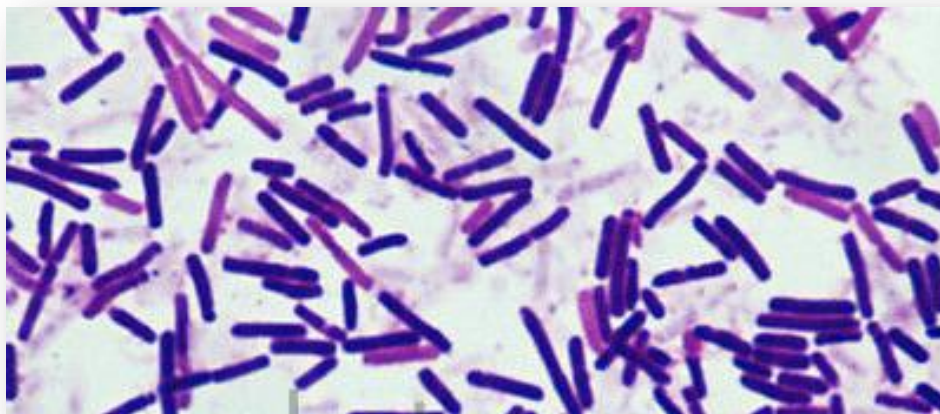


Figure shows *C botulinum* gram stained film

The clinical picture begins 18-24 hours after ingestion of the toxic food, with visual disturbances (incoordination of eye muscles, double vision), inability to swallow, and speech difficulty, and death occurs from respiratory paralysis or cardiac arrest.

The infants in the first months of life develop poor feeding, weakness, and signs of paralysis (*floppy baby*). Infant botulism may be one of the causes of sudden infant death syndrome. *C botulinum* and *botulinum toxin* are found in feces but not in serum.

Laboratory diagnosis carried out by detection of toxin and not the organism. Toxin can often be demonstrated in *serum, gastric secretions, or stool* from the patient, and toxin may be found in leftover food.

Clinical swabs or other specimens obtained from patients should be transported using *anaerobe containers*. Mice injected intraperitoneally with such specimens from these patients die rapidly. The antigenic type of toxin is identified by neutralization with specific antitoxin in mice. This mouse bioassay is the test of choice for the confirmation of botulism. In infant botulism, *C botulinum* and toxin can be demonstrated in bowel contents but not in serum. Other methods used to detect toxin include *Elisa and PCR*.

Clostridium tetani

C tetani which causes *tetanus* is worldwide in distribution in the soil and in the feces of horses and other animals. Several types of *C tetani* can be distinguished by specific flagellar antigens. All share a common *O (somatic) antigen*, which may be masked, and all produce the *same antigenic type of neurotoxin ((tetanospasmin))* which composed of two peptides linked by a disulfide bond. The larger peptide initially binds to receptors on the presynaptic membranes of motor neurons; it then migrates by the retrograde axonal transport system to the cell bodies of these neurons to the spinal cord and brainstem. The release of the *inhibitory glycine* and *GABA (γ -aminobutyric acid)* is blocked, and the motor neurons are not inhibited; so hyper-reflexia, muscle spasms, and spastic paralysis result.

Pathogenesis

C tetani is not an invasive organism, the infection remains strictly localized in the area of devitalized tissue (*wound, burn, injury, umbilical stump, surgical suture*) into which the spores have been introduced.



Figure shows *C tetani* gram film (tennis rackets or drumsticks)

The volume of infected tissue is small, and the disease is almost entirely a toxemia. Germination of the spore and development of vegetative organisms that produce toxin are aided by:

- 1- Necrotic tissue
- 2- Calcium salts
- 3- Associated pyogenic infections **all of which aid establishment of low oxidation-reduction potential**

The toxin released from vegetative cells reaches the central nervous system and rapidly becomes fixed to receptors in the spinal cord and brainstem and exerts the actions

The incubation period may range from 4-5 days up to 3 weeks. The disease is characterized by tonic contraction of voluntary muscles. Muscular spasms often involve first the area of injury and infection and then the muscles of the jaw (**trismus, lockjaw**), which contract so that the mouth cannot be opened. Gradually, other voluntary muscles become involved, resulting in **tonic spasms**. The patient is fully conscious, and pain may be intense. Death usually results from interference with the mechanics of respiration. The mortality rate in generalized tetanus is very high.

Diagnosis and tetanus prevention

Anaerobic culture of tissues from contaminated wounds may yield *C tetani*, but neither preventive nor therapeutic use of antitoxin should ever be withheld pending such demonstration. Proof of isolation of *C tetani* must rest on production of toxin and its neutralization by specific antitoxin.

Prevention of tetanus depends on:

- 1- Active immunization with toxoids
- 2- Aggressive wound care
- 3- Prophylactic use of antitoxin
- 4- Administration of penicillin

Active immunization with tetanus **toxoid** should accompany antitoxin prophylaxis; they are given very large doses of **antitoxin** (3000-10,000 units of tetanus immune globulin) intravenously in an effort to neutralize toxin that has not yet been bound to nervous tissue. Surgical debridement is vitally important because it removes the necrotic tissue that is essential for proliferation of the organisms. Penicillin strongly inhibits the growth of *C tetani* and stops further toxin production.

Tetanus toxoid is produced by detoxifying the toxin with formalin and then concentrating it. **Aluminum salt adsorbed toxoids** are used. Three injections comprise the initial course of immunization followed by another dose about 1 year later. Initial immunization should be carried out in all children during the first year of life. **“booster” injection of toxoid** is given upon entry into school. Thereafter, “boosters” can be spaced 10 years apart to maintain serum levels **In young children; tetanus toxoid is often combined with diphtheria toxoid and a cellular pertussis vaccine (DPT).**

Clostridia with tissue invasion

Many different toxin-producing *Clostridia* (*C perfringens* and related *Clostridia*) can produce invasive infection (including **myonecrosis and gas gangrene**) if introduced into damaged tissue.

About 30 species of *Clostridia* may produce such an effect, but the most common in invasive disease is *C perfringens* (90%). An **enterotoxin** of *C perfringens* is a common cause of **food poisoning**. The invasive

Clostridia produce a large variety of toxins and enzymes that result in a spreading infection. Many of these toxins have lethal, necrotizing, and hemolytic properties.

The **alpha toxin** of *C perfringens* type A is a **lecithinase**, and its lethal action is proportionate to the rate at which it splits lecithin (an important constituent of cell membranes). Alpha toxin also **aggregates platelets**, thereby leading to formation of thrombi in small blood vessels and adding to poor tissue perfusion and extending the consequences of anaerobiosis, namely, destruction of viable tissue (gas gangrene).

The **theta toxin** has similar hemolytic and necrotizing effects but is not a lecithinase that act by forming pores in cell membranes.

Epsilon toxin is a protein that causes edema, and hemorrhage is very potent. **DNase** and **hyaluronidase**, a **collagenase** that digests collagen of subcutaneous tissue and muscle, are also produced.

Some strains of *C perfringens* produce a powerful enterotoxin especially when grown in meat dishes. When more than 10^8 vegetative cells are ingested and sporulate in the gut. It induces intense diarrhea in 7-30 hours.

The action of *C perfringens* enterotoxin involves marked hypersecretion in the jejunum and ileum, with loss of fluids and electrolytes in diarrhea; this illness is similar to that produced by *B cereus* and tends to be self-limited.

Pathogenesis

In invasive *Clostridia* infections, spores reach tissue either by contamination of traumatized areas (**soil, feces**) or from the intestinal tract. The spores germinate at low oxidation-reduction potential; vegetative cells multiply, ferment carbohydrates present in tissue, and produce gas.

The distention of tissue and interference with blood supply, together with the secretion of necrotizing toxins and hyaluronidase, favor the spread of infection. Tissue necrosis extends, providing an opportunity for increased bacterial growth, hemolytic anemia, and, ultimately, severe toxemia and death.

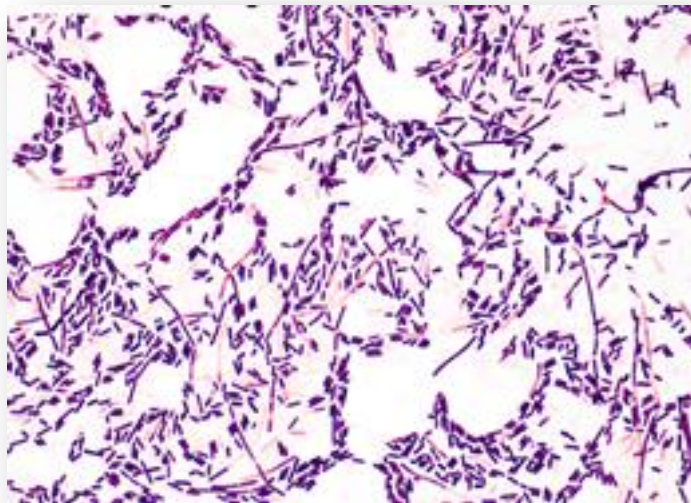


Figure shows C septicum gram stained slide from infected wound

In gas gangrene (*Clostridial myonecrosis*), a mixed infection is the rule. In addition to the toxigenic *Clostridia*, proteolytic *Clostridia* and various cocci and gram-negative organisms are also usually present. *C perfringens* occurs in the genital tracts of 5% of women.

In contaminated wound (e.g., **a compound fracture, postpartum uterus**), the infection spreads in 1-3 days to produce **crepitation** in the subcutaneous tissue and muscle, **foul-smelling discharge**, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death.

In the laboratory specimens consist of material from **wounds, pus, and tissue**. The presence of large gram-positive rods in Gram-stained smears suggests gas gangrene *Clostridia*; spores are not regularly present. Material is inoculated into **chopped meat glucose medium** and **thioglycolate medium** and onto blood agar plates incubated anaerobically.

After pure cultures have been obtained by selecting colonies from anaerobically incubated blood plates, they are identified by biochemical reactions (**various sugars in thioglycolate, action on milk**); **hemolysis, and colony morphology, lecithinase activity is evaluated by the precipitate formed around colonies on egg yolk media**.

Clostridium difficile

Pseudomembranous colitis is diagnosed by detection of one or both *C difficile* toxins in stool and by endoscopic observation of pseudomembrane or micro-abscesses in patients who have diarrhea and have been given antibiotics.

Plaques and micro-abscesses may be localized to one area of the bowel. The diarrhea may be watery or bloody, and the patient frequently has associated abdominal cramps, leukocytosis, and fever.

Many antibiotics have been associated with pseudomembranous colitis; the most common are **ampicillin** and **clindamycin** and, more recently, the **flouroquinilones**. The disease is treated by discontinuing administration of the offending antibiotic and orally giving metronidazole, vancomycin.

Fecal transplantation has become a successful and routine method for recurrent and refractory disease. This usually involves administration of the feces of a healthy related donor by way of colonoscopy or less commonly via a nasogastric tube into the gastrointestinal tract of the patient.

Administration of antibiotics results in proliferation of drug-resistant *C difficile* that produces two toxins:

- 1- **Toxin A** is a potent enterotoxin that also has some cytotoxic activity, binds to the brush border membranes of the gut at receptor sites
- 2- **Toxin B** is a potent cytotoxin

Both toxins are usually found in the stools of patients with pseudomembranous colitis. Not all strains of *C difficile* produce the toxins, and the toxin genes are found on a large, chromosomal pathogenicity island along with three other genes that regulate toxin expression.

Diagnosis is made clinically and supported by demonstration of toxin in the stool by a variety of methods that includes **anaerobic toxigenic culture, enzyme immunoassay, and molecular tests that detect the genes that encode toxins A or Antibiotic associated diarrhea**

The administration of antibiotics frequently leads to a mild to moderate form of diarrhea, termed antibiotic-associated diarrhea. This disease is generally less severe than the classic form of pseudomembranous colitis; 25% of cases of antibiotic-associated diarrhea are caused by *C difficile* infection, other *Clostridium* species such as *C perfringens* and *C sordellii* have also been implicated; but they are not associated with pseudomembranous colitis.

Mycobacterium

Mycobacteria are slender rods with lipid-rich cell walls that are resistant to penetration by chemical dyes such as those used in the Gram stain.

They stain poorly but, once stained, cannot be easily decolorized by treatment with acidified organic solvents. Therefore, they are termed “acid-fast”. *Mycobacteria* survive and replicate intracellularly. *Mycobacteria* infections generally result in the formation of slow-growing granulomatous lesions that are responsible for major tissue destruction.

Mycobacterium tuberculosis causes tuberculosis, the principal chronic bacterial disease in humans and a leading cause worldwide of death from infection. This organism has increasingly become a cause for special concern in immunocompromised patients.

Members of the genus *Mycobacterium* also cause **leprosy** as well as several tuberculosis-like human infections. This genus belongs to the order of organisms (***Actinomycetales***) that also includes the genera ***Actinomyces*** and ***Nocardia***. These organisms all cause granulomatous lesions with various clinical presentations.

Mycobacteria are nonmotile and do not form spores. *Mycobacteria* cell walls are unusual in that they are approximately 60 % lipid, including a unique class of very

Long-chain (75-90 carbons), β -hydroxylated fatty acids (***mycolic acids***).

These complexes with a variety of polysaccharides and peptides, creating a waxy cell surface that makes *Mycobacteria* strongly hydrophobic and accounts for their acid-fast staining characteristic. Their unusual cell walls make *Mycobacteria* impervious to many chemical disinfectants and convey resistance to the corrosive action of strong acids or alkalis, so decontaminating clinical specimens, such as sputum, in which non-*Mycobacteria* organisms are digested by such treatments. *Mycobacteria* are also resistant to drying but not to heat or ultraviolet irradiation. *Mycobacteria* are strictly aerobic. Most species grow slowly with **generation times of 8 to 24 hours**.

It is currently estimated that about one third of the world’s population is infected with *M. tuberculosis* (**tubercle bacillus**), with 30 million people having active disease.

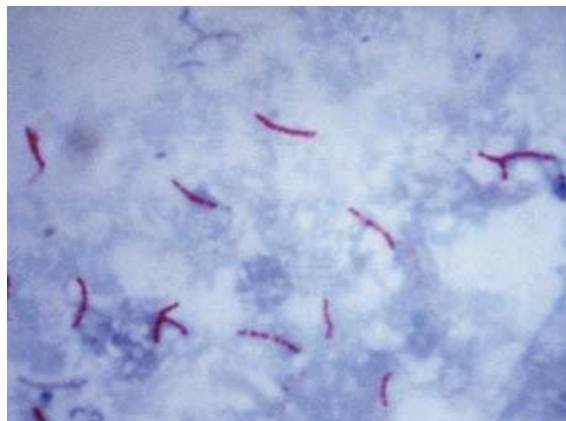


**Figure shows *Mycobacterium tuberculosis*
(Acid-fast stain of sputum from a
patient with tuberculosis)**

Patients with active pulmonary tuberculosis shed large numbers of organisms by coughing, creating aerosol droplet nuclei. Because of resistance to desiccation, the organisms can remain viable as droplet nuclei suspended in room air for at least

30 minutes. The principal mode of contagion is person-to-person transmission by inhalation of the aerosol.

A single infected person can pass the organism to numerous people in an exposed group, such as a family, classroom, or hospital ward without proper isolation.

After being inhaled, *Mycobacteria* reach the alveoli, where they multiply in the pulmonary epithelium or macrophages. Within 2-4 weeks, many bacilli are destroyed by the immune system, but some survive and are spread by the blood to extra - pulmonary sites.

The virulence of *M. tuberculosis* rests with its ability to survive and grow within host cells. Although the organism produces no demonstrable toxins, when engulfed by macrophages, **bacterial sulfolipids** inhibit the fusion of phagocytic vesicles with lysosomes. The ability of *M. tuberculosis* to grow even in immunologically activated macrophages and to remain viable within the host for decades is a unique characteristic of the pathogen.

M. tuberculosis stimulates both a humoral and a cell mediated immune response. Although circulating antibodies appear, they do not convey resistance to the organism. Instead, cellular immunity (CD4⁺ T cells) and the accompanying delayed hypersensitivity directed against a number of bacterial protein antigens develop in the course of infection and contribute to both the pathology of and immunity to the disease.

Primary tuberculosis occurs in a person who has had no previous contact with the organism. For the majority of cases (95%), the infection becomes arrested, and most people are unaware of this initial encounter.

The only evidence of tuberculosis may be a **positive tuberculin test**; the course of tuberculosis infection either **remaining dormant** or **progressing to clinical disease**.

About 10 % of those with an arrested primary infection develop clinical tuberculosis at some later time in their lives.

As primary tuberculosis is usually acquired by the respiratory tract, the initial lesion occurs in a small bronchiole or alveolus in the mid lung periphery. The organisms are engulfed by local mononuclear phagocytes, and their presence initiates an inflammatory reaction. The tubercle bacilli grow well in phagocytic cells; the bacteria proliferate and are carried by lymphatic drainage to the lymph nodes and beyond to set up additional foci.

This initial phase of the infection is usually mild or asymptomatic and results in exudative lesions in which fluid and polymorphonuclear leukocytes accumulate around the bacilli. A specific immune response develops after about 1 month, and this changes the character of the lesions.

Macrophages, activated by specific T lymphocytes, begin to accumulate and destroy the bacilli.

Tubercle formation: The productive (**granulomatous**) lesion that develops is known as a tubercle. It consists of a central area of large, multinucleate giant cells (macrophage syncytia) containing tubercle bacilli, a mid-zone of pale epithelioid cells, and a peripheral collar of fibroblasts and mononuclear cells.

Tissue damage is produced by the destruction of both bacilli and phagocytes, which results in the release of **degradative enzymes** and reactive oxygen species such as superoxide radicals. The center of the tubercle develops a characteristic expanding, **caseous (cheesy) necrosis**.

✚ Primary tuberculosis follows one of two courses:

- 1- If the lesion arrests, the tubercle undergoes fibrosis and calcification, although viable but non-proliferating organisms may persist.
- 2- If the lesion breaks down, the caseous material is discharged, and a cavity is created that can facilitate spread of the infection. The organisms are dispersed by the lymph and the bloodstream and can seed the lungs; regional lymph nodes; or various distant tissues, such as liver, spleen, kidneys, bone, or meninges. In progressive disease, one or more of the resulting tubercles may expand, leading to destruction of tissue and clinical illness of (**chronic pneumonitis, tuberculous osteomyelitis, and tuberculous meningitis**). In the extreme instance, active tubercles develop throughout the body, a serious condition known as **miliary (disseminated) tuberculosis**.

✚ Secondary disease reactivation:

It is caused by *M. tuberculosis* that has survived in a dormant primary tubercle lesion. Any of the preexisting tubercles may be involved, but pulmonary sites are most common, particularly the **lung apices** where high oxygen tension favors *Mycobacteria* growth. The resulting pathology is known as (**caseation necrosis**). Destruction of the lung tissue leads to **air-filled cavities** where the bacteria replicate actively. Bacterial populations in such lesions often become quite large, and many organisms are shed (in sputum). The patient again becomes capable of exposing others to the disease.

Reactivation is apparently caused by impairment in immune status, often associated with:

- a. **Malnutrition**
- b. **Alcoholism**
- c. **Advanced age**
- d. **Severe stress**
- e. **Immune-suppressive medication**
- f. **Diseases (such as diabetes and, particularly, AIDS)**

Tuberculin reaction

The tuberculin reaction test is a manifestation of delayed hypersensitivity to protein antigens of *M. tuberculosis*. Although such tests can be used to document contact with the tubercle bacillus, they do not confirm that the patient currently has active disease.

In the **Mantoux test**, purified protein derivative (PPD) is prepared from culture filtrates of the organism and biologically standardized. Activity is expressed in tuberculin units. In the routine procedure (Mantoux test), a measured amount of PPD is injected intradermally in the forearm. It is read 48-72 hours later for the presence and size of an **area of induration (hardening)** at the site of injection, which must be observed for the test to be positive. A positive reaction usually develops 4 to 6 weeks after initial contact with the organism. It remains positive for life, although it may wane after some years or in the presence of immune-suppression by medications or disease.

Laboratory Identification

A microscopic approach for acid-fast bacilli using techniques such as the **Ziehl-Neelsen stain** is the most rapid test for *Mycobacteria*. However, *M. tuberculosis* cannot be reliably distinguished on morphologic grounds from other pathogens in the genus, from some saprophytic *Mycobacteria* species that may contaminate glassware and reagents in the laboratory, or from those *Mycobacteria* that may be part of the normal flora. Therefore, a definitive identification of *M. tuberculosis* can only be obtained by culturing the organism or by using one of the molecular methods. **The conventional methods often require 6–8 weeks for identification.**

Specimens consist of **fresh sputum, gastric washings, urine, pleural fluid, cerebrospinal fluid, joint fluid, biopsy material, blood**, or other suspected material.

Decontamination and concentration of specimens from sputum and other non-sterile sites should be liquefied with **NaOH** (kills many other bacteria and fungi), neutralized with buffer, and concentrated by centrifugation. Specimens processed in this way can be used for acid-fast stains and for culture. Specimens from sterile sites, such as cerebrospinal fluid, do not need the decontamination procedure but can be directly centrifuged, examined, and cultured.

Smears Sputum, exudates, or other material is examined for acid-fast bacilli by staining. Stains of gastric washings and urine generally are not recommended because saprophytic *Mycobacteria* may be present and yield a positive stain. **Fluorescence** microscopy with **auramine-rhodamine stain** is more sensitive than traditional acid-fast stains, such as Ziehl-Neelsen, and is the preferred method for clinical material. If acid-fast organisms are found in an appropriate specimen, this is presumptive evidence of *Mycobacteria* infection.

Culture, identification, and susceptibility testing processed specimens from non-sterile sites and centrifuged specimens from sterile sites can be cultured directly onto selective media. The selective broth culture often is the most sensitive method and provides results most rapidly. A selective agar media (e.g., **Löwenstein-Jensen** or **Middle brook 7H10/7H11 bipate with antibiotics**) should be inoculated in parallel with broth media cultures.

Incubation is at 35-37°C in 5-10% CO₂ for up to 8 weeks. If culture results are negative in the setting of a positive acid-fast stain, then a set of inoculated media should be incubated at a lower temperature (e.g., 24-33°C) and both sets incubated for 12 weeks.

Blood for culture of *Mycobacteria* should be anticoagulated and processed by one of two methods:

- (1) Commercially available lysis centrifugation system
- (2) Inoculation into commercially available broth media specifically designed for blood cultures

Conventional methods for identification of *Mycobacteria* include observation of:

1. Rate of growth
2. Colony morphology
3. Pigmentation
4. Biochemical profiles

Nucleic acid amplification by molecular techniques is increasingly important in the diagnosis of tuberculosis because they have the potential to shorten the time required to detect and identify *M. tuberculosis* in clinical specimens. The amplified *M. tuberculosis* direct test uses enzymes that rapidly make copies of *M. tuberculosis* **16S ribosomal RNA**, which can be detected using genetic probes. The sensitivity of the test ranges from 75-100

%, with a specificity of 95-100 %, and it is used for patients whose clinical smears are positive for acid-fast bacilli and whose cultures are in progress.

The *polymerase chain reaction (PCR)*, amplifies a small portion of a predetermined target region of the *M. tuberculosis* DNA. Using human sputum, commercial PCR kits can confirm the diagnosis of tuberculosis within 8 hours, with a sensitivity and specificity that rivals culture techniques.

Also, PCR analysis facilitates DNA fingerprinting of specific strains, allowing studies of the progress of epidemics.



Mycobacterium tuberculosis colonies grown on Lowenstein-Jensen medium

The four groups of *Mycobacteria* (*M tuberculosis* complex, *M avium* complex, *M kansasii*, and *M gordonae*) make up 95% or more of clinical isolates of *Mycobacteria*. For species that cannot be identified by DNA probes, many laboratories with molecular capabilities have implemented 16S rRNA gene sequencing to rapidly identify probe-negative species or send such organisms to a reference laboratory with sequencing capability.

High-performance liquid chromatography (HPLC) has been applied to identification of *Mycobacteria*. The method is based on development of profiles of mycolic acids, which vary from one species to another.

Several chemotherapeutic agents are effective against *M. tuberculosis*. Because strains of the organism resistant to a particular agent emerge during treatment, multiple drug therapy is employed to delay or prevent emergence. Isoniazid, rifampin, ethambutol, streptomycin, and pyrazinamide are the principal or “first-line” drugs because of their efficacy and acceptable degree of toxicity.

Mutants resistant to each of these agents have been isolated even prior to drug treatment. Therefore, the standard procedure is to begin treatment with two or more drugs to prevent outgrowth of resistant strains. Sensitivity tests, administered as soon as sufficient cultured organisms are available, are an important guide to modifying treatment. In most parts of the United States, 8 to 14 % of *M. tuberculosis* strains are resistant to one or more of the primary drugs.

Brucellosis

a bacterial disease caused by organisms in the genus *Brucella* (The genus *Brucella* is aerobic, facultative intracellular, Gram negative coccobacilli), is an important zoonosis and a significant cause of reproductive losses in animals. The current members of this genus include *Brucella abortus*, *B. melitensis*, *B. suis* and *B. ovis* in livestock, *B. canis* in dogs, *B. ceti* and *B. pinnipedialis* in marine mammals, *B. neotomae* and *B. microti* in wild rodents, and a few additional organisms that are still incompletely understood. Most species of *Brucella* circulate in a limited number of reservoir hosts, but other animals can be infected, especially when they are in close

contact. People infected with brucellae may suffer from a debilitating nonspecific illness or localized involvement of various organs. Some unexpected agents identified recently in human brucellosis include *B. neotomae*, which was previously thought not to be zoonotic, and the novel organism *B. inopinata*.

Etiology

Brucellosis results from infection by members of the genus *Brucella*, a Gram negative coccobacillus in the family Brucellaceae (class Alphaproteobacteria). The currently recognized species include *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. ceti*, *B. pinnipedialis*, *B. neotomae*, *B. microti* and *B. inopinata*. Some of these organisms contain multiple biovars. *B. vulpis* and *B. papionis* have been proposed as new species, and several isolates from wild rodents in Australia, some of which were originally identified as *B. suis* biovar 3, might also be a novel species of *Brucella*.

Transmission

Brucellae are shed in birth products (placenta, fetus, fetal fluids), vaginal discharges, semen, urine and milk. They have also been reported occasionally in other secretions and excretions (e.g., saliva, feces, nasal or ocular secretions) that seem to have little or no role in transmission between domesticated animals. Females can shed brucellae whether they abort or carry a pregnancy to term, and reinvasion of the uterus can occur during subsequent pregnancies. Frogs can shed brucellae in urine and feces, and these organisms sometimes occur in large numbers on their skin.

Most mammals are thought to become infected by ingestion or contact with various mucous membranes, but brucellae can also be transmitted through broken skin.

Humans usually become infected with brucellae by ingesting organisms or via contaminated mucous membranes (including the conjunctiva and respiratory tract) and abraded skin. Foodborne sources of brucellae can include unpasteurized milk and other dairy products, undercooked meat and other animal products (e.g., bone marrow from caribou), and possibly undercooked fish or frogs. Routes implicated in rare instances of person-to person transmission of brucellae include blood transfusion, bone marrow transplantation, exposure to contaminated material while assisting at a delivery, sexual intercourse and nursing (infants). There is no indication that members of the genus *Brucella* are transmitted between people by casual contact under ordinary conditions.

Some species of *Brucella* are known or suspected to spread on fomites including feed and water. *Brucellae* have been reported to remain viable in the environment for periods ranging from less than a day to > 8 months, depending on factors such as temperature, humidity, exposure to sunlight and the presence of organic matter. Survival is longer when the temperature is low. Survival times of months have been reported for *brucellae* in ripened, fermented cheeses made from unpasteurized milk, and years for organisms in frozen meat. The environment does not seem to be an important reservoir for most *brucellae*, although they may remain viable for a time. However, *B. microti* seems to survive for unusually long periods in soil, and it is more metabolically active than most *brucellae*, growing rapidly on a variety of media. Some authors have speculated that soil might act as a reservoir for this organism.

Disinfection

Brucella spp. are readily killed by most commonly available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophors, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene. A 1% solution of citric acid was reported to be less effective. One study reported that xylene and calcium cyanamide decontaminated liquid manure after 2 to 4 weeks; however, some sources recommend storing such treated manure for much longer. Most *brucellae* are inactivated fairly quickly by acid pH < 3.5; however, *B. microti* seems to be more resistant to acidic conditions. *Brucellae* can also be destroyed by moist heat of 121°C (250°F) for at least 15 minutes, dry heat of 320-338°F (160-170°C) for at least 1 hour, gamma irradiation and pasteurization. Boiling for 10 minutes is usually effective for liquids.

Infections in Humans:**Incubation Period**

The acute symptoms of brucellosis often appear within 2-4 weeks, but the onset can be insidious, and some cases have been diagnosed as late as 6 months after exposure. Clinical Signs *Brucellae* can infect people asymptomatically or cause diverse syndromes that may appear insidiously or abruptly. Acute brucellosis is usually a febrile illness with nonspecific flu-like signs such as fever, chills, headache, malaise, back pain, myalgia and lymphadenopathy, which may be accompanied by splenomegaly and/or hepatomegaly. Patients may experience drenching sweats, particularly at night. Nonspecific gastrointestinal signs including anorexia, vomiting, diarrhea and constipation may also be seen. Some people recover spontaneously, while others develop persistent nonspecific symptoms (e.g., fever, weakness) that typically wax and wane. Localized infections in various organs and tissues can result in a wide range of syndromes. Fever may be absent or mild in these cases. Infections in bones and joints, the most common sites of localization, can appear as arthritis, spondylitis, sacroiliitis, osteomyelitis, bursitis and tenosynovitis. Brucellosis can also be characterized by neurological involvement (e.g., meningitis, meningoencephalitis, brain abscesses), ocular signs (uveitis, optic neuritis, endophthalmitis and other signs), anemia, thrombocytopenia, nephritis, cardiovascular complications (e.g., vasculitis, aneurisms, endocarditis), respiratory involvement (e.g., bronchopneumonia or pulmonary abscesses), peritonitis, pancreatitis, myelitis, and cutaneous rashes, ulcers or abscesses. Elevations in the liver enzyme alanine aminotransferase (ALT), with only mild increases in aspartate aminotransferase and no

unusual liver pathology, were reported to be common in people infected with *B. suis* on 2 islands in Polynesia. Epididymo-orchitis, prostatitis and seminal vesiculitis can be seen in males, and pregnant women may abort or give birth prematurely. Sepsis, pneumonia and other syndromes have been reported in congenitally infected infants, but some infected newborns are asymptomatic. Deaths are uncommon except in infants, and are usually caused by endocarditis or infections affecting the brain. After treatment, recovery may take a few weeks to months. Descriptions of brucellosis are mostly derived from cases caused by *B. melitensis*, *B. abortus* and *B. suis*. However, *B. canis* infections have been consistent with these descriptions, as were the four cases caused by brucellae from marine mammals. Two of these patients had neurological signs, one had spinal osteomyelitis, and the fourth had nonspecific signs of illness and severe sinusitis. Two people infected with *B. neotomae* developed neurological signs (e.g., recurrent headache, disorientation, hemiparesis), with additional symptoms that included intermittent fever, malaise, lethargy, myalgia, joint pain, weight loss, cough and anorexia. *B. inopinata* was isolated from an infected breast implant, possibly following a systemic infection. An organism that might also be *B. inopinata* was found in the lungs of a person with chronic destructive pneumonia.

Laboratory diagnosis:

A definitive diagnosis of brucellosis requires isolation of the organisms in cultures of blood, bone marrow, urine, abscesses, or other tissues. If processing will be delayed, the specimen may be held in the refrigerator.

It is essential that the clinical microbiology laboratory be notified whenever brucellosis is suspected:

- To ensure that specimens are cultivated in an appropriate manner for optimum recovery from clinical specimens
- To avoid accidental exposure of laboratory personnel handling the specimens, because *Brucella* spp. are considered class III pathogens (specimen labels should indicate that *Brucella* spp. are a potential pathogen)

Direct detection methods

Direct stains of clinical specimens are not particularly useful for the diagnosis of brucellosis. Conventional and real time polymerase chain reaction (PCR) assays are reliable and specific means of directly detecting *Brucella* organisms in clinical specimens.

Cultivation

Although most isolates of *Brucella* spp. grow on blood and chocolate agars (some isolates are also able to grow on MacConkey agar), more enriched agars and special incubation conditions generally are needed to achieve optimal recovery of these fastidious organisms from clinical specimens. *Brucella* agar or infusion base is recommended for specimen types other than blood. The addition of 5% heated horse or rabbit serum enhances growth on all media. Cultures should be incubated in 5% to 10% CO₂ in a humidified atmosphere; inoculated plates are incubated for up to 3 weeks before they are considered negative and discarded.

Commercial blood culture systems (e.g., BacT/Alert, BACTEC, and lysis-centrifugation systems) all have successfully detected brucellae in blood. Other blood culture bottles, such as those with brain-heart infusion and trypticase soy broth, also support the growth of brucellae if the bottles are continuously vented and placed in a CO₂ incubator. Most isolates can be detected within 5 to 7 days using commercial systems. Bottles need not be

incubated longer than 10 to 14 days. Culture bottles may not become turbid. All subculture plates should be held for a minimum of 7 days.

On culture, colonies appear small, convex, smooth, translucent, nonhemolytic, and slightly yellow and opalescent after at least 48 hours of incubation. Rough variants may be seen with *B. canis*. The colonies may become brownish with age.

Approach to identification

Because brucellosis is the most commonly reported laboratory-acquired bacterial infection, all handling and manipulations of suspected *Brucella* spp. should be performed in a class II or higher biologic safety cabinet. On Gram stain, the organisms are small coccobacilli that resemble fine grains of sand. *Brucella* spp. are catalase and urease positive, and most strains are oxidase positive. Other nonfermentative gram-negative coccobacilli that may be confused with brucellae are *Bordetella*, *Moraxella*, *Kingella*, and *Acinetobacter* spp. *Brucella* spp., however, are nonmotile, urease and nitrate positive, and strictly aerobic. The most rapid test for presumptive identification of *Brucella* spp. is the particle agglutination test with anti-smooth *Brucella* serum (Difco Laboratories, Detroit, Michigan). *Brucella* spp. are differentiated by the rapidity with which an organism hydrolyzes urea, its relative ability to produce hydrogen sulfide (H₂S), its requirements for CO₂, and its susceptibility to the aniline dyes thionine and basic fuchsin. For determination of the CO₂ requirement, identical plates of Brucella agar or brain-heart infusion agar should be given equal inocula (e.g., with a calibrated loop) of a broth suspension of the organism to be tested. One plate should be incubated in a candle jar and the other plate in air in the same incubator.

Most strains of *B. abortus* do not grow in air but show growth in the candle jar. Presumptive identification can be reported based on the colony's morphology and a positive catalase, oxidase, urease, and slide agglutination reaction (see next section). *Brucella* isolates should be sent to state or other reference laboratories for confirmation or definitive identification, because most clinical laboratories lack the necessary media and containment facilities.

Subtyping of biovars may be performed using a variety of molecular techniques, including pulsed-field electrophoresis, random amplification of polymorphic DNA, amplified fragment length polymorphism, various PCR techniques, and multilocus sequence typing.

Serodiagnosis

Because isolating brucellae is difficult, a serologic test is widely used (e.g., serum agglutination test [SAT] or microplate agglutination [MAT]). This technique detects antibodies to *B. abortus*, *B. melitensis*, and *B. suis*; however, the SAT does not detect *B. canis* antibodies. An indirect Coombs' test is performed after the SAT. This test detects nonagglutinating or incomplete antibodies in complicated and chronic cases of brucellosis.

The serology associated with *Brucella* infection follows the classic antibody response: IgM appears initially, followed by IgG. A titer of 1 : 160 or greater in the SAT is considered diagnostic if this result fits the clinical and epidemiologic findings. The SAT can cross-react with class M immunoglobulins with a variety of bacteria, such as *Francisella tularensis* and *Vibrio cholerae*. Enzyme-linked immunosorbent assays (ELISAs) also have been developed. Purified LPS or protein extracts are primarily used in ELISAs. However, currently no reference antigen exists; therefore, it is important to identify the antigen in the commercial antigen when

evaluating test results. In patients with neurobrucellosis, ELISA offers significant diagnostic advantages over conventional agglutination methods.

Additional serologic assays are commercially available, including a lateral flow dipstick for screening outbreaks and an immunocapture agglutination method. The immunocapture assay demonstrates sensitivity and specificity similar to a Coombs' test and is less cumbersome to perform. The dipstick test has a high degree of sensitivity

Antimicrobial susceptibility testing and therapy

Because of the fastidious nature of the brucellae and their intracellular localization, in vitro susceptibility testing is not reliable. To prevent relapse of infection, patients with brucellosis undergo prolonged treatment (6 weeks) with antibiotics that can penetrate macrophages and act in the acidic intracellular environment. For initial therapy, doxycycline or tetracycline in combination with streptomycin or rifampin is recommended. In some cases surgical drainage is also required to treat localized foci of infection.

Prevention

Successful vaccines against *Brucella* infection have been developed for livestock. However, the development of human vaccines has met with serious medical contraindications and low efficacy. The prevention of brucellosis in humans depends on elimination of the disease in domestic livestock.

Central Nervous System Diagnostic Microbiology

Meningitis is an infection of the membranes (meninges) surrounding the brain and spinal cord.

Meningitis is usually of multiple etiologies; bacterial, fungal or viral yet bacteria remain the common etiological agent.

Meningitis can be acute, with a quick onset of symptoms, or chronic, lasting a month or more, or can be mild or aseptic.

Bacterial meningitis continues to be a potentially life threatening emergency with significant morbidity and mortality throughout the world and is an even more significant problem in many other areas of the world, especially in developing countries

Types of bacteria that cause bacterial meningitis vary by age group. Currently, the average age of contracting meningitis is above 25 years with *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* being the most common pathogens.

Trauma to the skull gives bacteria the potential to enter the meningeal space. Similarly, individuals with a cerebral shunt or related device are at increased risk of infection through those devices. In these cases, infections with *Staphylococci*, *Pseudomonas aeruginosa* and other gram-negative bacilli are more likely.

Recurrent bacterial meningitis may be caused by persisting anatomical defects, either congenital or acquired, or by disorders of the immune system.

Tuberculous meningitis is common in those from countries where tuberculosis is common.

Viral meningitis is generally less severe and clears up without specific treatment. Viral (Aseptic) meningitis is serious but rarely fatal in people with normal immune systems. Usually, the symptoms last from 7-10 days and the patient recovers completely. Often, in early stages of viral meningitis and bacterial meningitis, the symptoms are almost similar

Fungal meningitis is rare, but can be life threatening. Although anyone can get fungal meningitis, people at higher risk are those who have AIDS, leukemia, or other forms of immunodeficiency.

The most common cause of fungal meningitis in HIV is *Cryptococcus* spp. In the last two decades, advancements in transplant procedures and concomitant use of immunosuppressive therapies as well as the pandemic spread of HIV, have increased the incidence of central nervous system (CNS) fungal infections. The clinical picture may mimic tuberculous meningitis.

The causes of non infectious meningitis include *cancers*, *systemic lupus erythematosus*, *drug induced*, *head trauma*, *brain surgery*.

Collection, transportation, receipt and storage of cerebrospinal fluid (CSF)

Direct testing of CSF is the most accurate way to confirm the diagnosis of bacterial meningitis.

CSF should be collected from all the cases with suspected meningitis before the antimicrobial therapy.

Petechial fluid can be another specimen in cases with meningococcal meningitis. Petechial lesions, if present, may be gently irrigated by injecting 0.2 ml of sterile saline solution using a small syringe with a fine needle and the fluid collected for smear and culture.

Early diagnosis is essential and is best established by laboratory examination of CSF. However, therapy should not be dependent or delayed pending lumbar puncture or laboratory results.

To initiate the definitive identification of a bacterium responsible for meningitis, CSF specimens should be obtained from patients with clinical signs and symptoms of meningitis and should be transported to the laboratory without delay.

“N.meningitidis, S.pneumoniae, and H.influenzae are fastidious organisms that may not survive long transit times”

The processing of a CSF specimen is one of the few clinical microbiology procedures that must be done immediately.

Laboratorians should always record the date and time a specimen was received. Usually, three or more tubes of CSF are collected during a lumbar puncture procedure .

The tubes should be numbered in sequential order with tube number one containing the first sample of CSF obtained.

The CSF in tubes 1, 2, and 3 most often are examined for chemistry, microbiology, and cytology . However, the contamination with skin flora and disinfectant will be ruled out after the first tube of CSF is collected.

The probabilities of detecting microorganisms by staining and by culturing are related to the volume of specimen that is concentrated and examined.

CSF volumes of 2 to 3 ml are usually sufficient to detect bacteria, but for mycological and mycobacterial investigations a minimum of 5 ml (preferably 10 to 15 ml) of CSF is required .

If only a small amount of CSF is received with requests for multiple assays, the order of priority of the tests is determined after discussion with the physician

“The specimen should not be refrigerated before subjecting to microbiological tests as it may prevent the recovery of the organisms; fastidious organisms may not survive variations in temperature”

CSF specimens should be stored at room temperature or at 37 °C if they cannot be processed immediately or till microscopy and bacterial cultures are performed, after which it can be refrigerated for further use.

Bacterial meningitis is a significant cause of mortality and morbidity worldwide, neurological outcome and survival depend largely on damage to CNS prior to effective antibacterial treatment

“Quick diagnosis and effective treatment is the key to success”

The diagnostic dilemma in acute pyogenic meningitis is due to large spectrum of signs and symptoms

Examination of CSF

The CSF should arrive still warm and either be examined immediately or placed in an incubator for examination within an hour, If delay is anticipated either in transportation to the laboratory or for examination, CSF should be divided into two tubes: one in a plain tube and the other in a tube having a few drops of glucose broth.

In the laboratory, CSF from the plain tube can be used for making smears for staining whereas cultures are done from tube having CSF in glucose broth

The residual CSF should be preserved frozen for further assessment and evaluation

An examination of CSF involves the following

1. Macroscopic examination
2. Cytological examination
3. Examination of Gram stained smear
4. Culture and antimicrobial susceptibility testing
5. Latex agglutination test for antigen detection

Macroscopic examination

By appearance, the CSF is normally clear like water; cloudy, purulent, bloody or pigmented CSF as per the disease states

Hazy, cloudy, turbid CSF indicates either metastatic spread of tumors into the CNS; Opalescent CSF may be suggestive of *Cryptococcal meningitis*

“The CSF is attributable to both the bacteria and leukocytes present”

In evaluating patients with suspected meningitis or encephalitis, a careful history along with biochemical and cellular analysis of CSF is required

CSF glucose

CSF glucose concentrations <45 mg/dl are indicative of bacterial meningitis. CSF glucose concentrations depend on serum concentrations and ***“should always be tested on paired samples”***

A CSF/serum ratio cut-off of <0.4 is helpful in distinguishing between bacterial and aseptic meningitis with a sensitivity and specificity of 91% and 96%, respectively

CSF protein

Despite typical CSF findings, the spectrum of CSF values in bacterial meningitis is so wide that the absence of one or more of the typical findings may not affect the diagnosis

In community-acquired bacterial meningitis only 50 % may have CSF glucose above 40 mg/dl, less than half cases may have a CSF protein below 200 mg/dl, CSF protein measurements of >55 mg/dl are diagnostic of bacterial, fungal and tubercular meningitis

Cytological examination

In untreated bacterial meningitis, the WBC count is elevated, usually in the range of 1000–5000 cells/mm³, although this range can be quite broad (<100 to >10, 000 cells/mm³)

Bacterial meningitis usually leads to a neutrophils predominance in CSF, typically between 80% and 95%; 10% of patients with acute bacterial meningitis present with a lymphocyte predominance (defined as >50% lymphocytes or monocytes) in CSF.

It is important to note that a false-positive elevation of the CSF WBC can be found after traumatic lumbar puncture, or in patients with intracerebral or subarachnoid hemorrhage in which both red blood cells and white blood cells are introduced into the subarachnoid space.

Examination of Gram stained smear

It is preferable to make a smear from CSF at the time of collection itself, for direct demonstration of organisms.

The Gram stained smear made either directly from the CSF or from the centrifuged deposit can reveal not only the Gram character of the causative organism, but can also clinch the diagnosis in some cases.

“Gram stain may not be interpretable in grossly blood stained samples”

Although Gram staining of CSF sediment is a very useful, cheap and fairly rapid method of identification of organism, the sensitivity in developing countries is only 25-40% when compared to 80-85% in developed countries.

CSF Gram staining may identify the causative microorganism for patients with suspected bacterial meningitis.

In 1/3 of the cases with bacterial meningitis defined by CSF parameters may have negative CSF cultures; around 50% of the CSF culture negative patients have a positive Gram stain with equal percent of patients being pretreated with antibiotics.

The Gram stain is positive in 10-15 percent of patients who have bacterial meningitis but with negative CSF culture.

In developing countries among suspected meningitis cases, CSF Gram staining can identify the causative organisms in 2/3rd, and CSF culture is positive in 1/10th of the pretreated patients.

“Gram staining correctly identifies the pathogen in 69 to 93% of patients with pneumococcal meningitis”

The reported sensitivities of CSF Gram staining vary considerably for different microorganisms. CSF Gram staining correctly identifies the organism in 50-65% of children and in 25-33% in adults with *H. influenzae meningitis*

The chances of recovery of bacteria in CSF Grams up to 100-fold, can be intensified by replacing conventional centrifugation with cytopsin centrifugation

This increase is comparable to the concentration of 100 ml of CSF to a volume of 1.0 ml by conventional centrifugation

Cytopsin-prepared smears not only increased the positivity of the smears, the morphology of the cells was well preserved with uniform distribution of the cells

Culture and antimicrobial susceptibility testing

Culture is the gold standard for determining the causative organism in meningitis.

After the receipt, specimen should be cultured at the earliest. CSF should also be inoculated into ***“enrichment medium like sodium thioglycollate broth”*** along with solid media like enriched, selective or differential media. Incubate in air plus 5-10% carbon dioxide.

Anaerobic culture may be important for post neurosurgical or posttraumatic meningitis or for the investigation of CSF shunt meningitis.

“Negative or inconclusive culture results may be seen in patients with partially-treated meningitis and those with atypical bacteria, and Mycobacterium tuberculosis”

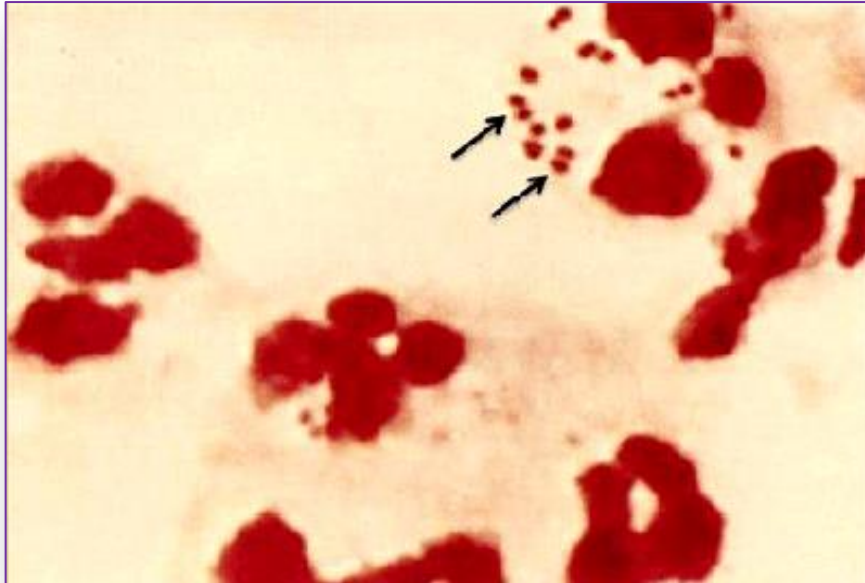


Figure shows Gram stain of N. meningitidis in CSF with associated PMNs

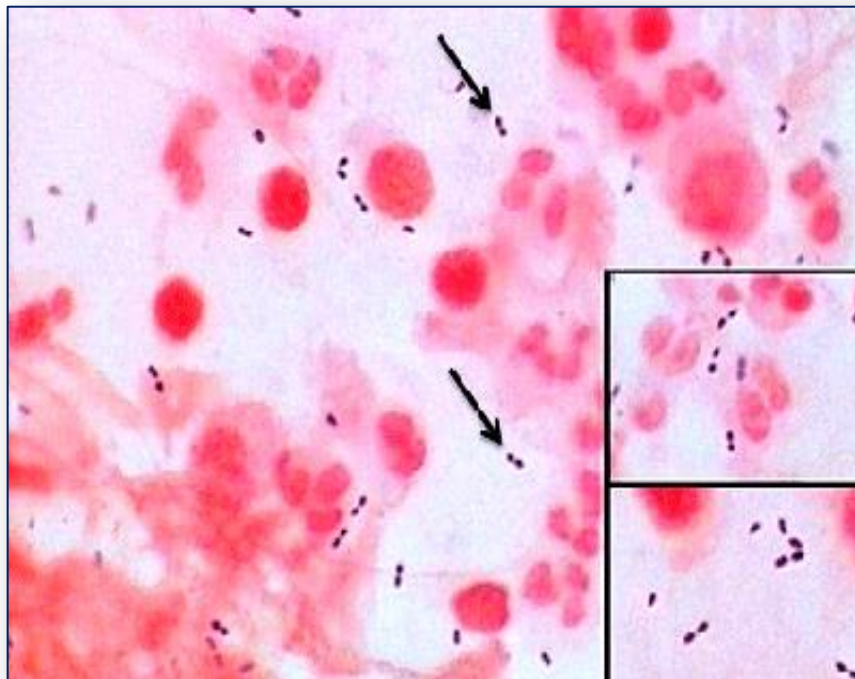


Figure shows Gram stain of S. pneumoniae with WBCs

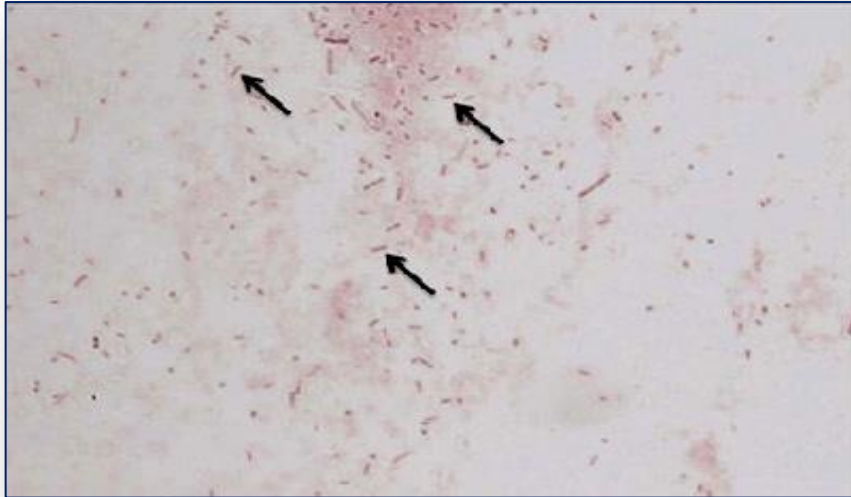


Figure shows H. influenzae are small, pleomorphic gram-negative rods or coccobacilli with random arrangements



Figure shows proper streaking and growth of N. meningitidis on a blood agar plate

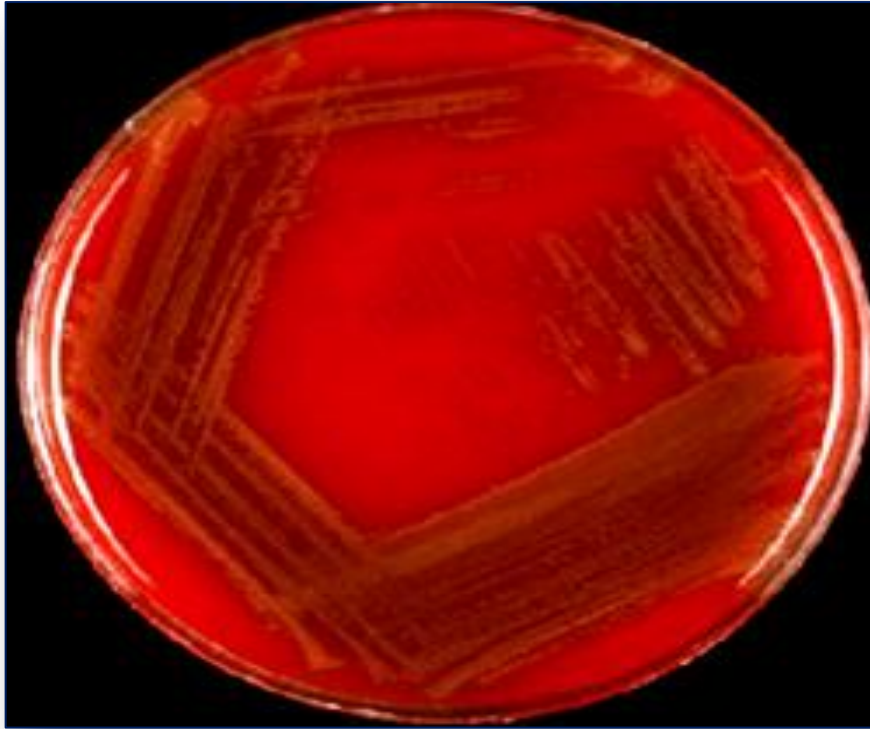


Figure shows proper streaking and growth of S. pneumoniae on a blood agar plate

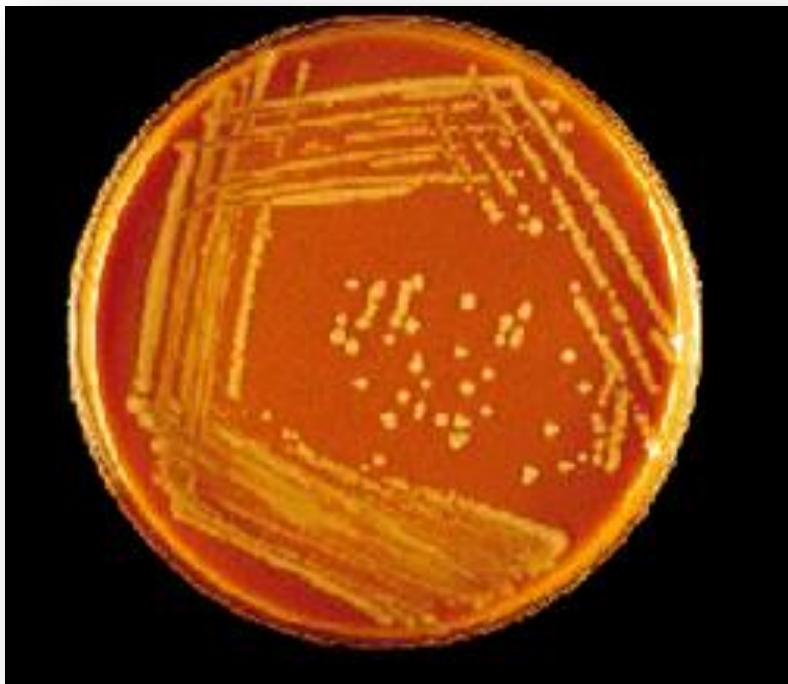


Figure shows proper streaking and growth of H. influenzae on a chocolate agar plate



Figure shows H. influenzae colonies on a chocolate agar plate

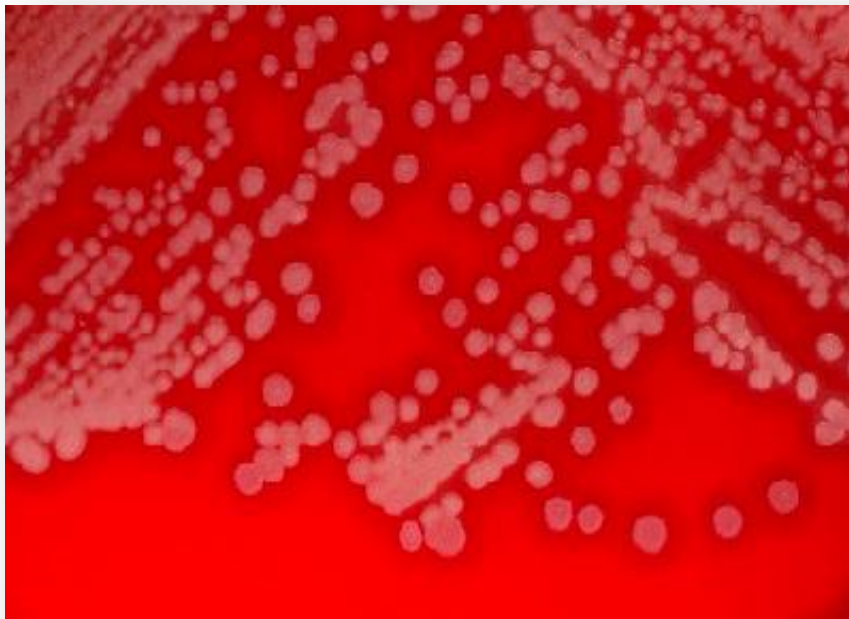


Figure shows N. meningitidis colonies on a blood agar plate

Gastrointestinal Tract Diagnostic Microbiology

- 1- Food and untreated water borne infectious diseases
 - A. *Salmonella, shigella, cholera*
 - B. Hepatitis A / E, Caliciviruses / Norwalk virus, poliomyelitis
 - C. *Giardia, cryptosporidium, Cyclospora*
- 2- Dairy (unpasteurized)
 - A- *Brucella, Listeria, Salmonella, Shigella*
- 3- Raw or undercooked food
 - A. Enteric bacteria (*Salmonella, Shigella, C. jejuni*)
 - B. Helminths (*Ascaris, Trichinella, Taenia*)
 - C. Protozoa (*Amoebiasis, Toxoplasma*)

Stool cultures are performed to detect enteric pathogens. Routine stool cultures should be examined for the presence for *Salmonella, Shigella*, and *Campylobacter* spp at minimum.

It should be noted that media routinely set to detect these pathogens will also detect *Aeromonas hydrophila* and *Plesiomonas shigelloides*. *Vibrio, Yersinia* and *E. coli* O157:H7.

Bloody stool specimens are routinely screened for *E. coli* O157:H7 or shiga-like producing strains of *E. coli*.

Recently, the CDC has recommended routine screening of stool culture for the presence of *E. coli* strains that produce a Shiga-like cytotoxin

Enterohemorrhagic *E. coli* (EHEC) has been isolated from patients who have hemorrhagic colitis and hemolytic-uremic syndrome (HUS).

One virulence trait of all EHEC strains is the ability to produce cytotoxin(s) called Shiga-like toxin (SLT) or verotoxin (VT)

SLT-I and SLT-II are the two most common toxins and individual EHEC strains have the ability to produce both or either, in varying quantities. Therefore, SLT production and not individual (O157:H7) serotype identification is a better diagnostic strategy for the determination of EHEC associated disease

Specimen Collection, Transport and Handling

Feces collected in sterile or non sterile clean container and sent to the laboratory for immediate processing. ***The pH of the specimen will begin to drop after collection and this could reduce the quantity of pathogens present.***

The number of stool specimens submitted to the laboratory is controversial. It is recommended if more than one specimen is submitted that ***it should be on different days***

Specimen transport and handling

Stool held at room temperature should be cultured within 1 hour of collection.

Refrigeration of stool specimens is not recommended as some pathogens, especially *Shigella* species, are very susceptible to lower temperatures and will die rapidly.

Specimens that cannot be sent immediately to the laboratory or processed shortly after collection should be placed in an appropriate enteric transport media (Cary-Blair transport swab or Amie's transport swab).

Specimens containing barium, mineral oil or urine should be rejected

Stool direct examination***Leukocyte evaluation***

1- Perform a Gram stain on the stool specimen to evaluate for the presence of leukocytes.

Direct Gram stain is not useful beyond its determination of the presence of leukocytes, as it will not differentiate suspected pathogens from normal microbial flora.

Place a drop of feces on a slide, spread out, and allow to air dry. Stain slide using Gram's stain procedure.

Evaluate slide under oil immersion for PMN's

2- ***Latex agglutination*** for detection of *lactoferrin* released by fecal leukocytes in diarrheal stool specimens.

Not appropriate for detection of leukocytes in breastfeeding babies. Lactoferrin present in breast milk

Culture Setup

- A. The purpose of a stool culture is to use selective and routine media to screen for the presence of stool pathogens.
- B. In many cases, full identification of a suspicious colony is not performed. Instead, screening tests are performed to rule out potential stool pathogens. If suspicious colonies are noted then full identification performed.
- C. Routine stool cultures should always include testing for the presence of *Salmonella* species, *Shigella* species, and *Campylobacter jejuni*
- D. It is recommended that routine stool cultures include testing for the presence of *Aeromonas* species, and *Plesiomonas* species. The CDC recommends routine screening of stool for *E. coli* strains that produce a Shiga-like cytotoxin.
- E. When required by the physician, media may also be included to detect *Yersinia* species or *Vibrio* species. However, in areas where these pathogens are common including media for these organisms is part of the routine culture
- F. The CDC recommends testing to detect shiga-like toxin producing strains of *E. coli* in addition to culture for *Escherichia coli* O157:H7.
- G. It is no longer recommended to do culture alone. Clinical sites may perform culture and toxin testing or toxin testing alone.
- H. A macroscopic exam is reported with every culture

Pathogens commonly isolated in stool

- a. *Salmonella* species
- b. *Shigella* species
- c. *Campylobacter jejuni*
- d. *Vibrio cholera*
- e. *Vibrio parahaemolyticus*
- f. *Yersinia enterocolitica*
- g. *Clostridium difficile*
- h. *Staphylococcus aureus*
- i. *Aeromonas* species
- j. *Plesiomonas* species

- k. Hemorrhagic *Escherichia coli* O157:H7
- l. Shiga-like toxin producing strains of *Escherichia coli*

Normal flora commonly isolated in stool

- a. Enteric organisms
- b. *Staphylococcus* species
- c. *Streptococcus* / *Enterococcus* species
- d. Anaerobic organisms

Inoculate media

Use a swab to inoculate plates using representative areas of the specimen. Inoculate the media making the first streak then use a sterile loop to streak for isolation. Non inhibitory media should always be inoculated first

Routine stool culture

- a. Blood agar
- b. MacConkey's agar
- c. Hektoen enteric (HE) agar or xylose-lysine deoxycholate (XLD) agar
- d. Selective agar for *Campylobacter* (i.e., CVA, Campy BAP, Skirrow's)
- e. GN broth (or Selenite F) is a selectively enhances the growth of *Salmonella* and *Shigella* while suppressing the growth of normal bowel flora. Also, used for the detection of shiga-like toxin producing strains of *E. coli*. This will increase the chances of isolating these pathogens when they are present in small numbers.

Yersinia culture

- 1. Cefsulodin Novobiocin (CN) agar or *Yersinia* Selective agar
- 2. Phosphate buffered saline (PBS) broth – suppresses the growth of normal bowel flora allowing easier detection of *Yersinia* species

Vibrio culture

- a. TCBS agar
- b. Alkaline peptone broth

Incubate media Temperature

- a. Campy BAP: 42°C
- b. PBS broth: 4°C
- c. CN plate: room temperature
- d. All other plates: 35°C

Atmosphere

- Campy BAP: microaerophilic (increased CO₂)
- b. BAP: either ambient air or CO₂
- c. All other plates: ambient air **Time: 24- 48 hours**

Culture Interpretation

A. Evaluation for Salmonella and Shigella

After 24 hours incubation, examine the HE and MAC plates for non-lactose fermenting colonies and H₂S producing colonies.

These colony types are suspicious for *Salmonella* species and *Shigella* species, but may also be normal enteric flora; so biochemical screens must be performed to rule in or out the presence of these pathogens.

For colonies that are suspicious for *Salmonella* or *Shigella* perform KIA slants.

If screen is positive perform biochemical ID panels such as API 20 E.

Colonies that appear to be *Salmonella* species or *Shigella* species are confirmed by performing serological agglutination tests. If enrichment broth (i.e., GN broth or Selenite F) is used, they are subcultured to HE at 24 hours.

Suspicious colonies from the enrichment broth subculture plates are screened biochemically.

A susceptibility test is performed only on any confirmed colonies of *Salmonella* and *Shigella* species when requested

Treatment with antibiotics is not recommended for *Salmonella* species because it may induce a carrier state in the patient. In most case treatment of the clinical symptoms such as dehydration is sufficient.

If no suspicious colonies are found or if all biochemical screens are negative, the report is sent out as: “**No *Salmonella* or *Shigella* isolated**”

B. Campylobacter jejuni

Campy plates are examined at 48 hours and 72 hours. Colonies growing on the original Campy BAP are tested for oxidase. Any oxidase positive colonies are Gram stained to look for the typical curved rods of *Campylobacter* species.

Oxidase positive curved gram-negative rods should be further tested to confirm the presence of *Campylobacter* species. A positive Sodium Hippurate test will confirm the species *Campylobacter jejuni*.

C. Aeromonas and Plesiomonas

After 24 hours incubation, examine the BAP for large gray colonies that are gram-negative rods in predominance.

Do an oxidase on each different colony type of gram-negative rods. If a colony type is oxidase positive and has a smooth morphology, perform a spot indole test. An oxidase positive indole positive gram-negative rod is suspicious for *Aeromonas* species or *Plesiomonas* species.

Confirm identification of suspicious organisms with various biochemical ID panels.

Aeromonas species are slightly beta hemolytic on the BAP but *Plesiomonas* species are nonhemolytic.

Do not use the MAC to screen for *Aeromonas* or *Plesiomonas* as these organisms can be lactose variable

D. Yersinia

After 24 hours incubation, examine the CN plate for dark red colonies with a “bull’s eye” center surrounded by transparent border colonies that indicate mannitol fermentation and is suspicious for *Yersinia*.

Confirm identification of suspicious organisms with various biochemical ID panels.

CN plates are held for 72 hours at room temperature.

E. Vibrio

After 8-24 hours of incubation, subculture alkaline peptone broth to a TCBS agar. Examine the TCBS agar for yellow colonies that indicate sucrose fermentation. Screen suspicious colonies biochemically.

F. *Staphylococcus aureus* or Yeast

After 24 hours incubation, examine the BAP for *Staphylococcus aureus* or yeast. If organism is in moderate to many amounts or as the predominant organism, work up the organism

G. *Escherichia coli* O157:H7

Setup stool culture with a MacConkey's agar with 1% D-sorbitol (instead of lactose).

After 24 hours of incubation, examine Mac-Sorbitol plate for colorless colonies (*E. coli* O157:H7 is sorbitol negative and most other normal flora strains of *E. coli* are sorbitol positive).

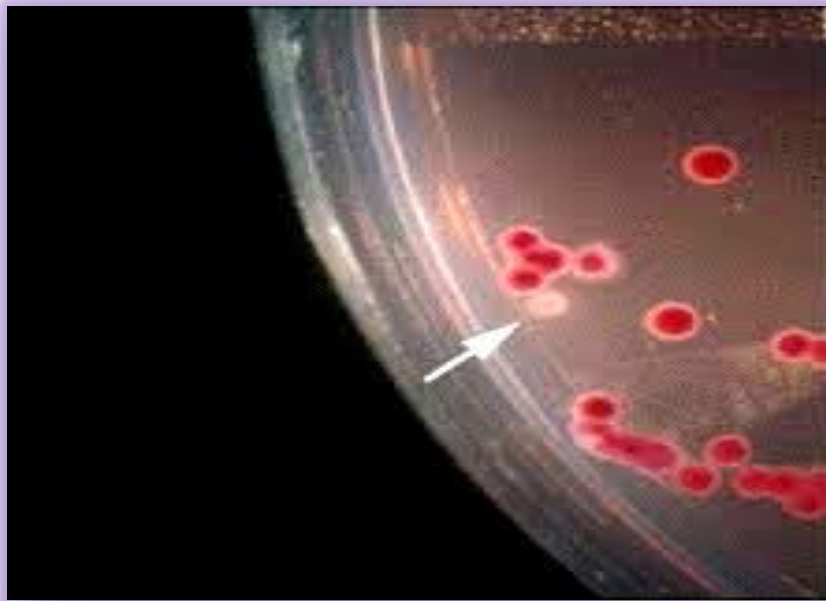


Figure shows E coli O157:H7 on a sorbitol-MacConkey's agar plate Arrow indicates distinctive colorless

Confirm identification of suspicious organisms with various biochemical ID panels and O157:H7 antisera.

✚ Shiga-like toxin producing strains of *E. coli* carry out EIA or molecular testing is performed to detect these strains.

✚ Serological Testing for *Salmonella* and *Shigella*

A. All isolates that biochemically resemble either *Salmonella* species or *Shigella* species must be confirmed by serological methods following site specific procedures.

B. For suspected *Shigella* species, test the following somatic (“O”) antigens:

1. Antigen A = *Shigella dysenteriae*
2. Antigen B = *Shigella flexneri*
3. Antigen C = *Shigella boydii*
4. Antigen D = *Shigella sonnei*

C. For suspected *Salmonella* species, test the following somatic (“O”) antigens:

1. Polyvalent A-E, Vi serum

2. Polyvalent F-I serum

Clostridium difficile

A. *Clostridium difficile* is an anaerobic gram-positive spore-forming rod that causes antibiotic-associated pseudomembranous enterocolitis.

Since *C. difficile* is found as part of the normal fecal flora in many individuals, isolation of the organism does not prove the presence of disease. In cases of *C. difficile* diarrhea, the patient's normal fecal flora is suppressed by prolonged antibiotic therapy allowing the *C. difficile* to multiply and produce a toxin

B. Detection of the presence of the toxin is used to diagnose the disease. Toxin may be detected by tissue culture assay, a latex agglutination test, or an ELISA test.

Stool specimens collected for toxin studies should be refrigerated until testing to preserve the toxin that is rapidly labile at higher temperatures.

Diagnostic Microbiology***Urinary tract infections (UTIs)***

Urinary tract infections (UTIs) are among the most common bacterial infections and account for a significant part of the workload in clinical microbiology laboratories.

Enteric bacteria (in particular, *Escherichia coli*) remain the most frequent cause of UTIs, although the distribution of pathogens that cause UTIs is changing.

More important is the increase in resistance to some antimicrobial agents, particularly the resistance to trimethoprim-sulfamethoxazole seen in *E. coli*. Physicians distinguish UTIs from other diseases that have similar clinical presentations with use of a small number of tests, none of which, if used individually, have adequate sensitivity and specificity. Among the diagnostic tests, urinalysis is useful mainly for excluding bacteriuria. Urine culture may not be necessary as part of the evaluation of outpatients with uncomplicated UTIs, but it is necessary for outpatients who have recurrent UTIs, experience treatment failures, or have complicated UTIs, as well as for inpatients that develop UTIs.

Urinary tract infections (UTIs) are among the most common bacterial infections. It has been estimated that symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually. UTIs have become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and they are the second most common cause of bacteremia in hospitalized patients.

UTIs are challenging, not only because of the large number of infections that occur each year, but also because the diagnosis of UTI is not always straightforward. Physicians must distinguish UTI from other diseases that have a similar clinical presentation, some UTIs are asymptomatic or present with atypical signs and symptoms, and the diagnosis of UTIs in neutropenic patients (who do not typically have pyuria) may require different diagnostic criteria than those used for the general patient population. Because of these factors, physicians frequently rely on a small number of imperfect laboratory tests to augment clinical impressions; even when clinical diagnosis are unequivocal, physicians may order laboratory tests to identify the cause of the infection and/or to provide isolates for antimicrobial susceptibility testing. It therefore comes as no surprise that the laboratory examination of urine specimens accounts for a large part of the workload in many hospital-based laboratories.

In fact, in many clinical laboratories, urine cultures are the most common type of culture, accounting for 24%–40% of submitted cultures; as many as 80% of these urine cultures are submitted from the outpatient setting.

Causes of UTIS

The etiological agents of community-acquired and hospital-acquired UTIs differ. Enteric bacteria (in particular, *Escherichia coli*) have been and remain the most frequent cause of UTI, although there is some evidence that the percentage of UTIs caused by *E. coli* is decreasing. In contrast, significant changes in the causes of nosocomial UTI have been reported since 1980; from 1980 through 1991, the percentage of UTIs caused by *E. coli*, *Proteus* species, and *Pseudomonas* species decreased, whereas the percentage of UTIs caused by yeasts, group B streptococci, and *Klebsiella pneumoniae* increased.

Different changes in the causative agents of UTI, with a decrease in the percentage of UTIs caused by *Enterobacter* species, but with an increase in the percentage of UTIs caused by *Acinetobacter* species and *Pseudomonas aeruginosa*. *Candida albicans* is

the most common cause of funguria, followed by *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, and other yeasts.

Specimen collection

Suprapubic aspiration is the best method to avoid contamination of specimens with bacteria in the distal urethra. This collection method is used infrequently because it is not indicated clinically (except in rare circumstances), it is invasive and uncomfortable, and it requires too much time and too many resources to be practical. Collection of urine by use of a single catheter (straight catheter technique) is the next best technique for obtaining urine specimens with minimal contamination, but, again, it is not indicated clinically for most patients because it is too labor intensive and costly for routine use and it is invasive.

It has added disadvantages, because the process of inserting a catheter through the urethra can introduce bacteria into the bladder (and thereby cause UTI), and rare complications have been reported.

Most urine specimens are obtained from adult patients via the ***clean-catch midstream technique***. This technique has the following advantages: it is neither invasive nor uncomfortable, it is simple and inexpensive, it can be performed in almost any clinical setting, there is no risk of introducing bacteria into the bladder by catheterization, and there is no risk of complications.

Colony counts from urine specimens collected by this method correlate reasonably well with those of specimens collected via suprapubic aspiration or straight catheterization.

The obvious disadvantage of this technique is that the urine sample passes through the distal urethra and can become contaminated with commensal bacteria. Simple procedures that have been developed to decrease the contamination rate include cleansing of skin and mucous membranes adjacent to the urethral orifice before micturition, allowing the first part of the urine stream to pass into the toilet, and collecting urine for culture from the midstream.

Although the clean-catch midstream method is accepted and used widely, the available evidence suggests that the cleansing procedures may not decrease urine contamination rates significantly and, therefore, may be unnecessary as a routine. There may be difficulties with proper collection of samples from elderly patients, as well as from those patients who have physical or other types of impairments, which adds to the importance of collecting specimens properly to avoid contamination.

Correct processing and handling of urine specimens, as well as correct interpretation of test results, is dependent on the method used to collect the specimen. It is, therefore, of obvious importance for clinicians to specify the method of collection on the test requisition. Other information that should be included on the test requisition includes the date and time of specimen collection, patient demographic information, and any clinically relevant information (e.g., whether the patient was treated with antimicrobial agents or whether anatomic abnormalities, stones, or an indwelling urinary catheter were present).

Specimen transportation

Urine specimens must be plated within 2 hours after collection and if plated after delayed for 24 hours then plated so the culture results were compared to determine whether delays in plating resulted in an increase in colony counts. In each of the situation, some of the cultures that were delayed showed increases in the number of colony forming units (CFU) per ml to $>10^5$ CFU/ml; thereby leading to false-positive results.

It should be noted that recent studies were performed for interpreting quantitative urine cultures which regarded colony counts of 10^2 - 10^3 CFU/ml were used to define probable infection in specific patients. On the basis of these results, it is currently

recommended that urine specimens should be plated within 2 h after collection unless specimens have been refrigerated or kept in a preservative.

Specimen processing

Routine urine cultures should be plated using calibrated loops for the semi-quantitative method. This method has the advantage of providing information regarding the number of CFU/ml, also providing isolated colonies for identification and susceptibility testing. The types of media used for routine cultures should be limited to blood agar and MacConkey's agar.

For urine specimens obtained from outpatients, it is not necessary to routinely inoculate a medium that is selective for gram-positive bacteria, because nearly all UTIs in outpatients are caused by aerobic and facultative gram-negative bacteria.

Even in patient populations in which *Staphylococcus saprophyticus* is a common cause of UTIs, it is not necessary to use selective media and urine specimens which obtained from hospitalized patients are likely to contain *Enterococci*, which have emerged as the second most common cause of nosocomial infections.

Urine cultures should be incubated overnight at 35°C-37°C in ambient air before being read. There is no added benefit to incubating routine urine cultures for 48 h, provided that specimens are incubated for a full 24 h and that urine specimens containing $<10^4$ uropathogens or specimens from patients with suspected funguria are incubated for 48 hours.

Most pathogenic yeasts grow well on blood agar plates, so it is unnecessary to use selective fungal media for urine cultures, even for samples obtained from patients with suspected funguria. Selective fungal media can be used in those rare instances in which there is a high clinical probability that a UTI is caused by a more fastidious yeast or mold. Urine specimens obtained from patients with suspected mycobacterial UTIs should be processed and plated to the appropriate mycobacterial media

Detection of bacteriuria by urine microscopy

Bacteriuria can be detected microscopically using Gram staining of uncentrifuged urine specimens, Gram staining of centrifuged specimens, or direct observation of bacteria in urine specimens. Gram stain of uncentrifuged urine specimens is a simple method. A volume of urine is applied to a glass microscope slide, allowed to air dry, stained with Gram stain, and examined microscopically.

The performance characteristics of the test are not well-defined, because different criteria have been used to define a positive test result. In one study, the test was found to be sensitive for the detection of $\geq 10^5$ CFU/ml but insensitive for the detection of lower numbers of bacteria.

The urine Gram stain test has the important advantage of providing immediate information as to the nature of the infecting bacterium or yeast and thereby guiding the physician in selecting empiric antimicrobial therapy. This is of importance in some settings, but the Gram stain test has 3 disadvantages that limit its usefulness in most clinical settings:

- 1- It is an insensitive test, being reliably positive only if the concentration of bacteria in the urine is $\geq 10^5$ CFU/ml; infections with bacterial concentrations of 10^2 - 10^3 CFU/ml may not be detected by this test.
- 2- The test is too labor intensive for it to be practical for most clinical microbiology laboratories to offer it on more than a select basis.

3- Because it may not detect bacteria at concentrations of 10^2 - 10^3 CFU/ml, it should not be used in the outpatient setting for patients with uncomplicated UTIs.

Because of these limitations, its use should be limited to patients with cases of acute pyelonephritis, patients with invasive UTIs, or other patients for whom it is important to have immediate information as to the nature of the infecting pathogen.

Detection of bacteriuria by nitrite test

Bacteriuria can be detected chemically when bacteria produce nitrite from nitrate. The biochemical reaction that is detected by the nitrite test is associated with members of the family *Enterobacteriaceae* (the pathogens most commonly responsible for UTIs), but the usefulness of the test is limited because nitrite production is not associated with urinary-tract pathogens such as *S. saprophyticus*, *Pseudomonas* species, or *Enterococci*.

Another limitation to the test is that it requires testing a specimen of the first urine produced in the morning, as ≥ 4 h are required for bacteria to convert nitrate to nitrite at levels that are reliably detectable.

Detection of pyuria by urine microscopy

Pyuria can be detected and quantified microscopically by measuring the urinary leukocyte excretion rate, counting leukocytes with a hemocytometer, counting leukocytes in urine specimens using Gram staining, or counting leukocytes in a centrifuged specimen.

The advantages to urine microscopy are that leukocytes, leukocyte casts, and other cellular elements are observed directly. One disadvantage to urine microscopy is that leukocytes deteriorate quickly in urine that is not fresh or that has not been adequately preserved. Because of these disadvantages, urine microscopy should be limited to patients in whom pyelonephritis or other more serious infections are suspected.

The most accurate microscopic method for quantitating pyuria is to measure the urinary leukocyte excretion rate. Patients with symptomatic UTIs have urinary leukocyte excretion rates of $\geq 400,000$ leukocytes/hour. The test is impractical for clinical use, however, making it necessary for laboratories to use other methods. A simple and inexpensive alternative is to count urine leukocytes with a hemocytometer. Comparison of hemocytometer counts with urinary leukocyte excretion rates has shown that a hemocytometer count of ≥ 10 leukocytes/mm³ correlates with a urinary leukocyte excretion rate of $\geq 400,000$ leukocytes/h.

Moreover, the correlation of hemocytometer counts with urine colony counts has shown that patients with symptomatic UTIs and bacterial concentrations of $>10^5$ CFU/ml have urine leukocyte counts of ≥ 10 leukocytes/mm³. Although using a hemocytometer to count leukocytes is easier than measuring urinary leukocyte excretion rates, it is impractical for clinical laboratories to use a hemocytometer to count leukocytes on a routine basis. The most practical microscopic method involves counting the number of leukocytes in the sediment of centrifuged urine specimens; this method is inaccurate because of inadequate standardization of the method. For these reasons, and to facilitate the processing of large numbers of specimens, most laboratories use rapid tests for leukocyte esterase as a surrogate for microscopic leukocyte counts.

Detection of pyuria by leukocyte esterase tests

Leukocyte esterase tests are based on the hydrolysis of ester substrates by proteins with esterolytic activity; human neutrophils produce as many as 10 proteins with esterolytic activity. These proteins react with ester substrates to produce alcohols and acids that then react with other chemicals to produce a color change that is proportional to the amount of esterase in the specimen.

These tests have the advantage of detecting both esterases in intact leukocytes and esterases released after cell lysis; therefore, even specimens that have not been preserved properly may yield a positive test result.

Leukocyte esterase tests can yield false-positive test results when the urine is contaminated with bacteria present in vaginal fluid; when the specimen contains eosinophils or *Trichomonas* species, both of which can act as sources of esterases; and when oxidizing agents or formalin react with the test strips to generate false-positive test results.

Leukocyte esterase tests may show a decrease in positive test results when the specimen has an elevated specific gravity and/or elevated levels of protein and glucose; when boric acid preservatives are present; when large amounts of ascorbic or oxalic acid are present; and when the patient has received antimicrobial agents, such as Cephalothin, Cephalexin, or Tetracycline.

Routine bacterial urine cultures

Urine culture may not be necessary as part of the evaluation of outpatients with uncomplicated UTIs. However, urine cultures are necessary for outpatients who have recurrent UTIs, experience treatment failures, or have complicated UTIs.

Urine cultures are also necessary for inpatients that develop UTIs. The bacterial culture remains an important test in the diagnosis of UTI, not only because it helps to document infection, but also because it is necessary for determination of the identity of the infecting microorganism(s) and for antimicrobial susceptibility testing. This is particularly true because of the increased incidence of antimicrobial resistance.

The most commonly used criterion for defining significant bacteriuria is the presence of $\geq 10^5$ CFU per milliliter of urine. This criterion was established only for women with acute pyelonephritis or women who were asymptomatic but had multiple urine cultures that yielded this number of bacteria; however, the criterion is often applied to other patient populations. Most patients with UTIs, however, do not fall into either category, and 30%-50% of patients with acute urethral syndrome will have colony counts of $< 10^5$ CFU/ml. For this reason, many laboratories have opted to use lower colony counts as a criterion for interpreting and reporting results. One common criterion is a colony count of 10^4 CFU/ml, which would be expected to increase the sensitivity of the test without making the test impractical for clinicians and laboratories to use.

Catheterized patients (who may have low concentrations of bacteria that can progress to higher concentrations) and many patients with infections of the lower urinary tract have colony counts much lower than 10^5 CFU/ml if the specimens are obtained via suprapubic aspirate or catheterization. Accordingly, the most appropriate diagnostic criterion for urine culture specimens obtained via suprapubic aspirate or catheterization is a bacterial concentration of $\geq 10^2$ CFU/ml.

Follow-up cultures are recommended for patients with infections that do not respond to therapy, patients who have recurrent UTIs, patients who have anatomic or functional abnormalities of the urinary tract, or patients who continue to have unexplained abnormal urinalysis findings.

Interpretation of urine culture results

Microbiologists need to interpret the microbiologic relevance of growth on culture plates to determine whether further identification and antimicrobial susceptibility testing are necessary. Most culture results can be interpreted readily; no growth and gross contamination are both unambiguous results, as are pure cultures of common pathogens growing in a quantity of $>10^5$ CFU per milliliter of urine.

The interpretation of cultures that yield pure growth in lower quantities is also clear for specimens obtained via suprapubic aspiration or straight catheterization. On the other hand, interpretation of urine cultures that yield mixed flora in varying quantities can be difficult. Test reports for cultures that yield mixed flora in varying quantities should specify the microorganisms that were recovered, the quantity of each microorganism, and the probable clinical importance of each isolate.

Antimicrobial susceptibility testing

Each laboratory should have guidelines by which pathogens are tested for antimicrobial susceptibility. These guidelines should be developed and antimicrobial susceptibility tests should be performed and reported according to the most recent version of the NCCLS guidelines. Bacterial or fungal isolates of uncertain clinical importance should not be tested for antimicrobial susceptibility for purposes of routine patient care.

Most patients with uncomplicated acute cystitis have cases that are clinically straightforward, and they may not require any laboratory testing beyond urinalysis. For a significant number of patients, however, the clinical history and physical findings alone may be insufficient to make a definitive diagnosis of UTI.

For those patients and for patients with complicated UTIs, laboratory tests are necessary to make the diagnosis and to provide specific information regarding the identity and the antimicrobial susceptibility pattern of pathogens. Both the laboratory diagnosis and the clinical diagnosis of laboratory test results must be made in light of the method of collection used; clinicians should specify the method of collection on test requisition forms.

Of the available laboratory tests, urinalysis is helpful primarily as a means of excluding bacteriuria, but it is not a surrogate for culture. Although cultures identify pathogens, the accurate interpretation of culture results requires clinical information that is usually available only to the clinician.

Bloodstream Infections

Invasion of the bloodstream by microorganisms constitutes one of the most serious situations in infectious disease. Microorganisms present in the circulating blood—whether continuously, intermittently, or transiently—are a threat to every organ in the body.

The suffix *emia* is derived from the Greek word meaning “blood” and refers to the presence of a substance in the blood; *bacteremia* refers to the presence of bacteria in the blood, *fungemia* refers to the presence of fungi in the bloodstream, and *septicemia* indicates bacteria are present in the blood, producing an infection and reproducing within the bloodstream.

Microbial invasion of the bloodstream resulting from any organism can have serious immediate consequences, including shock, multiple organ failure, disseminated intravascular coagulation (DIC), and death. Approximately 200,000 cases of bacteremia and fungemia occur annually, with mortality rates ranging from 20% to 50%. Timely detection and identification of blood-borne pathogens are two of the most important functions of the microbiology laboratory.

Pathogens of all four major groups of microbes—bacteria, fungi, viruses, and parasites—may be found circulating in blood during the course of many diseases. Positive blood cultures may help provide a clinical diagnosis, as well as a specific etiologic diagnosis.

General considerations:

The successful recovery of microorganisms from blood by the laboratory depends on many, often complex, factors: the type of bacteremia, the specimen collection method, the blood volume, the number and timing of blood cultures, the interpretation of results, and the type of patient population being served by the laboratory.

All of these parameters must be considered in the development of the blood culture protocol within the laboratory in order to maximize the detection and recovery of microorganisms and ensure quality patient care.

Etiology:

As previously mentioned, all major groups of microbes can be present in the bloodstream during the course of many diseases.

Bacteria:

The organisms most commonly isolated from blood are gram-positive cocci, including coagulase-negative staphylococci, *Staphylococcus aureus*, and *Enterococcus* spp., and other organisms likely to be inhabitants of the hospital environment that colonize the skin, oropharynx, and gastrointestinal tract of patients. Some of the most common, clinically significant bacteria isolated from

blood cultures are listed in Table 1. (Table 1 Organisms Commonly Isolated from Blood Cultures)

Organisms Commonly Isolated from Blood Cultures:

Staphylococcus aureus

Escherichia coli

Coagulase-negative staphylococci

Enterococcus spp.

Candida albicans
Pseudomonas aeruginosa
Klebsiella pneumoniae
Viridans streptococci
Streptococcus pneumoniae
Enterobacter cloacae
Proteus spp.
Beta-hemolytic streptococci
Anaerobic bacteria: *Bacteroides* and *Clostridium* spp.

In general, the number of fungi and coagulase-negative staphylococci has increased, whereas the number of clinically significant anaerobic isolates has decreased since the early 2000s.

Of importance, the laboratory isolation of certain bacterial species from blood can indicate the presence of an underlying, occult, or undiagnosed neoplasm. Alterations in local conditions at the site of the neoplasm allowing bacteria to proliferate and seed the bloodstream have been suggested as a potential mechanism for the association between bacteremia and cancer. Another possible mechanism is reduced killing of bacterial cells by the host phagocytes. Organisms associated with neoplastic disease include *Clostridium septicum* and other uncommonly isolated clostridial species, *Streptococcus gallolyticus*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, and *Campylobacter* spp. Finally, if *Streptococcus anginosus* group bacteria are isolated from blood, the possibility of an abscess should be considered.

Fungi:

Fungemia (the presence of fungi in blood) is usually a serious condition, occurring primarily in immunosuppressed patients and in those with serious or terminal illness. *Candida albicans* is by far the most common species, but *Malassezia furfur* can often be isolated in patients, particularly neonates, receiving lipid-supplemented parenteral nutrition. *Candida* spp. account for approximately 8% to 10% of all nosocomial bloodstream infections. Except for *Histoplasma*, which multiply in leukocytes (white blood cells), fungi do not invade blood cells, but their presence in the blood usually indicates a focus of infection elsewhere in the body.

Fungi in the bloodstream can disseminate (be carried) to all organs of the host, where they may grow, invade normal tissue, and produce toxic products. Fungi gain entrance to the circulatory system via loss of integrity of the gastrointestinal or other mucosa; through damaged skin; from primary sites of infection, such as the lung or other organs; or by means of intravascular catheters. Systemic fungal infections begin as pneumonia and may disseminate from the lungs, which serve as the portal of entry. Arthroconidia of *Coccidioides immitis* and microconidia of *Histoplasma capsulatum* and *Blastomyces dermatitidis* are ingested by alveolar macrophages in the lung.

These macrophages carry the fungi to nearby lymph nodes, usually the hilar nodes. The fungi multiply within the node tissue and ultimately are released into the circulating blood, from which they are capable of seeding

other organs or are destroyed by the body's defenses. Molds are particularly insensitive to host defenses such as antibody and phagocytic cells because of their large size and their sterol containing cell wall structure.

Parasites:

Eukaryotic parasites may be found transiently in the bloodstream as they migrate to other tissues or organs. Their presence, however, cannot be considered consistent with a state of good health. For example, tachyzoites of the parasite *Toxoplasma gondii* may be found in circulating blood. They invade cells within lymph nodes and other organs, including the lungs, liver, heart, brain, and eyes. The resulting cellular destruction accounts for the manifestations of toxoplasmosis. Also, microfilariae are

seen in peripheral blood during infection with *Dipetalonema*, *Mansonella*, *Loa loa*, *Wuchereria*, or *Brugia*.

Malarial parasites invade host erythrocytes and hepatic parenchymal cells. The significant anemia and subsequent tissue hypoxia (reduction in oxygen levels) may result from destruction of red blood cells by the parasite. Vascular trapping of normal erythrocytes by the infected red blood cells, which are less flexible and tend to clog small capillaries, is a major cause of morbidity. The host's immunologic response is to remove the parasites and damaged red blood cells; the immune response may also

have deleterious effects. Parasites in the bloodstream are usually detected by direct visualization. Those parasites for which traditional diagnosis is dependent on observation of the organism

in peripheral blood smears include *Plasmodium*, *Trypanosoma*, and *Babesia*. Patients with malaria or filariasis may display a periodicity in their episodes of fever that allows the physician to time the collection of blood for microscopic examination intended for optimal detection. Rapid serological methods and molecular methods are currently used to detect malaria, babesiosis, and trypanosomiasis.

Viruses:

Although many viruses do circulate in the peripheral blood at some stage of disease, the primary pathology relates to infection of the target organ or cells. Those viruses that preferentially infect blood cells are Epstein-Barr virus (invades lymphocytes), cytomegalovirus (invades monocytes, polymorphonuclear cells, and lymphocytes), and human immunodeficiency virus (HIV)

(involves only certain T lymphocytes and perhaps macrophages) and other human retroviruses that attack lymphocytes. The pathogenesis of viral diseases of the blood is the same as that for viral diseases of any organ; by diverting the cellular machinery to create new viral components

or by other means, the virus may prevent the host cell from performing its normal function. The cell may be destroyed or damaged by viral replication, and immunologic responses of the host may also contribute to the pathogenesis. Although many viral diseases have a viremic stage, recovery of virus particles or detection of circulating viruses is used in the diagnosis of only a few diseases.

Types of bacteremia:

Bacteremia may be transient, continuous, or intermittent. Most people have experienced transient bacteremia; teething infants and people having dental procedures have had oral flora gain entry to the bloodstream through breaks in the gums. Other conditions in which bacteria are only transiently present in the bloodstream include manipulation of infected tissues, devices or instrumentation inserted through contaminated mucosal surfaces,

and surgery involving nonsterile sites. These circumstances may also lead to significant septicemia, although normally the bacteria are cleared from the blood by scavenging

leukocytes, resulting in no infection. Septicemia can occur when the bacteria multiply more rapidly than the immune system is capable of killing and removing the organism. In septic shock, bacterial endocarditis, and other endovascular infections, organisms are released into the bloodstream at a fairly constant rate (continuous bacteremia). Also, during the early stages of specific infections, including typhoid fever, brucellosis, and leptospirosis, bacteria are continuously present in the bloodstream.

In most other infections, such as in patients with undrained abscesses, bacteria can be found intermittently in the bloodstream. Of note, the causative agents of meningitis, pneumonia, pyogenic arthritis, and osteomyelitis are often recovered from blood during the early course of these diseases. In the case of transient seeding of the blood from a sequestered focus of infection, such as an

abscess, bacteria are released into the blood approximately 45 minutes before a febrile episode.

The symptoms of septicemia are fever, chills, and malaise; these are caused by the presence of the invading microorganism and the toxins produced by these microorganisms. The older the patient is, the greater the risk and the rate of mortality as a result of septicemia.

Types of blood stream infections:

The two major categories of bloodstream infections are intravascular (those that originate within the cardiovascular system) and extravascular (those that result from bacteria entering the blood circulation through the lymphatic system from another site of infection). Of note, other organisms, such as fungi, may also cause intravascular or extravascular infections. However, because bacteria account for the majority of significant vascular infections, these types of bloodstream infections are discussed in more detail. Factors contributing to the initiation of bloodstream infections are immunosuppressive agents, widespread use of broad-spectrum antibiotics that suppress the normal flora and allow the emergence

of resistant strains of bacteria, invasive procedures allowing bacteria access to the interior of the host, more extensive surgical procedures, and prolonged survival of debilitated and seriously ill patients.

1) Intravascular Infections:

Intravascular infections include infective endocarditis, mycotic aneurysm, suppurative thrombophlebitis, and intravenous (IV), catheter-associated bacteremia. Because these infections are within the vascular system, organisms are present in the bloodstream at a fairly constant rate (i.e., a continuous bacteremia). These infections in the cardiovascular system are extremely serious and considered life threatening.

Infective Endocarditis. The development of infective endocarditis (infection of the endocardium most commonly caused by bacteria) is believed to involve several independent events. Cardiac abnormalities, such as congenital valvular diseases that lead to turbulence in blood flow or direct trauma from IV catheters, can damage cardiac endothelium. This damage to the endothelial

surface results in the deposition of platelets and fibrin. If bacteria transiently gain access to the bloodstream (this can occur after an innocuous procedure such as brushing the teeth) after alteration of the capillary endothelial cells, the organisms may stick to and then colonize the damaged cardiac endothelial cell surface.

After colonization, the surface will rapidly be covered with a protective layer of fibrin and platelets. This protective environment is favorable to further bacterial multiplication. This web of platelets, fibrin, inflammatory cells, and entrapped organisms is called a vegetation (Figure -1).

The resulting vegetations ultimately seed bacteria into the blood at a slow but constant rate. The primary causes of infective endocarditis are the viridans streptococci, comprising several species (Box 68-2). These organisms are normal inhabitants of the oral cavity, often gaining entrance to the bloodstream as a result of gingivitis, periodontitis, or dental manipulation. Heart valves, especially those previously damaged, present convenient surfaces for attachment of these bacteria. *Streptococcus sanguis* and *Streptococcus mutans* are frequently isolated in streptococcal endocarditis.

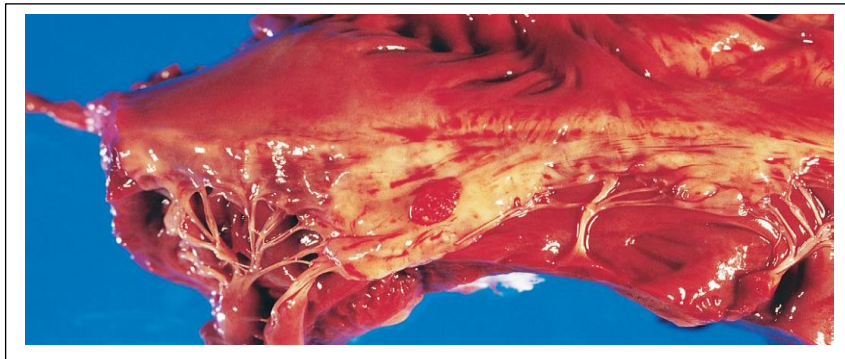


Figure 1 Vegetations of bacterial endocarditis. Arrow indicates the vegetations.

(Courtesy Celeste N. Powers, MD, PhD, Virginia Commonwealth

University Medical Center, Medical College of Virginia Campus, Richmond, Va.

Gram-negative bacilli, known as the AACEK group, *Aggregatibacter aphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*, can also be associated with endocarditis.

With the ever-increasing use of IV catheters, arterial lines, and vascular prostheses, organisms considered normal or hospital-acquired inhabitants of the human skin are able to gain access to the bloodstream and attach to various surfaces, including heart valves and vascular endothelium. It has been estimated that more than 200,000 nosocomial infections (bloodstream) occur annually in the United States in adults and children. The majority of these infections are caused by the use of intravascular catheters. *Staphylococcus epidermidis* and other coagulase-negative staphylococci have been increasingly implicated as the cause of infection associated with intravascular catheters. *S. epidermidis* is the most common etiologic agent identified in prosthetic valve endocarditis, with *S. aureus* being the second most common. *S. aureus* is an important cause of septicemia without endocarditis and is found in association with other foci, such as abscesses, wound infections, and pneumonia, as well as sepsis related to indwelling intravascular catheters.

Table 2 Agents of Infective Endocarditis

Agents of Infective Endocarditis:
<i>Aggregatibacter aphrophilus</i> Viridans streptococci* Nutritionally deficient streptococci (<i>Abiotrophia</i> spp. and <i>Granulicatella</i> spp.) Enterococci* <i>Streptococcus bovis</i> <i>Staphylococcus aureus</i>* Staphylococci (coagulase-negative) Enterobacteriaceae <i>Pseudomonas</i> spp. (usually in drug users) <i>Haemophilus</i> spp. Unusual gram-negative bacilli (e.g., <i>Actinobacillus</i>, <i>Cardiobacterium</i>, <i>Eikenella</i>, <i>Coxiella burnetii</i>) Yeast Other (including polymicrobial infectious endocarditis) *Most common organisms associated with native valve endocarditis in non-drug-using adults.

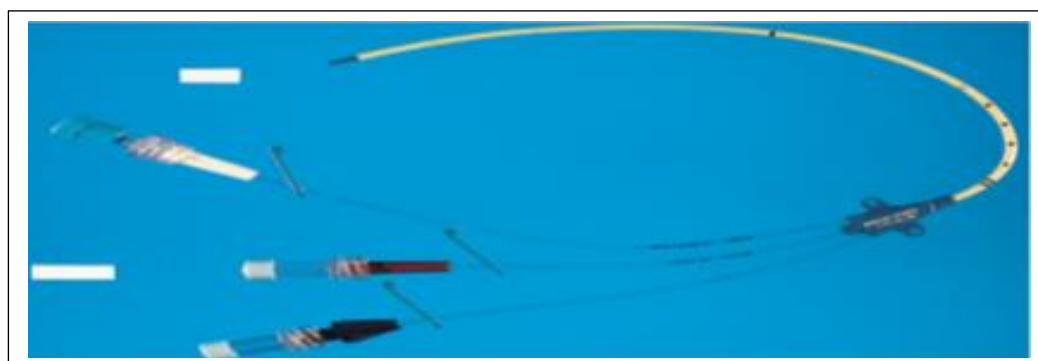


Figure 2 Short-term, triple-lumen central venous catheter. The ends from which the catheter is accessed are usually referred to as the hubs. After the catheter is inserted, the tip resides within the bloodstream.

Table -3 Common Agents of IV Catheter–Associated Bacteremia:
<i>Staphylococcus epidermidis</i> Other coagulase-negative staphylococci <i>Staphylococcus aureus</i> Enterobacteriaceae <i>Pseudomonas aeruginosa</i>

Candida spp.
Corynebacterium spp.
Other gram-negative rods

2) Extravascular Infections:

Except for intravascular infections, bacteria usually enter the circulation through the lymphatic system. Most cases of clinically significant bacteremia are a result of extravascular infection. When organisms multiply at a local site of infection such as the lung, they are drained by the lymphatics and reach the bloodstream. In most individuals, organisms in the bloodstream are effectively and rapidly removed by the reticuloendothelial system in the liver, spleen, and bone marrow and by circulating phagocytic cells. Depending on the extent of immunologic control of the infection, the organism may be circulated more widely, thereby causing a bacteremia or fungemia..

The most common portals of entry for bacteremia are the genitourinary tract (25%), respiratory tract (20%), abscesses (10%), surgical wound infections (5%), biliary tract (5%), miscellaneous sites (10%), and uncertain sites (25%). For the most part, the probability of bacteremia occurring from an extravascular site depends on the site of infection, its severity, and the organism. For example, any organism producing meningitis is likely to produce bacteremia at the same time.

Of importance, certain organisms causing extravascular infections commonly invade the bloodstream; In addition to these organisms, a large number of other bacteria and fungi that cause extravascular infections are also capable of invading the bloodstream. Whether these organisms invade the bloodstream depends on the host's ability to

control the infection and the organism's pathogenic potential. Some of the organisms associated with potential bloodstream infections from a localized site include members of the family Enterobacteriaceae, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*,

anaerobic cocci, *Bacteroides*, *Clostridium*, beta-hemolytic streptococci, and *Pseudomonas*. These are only some of the organisms frequently isolated from blood. Almost every known bacterial species and many fungal species have been implicated in extravascular bloodstream infection.

Clinical manifestations:

bacteremia may indicate the presence of a focus of disease, such as intravascular infection, pneumonia, or liver abscess, or it may represent transient release of bacteria into the bloodstream. Septicemia or sepsis indicates a condition in which bacteria or their products (toxins) are causing harm to the host. Unfortunately, clinicians often use the terms *bacteremia*

and *septicemia* interchangeably. Signs and symptoms of septicemia may include fever or hypothermia (low body temperature), chills, hyperventilation (abnormally increased breathing leading to excess loss of carbon dioxide from the body) and subsequent respiratory alkalosis (a condition caused by the loss of acid leading to an increase in pH), skin lesions, change in mental status, and diarrhea. More serious manifestations include hypotension or shock, DIC, and major organ system failure.

The syndrome known as septic shock, characterized by fever, acute respiratory distress, shock, renal failure, intravascular coagulation, and tissue destruction, can be initiated by either exotoxins or endotoxins.

Septic shock is mediated by the production of cytokines from activated mononuclear cells, such as tumor necrosis factor and interleukins. Shock is the gravest complication of septicemia. In septic shock, the presence of bacterial products and the host's response act to shut down major host physiologic systems.

Clinical manifestations include a drop in blood pressure, increase in heart rate, functional impairment in vital organs (brain, kidney, liver, and lungs), acid base alterations, and bleeding problems. Gram-negative bacteria contain a substance in their cell walls, called endotoxin, which has a strong effect on several physiologic functions. This substance, a lipopolysaccharide (LPS) comprising part of the cell wall structure, may be released during the normal growth cycles of bacteria or after the destruction of bacteria by host defenses. Endotoxin (or the core of the LPS, lipid A) has been shown to mediate numerous systemic reactions, including a febrile response, and the activation of complement and certain blood-clotting factors. Although gram-positive bacteria do not contain the lipid A endotoxin, many produce exotoxins, and the effects of their presence in the bloodstream may be equally devastating to the patient.

Disseminated intravascular coagulation (DIC) is a complication of sepsis. DIC is characterized by numerous small blood vessels becoming clogged with blood clots and bleeding as a result of the depletion of coagulation factors. DIC can occur with septicemia involving any circulating pathogen, including parasites, viruses, and fungi, although it is most often a consequence of gram-negative bacterial sepsis.

Immunocompromised patients:

One of the greatest challenges facing microbiologists is the handling of blood cultures from immunocompromised patients. The number of immunocompromised patients has steadily increased in recent years in large part as the result of advances in medicine. People undergoing organ transplantation, elderly persons, individuals with malignant disease (e.g., malignancies and cancer), and those receiving therapy for the malignancy are examples of immunosuppressed patients. Acquired immunodeficiency syndrome (AIDS) has also contributed to the increase in the number of immunocompromised individuals.

The marked immunosuppression brought about by infection with the human immunodeficiency virus (HIV) in patients with AIDS is a result of this virus' profound impairment of cellular immunity. Patients with AIDS have the greatest diversity of pathogens recovered from blood, including mycobacterial species, *Bartonella henselae*, *Corynebacterium jeikeium*, *Shigella flexneri*, unusual *Salmonella* species, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and cytomegalovirus.

As is typically observed in other hospitalized patients, organisms such as gram-positive aerobic bacteria (e.g., *Staphylococcus aureus*, *Enterococcus*) and gram-negative aerobic bacteria (e.g., Enterobacteriaceae, *Pseudomonas aeruginosa*) are common causes of bloodstream infections in immunocompromised patients. In addition, bloodstream infections in immunocompromised patients are frequently caused by either unusual pathogens whose recovery from blood requires special techniques or by organisms normally considered contaminants when isolated from blood cultures.

Therefore, microbiologists must be aware of the potential pathogenicity of organisms in immunosuppressed patients that are typically considered as probable blood culture contaminants.

Without this knowledge, aerobic gram-positive rods isolated from blood cultures may be dismissed as contaminating diphtheroids, when, in fact, the organism is *C. jeikeium*, known to cause bacteremia in immunosuppressed patients. Microbiologists must be familiar with the unusual pathogens isolated from blood cultures obtained from immunocompromised patients and organisms that require special techniques for isolation.

Specimen collection:

Antisepsis. Once a vein is selected, the skin site is defatted (fat removal) with 70% isopropyl alcohol and an antiseptic is applied to kill surface and subsurface bacteria. Regardless of the antiseptic used, it is critical to follow the manufacturer's recommendation for the length of time the antiseptic is allowed to remain on the skin. Available data indicate that iodine tincture (iodine in alcohol) and chlorhexidine are equivalent for skin preparation before drawing blood cultures.

Drawing Blood for Culture: Organisms found in circulating blood can be enriched in culture for isolation and further studies. Blood for culture must be obtained aseptically. Once removed from the circulation, unclotted blood must be diluted in growth media. Universal precautions require that phlebotomists wear gloves for this procedure:

1. Choose the vein to be drawn by touching the skin before it has been disinfected.
2. Using 70% alcohol, cleanse the skin over the venipuncture site in a circle approximately 5 cm in diameter, rubbing vigorously. Allow to air-dry.
3. Starting in the center of the circle, apply 2% tincture of iodine (or povidone-iodine) in ever-widening circles until the entire circle has been saturated with iodine. Allow the iodine to dry on the skin for at least 1 minute. The timing is critical; a watch or timer should be used.
4. If the phlebotomist must touch the site after preparation, the phlebotomist must disinfect the gloved fingers used for palpation in identical fashion.
5. Insert the needle into the vein and withdraw blood. Do not change needles before injecting the blood into the culture bottle.
6. After the needle has been removed, the site should be cleansed with 70% alcohol again, because many patients are sensitive to iodine.

Specimen Volume:

Adults. There is a direct relationship between the volume of blood and an increased probability that the laboratory will isolate the infecting organism. Therefore, collection of two sets of cultures using 10 to 20 mL of blood per culture is strongly recommended for adults.

Children. For infants and small children, only 1 to 5 mL of blood should be drawn for bacterial culture. Blood culture bottles are available designed specifically for the pediatric patient. Because blood specimens from septic children may yield fewer than 5 CFU/mL of the organism, quantities less than 1 mL may not be adequate to detect pathogens. Nevertheless, smaller volumes should still be cultured because high levels of bacteremia (more than 1000 CFU/mL of blood) are detected in some infants.

Number of Blood Cultures: usually two or three blood cultures are sufficient to achieve the optimum blood culture sensitivity.

Timing of Collection:

The timing of cultures is not as important as other factors in patients with intravascular infections because organisms are released into the bloodstream at a fairly constant rate. Because the timing of intermittent bacteremia is unpredictable, it is generally accepted that two or three blood cultures be spaced an hour apart. blood should be transported immediately to the laboratory and placed into the incubator or instrument as soon as possible. With blood culture instrumentation, a delay beyond 2 hours can delay the detection of positive cultures.

Miscellaneous Matters:

Anticoagulation. Blood drawn for culture must not be allowed to clot. If bacteria become entrapped within a clot, their presence may go undetected. Thus, blood drawn for culture may be either inoculated directly into the blood culture broth media .

Heparin, ethylenediaminetetraacetic acid (EDTA), and citrate inhibit numerous organisms and are not recommended for use. Sodium polyanethol sulfonate (SPS, Liquoid) in concentrations of 0.025% to 0.03% is the best anticoagulant available for blood cultures. As a result, the most commonly used preparation in blood culture media today is 0.025% to 0.05% SPS. In addition to its anticoagulant properties, SPS is also anticomplementary and antiphagocytic, and interferes with the activity of some antimicrobial agents, notably aminoglycosides.

SPS, however, may inhibit the growth of a few microorganisms, such as some strains of *Neisseria* spp., *Gardnerella vaginalis*, *Streptobacillus moniliformis*, and all strains of *Peptostreptococcus anaerobius*.

Blood Culture Media: The diversity of bacteria recovered from blood requires an equally diverse and large number of media to enhance the growth of these bacteria. Basic blood culture media contain a nutrient broth and an anticoagulant. Several different broth formulations are commercially available. Most blood culture bottles available commercially contain trypticase soy broth, brain-heart infusion broth, supplemented peptone, or thioglycolate broth. More specialized broth bases include Columbia or Brucella broth.

BacT/ALERT has a blood culture bottle with supplemented brain heart infusion (BHI) broth containing activated charcoal particles that significantly increase the yield of microorganisms over standard blood culture media. In addition, resins or charcoal may be added to commercial media to absorb and inactivate antimicrobial agents within the patient's blood.

Special blood culture broth systems are available for the isolation of mycobacteria. The systems are useful in detecting disseminated infections caused by *Mycobacterium tuberculosis* and non-tuberculosis mycobacteria. To subculture, the procedure that does not require opening the bottle, the large volume of broth subcultured and the enclosed method provide faster detection for many organisms than is possible with conventional systems

Incubation Conditions. The atmosphere in commercially prepared blood culture bottles is usually permitting the growth of most facultative

and some anaerobic organisms. To encourage the growth of obligate (strict) aerobes, such as yeast and *Pseudomonas aeruginosa*, transient venting of the bottles with a sterile, cotton-plugged needle may be

necessary. Constant agitation of the bottles during the first 24 hours of incubation also enhances the growth of most aerobic bacteria.

BACTEC Systems: Many laboratories use the BACTEC system (Becton Dickinson Microbiology Systems, Sparks, Maryland), which measures the production of carbon dioxide (CO₂) by metabolizing organisms. Blood or sterile body fluid for routine culture is inoculated into bottles containing appropriate substrates.

The first BACTEC systems were semiautomated. Vials, containing ¹⁴C-labeled substrates (glucose, amino acids) were incubated and often agitated on a rotary shaker. At predetermined time intervals thereafter, the bottles were placed into the monitoring module, where they were automatically moved to a detector. The detector inserted two needles through a rubber septum seal at the top of each bottle and withdrew the accumulated gas above the liquid medium and replaced it with fresh gas of the same mixture (aerobic or anaerobic). Any amount of radiolabeled CO₂, the final end product of metabolism of the ¹⁴C-labeled substrates (above a preset baseline level), was considered to be suspicious for microbial growth. Microbiologists retrieved suspicious bottles and worked them up (performed subcultured and identification procedures) for possible microbial growth.

Other laboratories use the BacT/Alert System (bioMérieux, Durham, North Carolina), which measures CO₂-derived pH changes with a colorimetric sensor in the bottom of each bottle (see Figure 3). The sensor is separated from the broth medium by a membrane permeable to CO₂. As organisms grow, they release CO₂, which diffuses across

the membrane and is dissolved in water present in the matrix of the sensor. As CO₂ is dissolved, free hydrogen ions are generated. These free hydrogen ions cause a color change in the sensor (blue to light green to yellow as the pH decreases); a sensor in the instrument reads this color change.

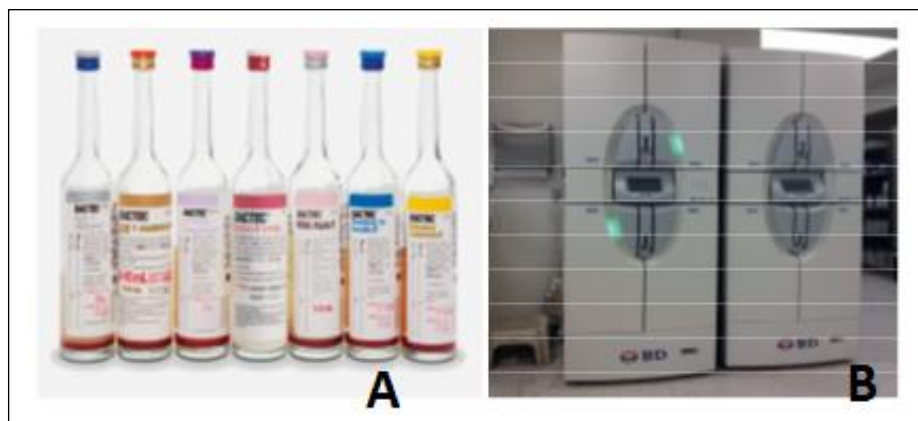


Figure 3 A, Blood culture bottles for the BACTEC 9240, 9120, and 9050 continuous monitoring instruments. B, The BD BACTEC FX continuous monitoring blood culture system

Handling Positive Blood Cultures:

Most laboratories use a broth-based automated blood culture method. When a positive culture is indicated according to the automated detection system, or manual technique: a Gram stained smear of an air-dried drop of

medium should be performed. Methanol fixation of the smear preserves bacterial and cellular morphology, which may be especially

valuable for detecting gram-negative bacteria among red cell debris. Designed to maximize sensitivity, detection algorithms of automated blood culture instruments lead to a certain percentage of false-positive results. Thus, in addition to performance of a Gram stain using methanol fixation, As soon as a morphologic description can be tentatively assigned to an organism detected in blood, the physician should be contacted and given all available information.

Determining the clinical significance of an isolate is the physician's responsibility. If no organisms are seen on microscopic examination of a bottle that appears positive, subcultures should be performed anyway.

Subcultures from blood cultures suspected of being positive, whether proved by microscopic visualization or not, should be made to various media that would support the growth of most bacteria, including anaerobes. Initial subculture may include chocolate agar, 5% sheep blood agar, MacConkey agar, and supplemented anaerobic blood agar.

In addition, some laboratories are subculturing to specialized chromogenic agar for the isolation of specific pathogenic organisms such as MNSA, yeast (*Candida* spp.). The incidence of polymicrobial bacteremia or fungemia ranges from 3% to 20% of all positive blood cultures. For this reason, samples must be resubcultured for isolated colonies.

Numerous rapid tests for identification and presumptive antimicrobial susceptibilities can be performed from the broth blood culture if a monomicrobial infection is suspected (based on microscopic evaluation). A suspension of the organism that approximates the turbidity of a 0.5 McFarland standard, obtained directly from the broth or by centrifuging the broth and resuspending the pelleted bacteria, can be used to perform either disk diffusion (qualitative) or broth dilution (quantitative) antimicrobial susceptibility tests. These suspensions may also be used to perform preliminary tests such as coagulase, thermostable nuclease, esculin hydrolysis, bile solubility, antigen detection by fluorescent-antibody stain or agglutination procedures for gram-positive bacteria, oxidase, and commercially available rapid identification kits for gram-negative bacteria. Presumptive results must be verified with conventional procedures using pure cultures. In addition to these approaches, the introduction of a number of molecular methods, including conventional and peptide nucleic acid hybridization assays using specific probes, conventional and real-time polymerase chain reaction assays and microarrays have been used to directly identify microorganisms in blood culture bottles.

In the event of possible future studies (e.g., additional susceptibility testing), all isolates from blood cultures should be stored for a minimum of 6 months by freezing. It is often necessary to compare separate isolates from the same patient or isolates of the same species from different patients months.

On one hand, contaminants may lead to unnecessary antibiotic therapy, additional testing and consultation, and increased length of hospital stay. Costs related to false-positive blood culture results (i.e., contaminants) are associated with 40% higher charges for IV antibiotics and microbiology testing. On the other hand, failure to recognize and appropriately treat indigenous microflora can have dire consequences.

Guidelines that can assist in distinguishing probable pathogens from contaminants are as follows:

❖ Probable contaminant

- Growth of *Bacillus* spp., *Corynebacterium* spp., *Propionibacterium acnes*, or coagulase-negative staphylococci in one of several cultures

Note: *Bacillus anthracis* must be ruled out before dismissing *Bacillus* species as a probable contaminant.

- Growth of multiple organisms from one of several cultures (polymicrobial bacteremia is uncommon)
- The clinical presentation or course is not consistent with sepsis (physician-based, not laboratory-based criteria)
- The organism causing the infection at a primary site of infection is not the same as that isolated from the blood culture.

❖ Probable pathogen

- Growth of the same organism in repeated cultures obtained either at different times or from different anatomic sites
- Growth of certain organisms in cultures obtained from patients suspected of endocarditis, such as enterococci, or gram-negative rods in patients with clinical gram-negative sepsis
- Growth of certain organisms such as members of Enterobacteriaceae, *Streptococcus pneumoniae*, gram-negative anaerobes, and *Streptococcus pyogenes*
- Isolation of commensal microbial flora from blood cultures obtained from patients suspected to be bacteremic (e.g., immunosuppressed patients or those having prosthetic devices).

Normally Sterile Body Fluids, Bone and Bone Marrow, and Solid Tissues

The human body is divided into five main body cavities: cranial, spinal, thoracic, abdominal, and pelvic. Each cavity is lined with membranes, and within the body wall and these membranes, or between the membranes and organs, are small spaces filled with minute amounts of fluid. The purpose of this fluid is to bathe the organs and membranes, reducing the friction between organs.

Bacteria, fungi, virus, or parasite can invade any body tissue or sterile body fluid site. Although from different areas of the body, all specimens discussed in this chapter are considered normally sterile. Therefore, even one colony of a potentially pathogenic microorganism may be significant.

Specimens from sterile body sites:

FLUIDS :In response to infection, fluid may accumulate in any body cavity. Infected solid tissue often presents as cellulitis or with abscess formation. Areas of the body from which fluids are typically sent for microbiologic studies include those in (Table -1.)

Pleural Fluid : Lining the entire thoracic cavity of the body is a serous membrane called the parietal pleura. Covering the outer surface of the lung is another membrane called the visceral pleura . Within the pleural space between the lung and chest wall is a small amount of fluid called pleural fluid that lubricates the surfaces of the pleura (the membranes surrounding the lungs and lining the chest cavity). Normally, equilibrium exists among the pleural membranes, but in certain disease states, such as cardiac, hepatic, or renal disease, excess amounts of this fluid can be produced and accumulates in the pleural space; this is known as a pleural effusion. Pleural effusions can either be exudative or transudative. Exudative pleural effusions are caused by inflammation,

infection, and cancer, whereas transudative effusions are due to systemic changes, such as congestive heart failure.

Normal pleural fluid contains few or no cells and has a consistency similar to serum, but with a lower protein count. Pleural fluid containing numerous white blood cells is indicative of infections. Pleural fluid specimens are collected by thoracentesis, a procedure in which a needle is inserted through the chest wall into the pleural space and the excess fluid aspirated. This fluid is then submitted to the laboratory as thoracentesis fluid, pleural fluid, or empyema fluid. The fluid, or effusion, can then

be analyzed for cell count, total protein, glucose, lactate dehydrogenase, amylase, cytology, and culture.

The total protein and glucose results determine if the effusion is transudate or exudate. The patient's serum or plasma glucose level is needed to compare with the results indicated in the body fluid. Several characteristics can be used to determine whether a fluid is a transudate or exudate (Table 2).

When effusions are extremely purulent or full of pus, the effusion is referred to as an empyema. Empyema often arises as a complication of pneumonia, but other infections near the lung (e.g., subdiaphragmatic infection) may seed microorganisms into the pleural cavity. It has been estimated that 50% to 60% of patients develop empyema as a complication of pneumonia.

Peritoneal Fluid:

The peritoneum is a large, moist, continuous sheet of serous membrane lining the walls of the abdominalpelvic cavity and the outer coat of the organs contained within the cavity . In the abdomen, these two membrane linings are separated by a space called the peritoneal cavity, which contains or abuts the liver pancreas, spleen, stomach and intestinal tract, bladder, and fallopian tubes and ovaries. The kidneys occupy a retroperitoneal (behind the peritoneum) position. Within the healthy human peritoneal cavity is a small amount of fluid that maintains the surface moisture of the peritoneum. Normal peritoneal fluid may contain as many as 300 white blood cells per milliliter, but the protein content and specific gravity of the fluid are low.

During an infectious or inflammatory process, increased amounts of fluid accumulate in the peritoneal cavity, a condition called ascites. Most cases of ascites are due to liver disease, and in severe cases, the abdomen is often distended. The fluid can be collected for testing by paracentesis (the insertion of a needle into the abdomen and removal of fluid). The peritoneal or ascites fluid can then be analyzed for amylase, protein, albumin, cell count, culture, and cytology. Often ascitic fluid contains

an increased number of inflammatory cells and an elevated protein level.

Agents of infection gain access to the peritoneum through a perforation of the bowel, through infection within abdominal viscera, by way of the bloodstream, or by external inoculation (as in surgery or trauma). On occasion, as in pelvic inflammatory disease (PID).

(Table-1):Microbiology Laboratory Body Fluid Collection Sites:

Body Area	Fluid Name(s)
Thorax	Thoracentesis or pleural or empyema fluid
Abdominal cavity	Paracentesis or ascitic or peritoneal fluid
Joint	Synovial fluid
Pericardium	Pericardial fluid

organisms travel through the natural channels of the fallopian tubes into the peritoneal cavity.

Primary Peritonitis. Peritonitis results when the peritoneal membrane becomes inflamed and can be either primary or secondary. Primary peritonitis is rare and results when infection spreads from the blood and lymph nodes with no apparent evidence of infection. The organisms likely to be recovered from patient specimens.

(Table 2): Pleural Fluid Effusion Characteristics:

	Transudate	Exudate
Appearance	Clear	Cloudy
Specific Gravity	<1.015	>1.015
Total Protein	<3.0 mg/dL	>3.0 mg/dL
LD Fluid: Serum Ratio	<0.6	>0.6
Cholesterol	<60 mg/dL	>60 mg/dL
Cholesterol Fluid: Serum Ratio	<0.3	>0.3
Bilirubin Fluid:Serum Ratio	<0.6	>0.6
Total Protein Fluid: Serum Ratio	<0.5	
White Blood Cells	<1000/μL (all white blood cell types, all <50%)	>1000/μ
Red Blood Cells	<10,000/μL = because of traumatic tap	>100,000/μL
Clotting	Will not clot	May clot

with primary peritonitis vary with the patient's age. The most common etiologic agents in children are *Streptococcus pneumoniae* and group A streptococci, Enterobacteriaceae, other gram-negative bacilli, and staphylococci. In adults, *Escherichia coli* is the most common bacterium, followed by *S. pneumoniae* and group A streptococci. Polymicrobial peritonitis is unusual in the absence of bowel perforation or rupture. Among sexually active young women, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are common etiologic agents

of peritoneal infection, often in the form of a perihepatitis (inflammation of the surface of the liver, called Fitz-Hugh–Curtis syndrome).

Fungal causes of peritonitis are not common, but *Candida* spp. may be recovered from immunosuppressed patients and patients receiving prolonged antibacterial therapy.

Secondary Peritonitis. Secondary peritonitis is a complication of a perforated viscus (organ), surgery, traumatic injury, loss of bowel wall integrity following a destructive disease (e.g., ulcerative colitis, ruptured appendix, carcinoma), obstruction, or a preceding infection (liver abscess, salpingitis, septicemia). The nature, location, and etiology of the underlying process govern the agents recovered from peritoneal fluid. With PID as the background, gonococci, anaerobes, or chlamydiae are isolated. With peritonitis or intra-abdominal abscess, anaerobes generally are found in peritoneal fluid, usually together with Enterobacteriaceae and enterococci or other streptococci. In patients whose bowel flora has

been altered by antimicrobial agents, more resistant gram-negative bacilli and *Staphylococcus aureus* may be encountered. Because anaerobes outnumber aerobes in the bowel by 1000-fold, it is not surprising that anaerobic organisms play a prominent role in intra-abdominal infection, perhaps acting synergistically with facultative bacteria. The organisms likely to be recovered include *E.coli*, the *Bacteroides fragilis* group, enterococci and other streptococci, *Bilophila* spp., other anaerobic gramnegative bacilli, anaerobic gram-positive cocci, and clostridia.

Peritoneal Dialysis Fluid:

Continuous ambulatory peritoneal dialysis (CAPD). the number of organisms is usually too low for detection on Gram stain of the peritoneal fluid sediment unless a concentrating technique is used; fungi are more readily detected. Many recent studies show that improved sensitivity can be achieved by using automated blood culture systems in which 10 mL of fluid is inoculated into culture bottles.

Most infections originate from the patient's own skin flora; *Staphylococcus epidermidis* and *S. aureus* are the most common etiologic agents, followed by streptococci, aerobic or facultative gram-negative bacilli, *Candida* spp., *Corynebacterium* spp., and others. The oxygen content of peritoneal dialysate is usually too high for the development of anaerobic infection. Among the gram-negative bacilli isolated, *Pseudomonas* spp., *Acinetobacter* spp., and the Enterobacteriaceae are frequently observed.

Pericardial Fluid:

The heart and contiguous major blood vessels are surrounded by the pericardium, a protective tissue. The area between the epicardium, which is the membrane surrounding the heart muscle, and the pericardium is called the pericardial space and normally contains 15 to 20 mL of clear fluid. If an infectious agent is present within the fluid, the pericardium may become distended and tight, and eventually tamponade (interference with cardiac function and circulation) can ensue. Up to 500 mL of fluid can accumulate during infection, which may seriously complicate cardiac function.

Agents of pericarditis (inflammation of the pericardium) are usually viruses, especially Coxsackie virus. Parasites, bacteria, certain fungi, and noninfectious causes are also associated with this disease.

Myocarditis (inflammation of the heart muscle itself) may accompany or follow pericarditis. The pathogenesis of disease involves the host inflammatory response contributing to fluid buildup as well as cell and tissue damage.

Common causes of myocarditis include viral infections with Cocksackie virus, echoviruses, or adenovirus. The most common etiologic agents of pericarditis and myocarditis are listed in Table 3. Other bacteria, fungi, and parasitic agents have been recovered from pericardial effusions.

Patients who develop pericarditis resulting from agents other than viruses are often immunocompromised or suffering from a chronic disease. An example is infective endocarditis, in which a myocardial abscess develops and then ruptures into the pericardial space.

Joint Fluid Arthritis is an inflammation in a joint space. Infectious arthritis may involve any joint in the body. Infection of the joint usually occurs secondary to hematogenous spread of bacteria or, less often, fungi, as a direct extension of infection of the bone. It may also occur after

injection of material, especially corticosteroids, into joints or after insertion of prosthetic material (e.g., total hip replacement). Although infectious arthritis usually occurs at a single site (monoarticular), a preexisting bacteremia or fungemia may seed more than one joint to establish polyarticular infection, particularly when multiple joints are diseased, such as in rheumatoid arthritis.

In bacterial arthritis, the knees and hips are the most commonly affected joints in all age groups.

In addition to active infections associated with viable microorganisms within the joint, sterile, self-limited arthritis caused by antigen-antibody interactions may follow an episode of infection, such as meningococcal meningitis. When an etiologic agent cannot be isolated from an inflamed joint fluid specimen, either the absence of viable agents or inadequate transport or culturing procedures may be the cause. For example, even under the best circumstances, *Borrelia burgdorferi* is isolated from the joints of fewer than 20% of patients with Lyme disease. Nonspecific test results, such as increased white blood cell count, decreased glucose, or elevated protein, may indicate that an infectious agent is present but inconclusive.

Overall, *Staphylococcus aureus* is the most common etiologic agent of septic arthritis, accounting for approximately 70% of infections. In adults younger than 30 years of age, however, *Neisseria gonorrhoeae* is isolated most frequently. *Haemophilus influenzae* has been the most common agent of bacteremia in children younger than 2 years of age, and consequently it has been the most frequent cause of infectious arthritis in these patients, followed by *S. aureus*. The widespread use of *H. influenza* type B vaccine should contribute to a change in this pattern. Streptococci, including groups A (*Streptococcus pyogenes*) and B (*Streptococcus agalactiae*), pneumococci, and viridans streptococci, are prominent among bacterial agents associated with infectious arthritis in patients

of all ages. Among anaerobic bacteria, *Bacteroides*, including *B. fragilis*, may be recovered and *Fusobacterium necrophorum*, which usually involves more than one joint in the course of sepsis. Among people living in certain endemic areas of the United States and Europe,

(Table 3) :Common Etiologic Agents of Pericarditis and Myocarditis:

Viruses:

Enteroviruses (primary Coxsackie A and B and, less frequently, echoviruses)

Adenoviruses

Influenza viruses

Bacteria (relatively uncommon):

Mycoplasma pneumoniae

Chlamydia trachomatis

Mycobacterium tuberculosis

Staphylococcus aureus

Streptococcus pneumoniae

Enterobacteriaceae and other gram-negative bacilli

Fungi (relatively uncommon):

Coccidioides immitis

Aspergillus spp.

Candida spp.

Cryptococcus neoformans

Histoplasma capsulatum

Parasites (relatively uncommon):

Entamoeba histolytica

Toxoplasma gondii

(Table 4): Most Frequently Encountered Etiologic Agents of Infectious Arthritis:

Bacterial:

Staphylococcus aureus

Beta-hemolytic streptococci

Streptococci (other)

Haemophilus influenzae

Haemophilus spp. (other)

Bacteroides spp.

Fusobacterium spp.

Neisseria gonorrhoeae

Pseudomonas spp.

Salmonella spp.

Pasteurella multocida

Moraxella osloensis

Kingella kingae

Moraxella catarrhalis

Capnocytophaga spp.

Corynebacterium spp.

Clostridium spp.

Peptostreptococcus spp.

Eikenella corrodens

Actinomyces spp.

Mycobacterium spp.

Mycoplasma spp.

Ureaplasma urealyticum

Borrelia burgdorferi

Fungal:

Candida spp.

Cryptococcus neoformans

Coccidioides immitis

Sporothrix schenckii

Viral:

Hepatitis B

Mumps

Rubella

Other viruses (rarely)

infectious arthritis is a prominent feature associated with Lyme disease. Chronic monoarticular arthritis is frequently due to mycobacteria, *Nocardia asteroides*, and fungi. Some of the more frequently encountered etiologic agents of infectious arthritis are listed in (Table 4)

These agents act to stimulate a host inflammatory response, which is initially responsible for the pathology of the infection. Arthritis is also a symptom associated with infectious diseases caused by certain agents, such as *Neisseria meningitidis*, group A streptococci (rheumatic fever), and *Streptobacillus moniliformis*, in which the agent cannot be recovered from joint fluid.

Presumably, antigen-antibody complexes formed during active infection accumulate in a joint, initiating an inflammatory response that is responsible for the ensuing damage.

Infections in prosthetic joints are usually associated with somewhat different etiologic agents than those in natural joints. After insertion of the prosthesis, organisms that gained access during the surgical procedure slowly multiply until they reach a critical mass and produce a host response. This may occur long after the initial surgery; approximately half of all prosthetic joint infections occur more than 1 year after surgery. Skin flora is the most common etiologic agent, with *Staphylococcus epidermidis*, other coagulase-negative staphylococci, *Corynebacterium* spp., and *Propionibacterium* spp. as the most common. However,

Staphylococcus aureus is also a major pathogen in this infectious disease. Alternatively, organisms may reach joints during hematogenous spread from distant, infected sites.

Diagnosis of joint infections requires an aspiration of joint fluid for culture and microscopic examination. Inoculating the fluid directly into blood culture bottles may prevent the fluid from clotting. Some of the fluid may be Gram stained and inoculated onto blood as well as chocolate and anaerobic media. The use of AFB (acid fast bacteria) and fungal media must also be considered.

BONE:

Bone Marrow Aspiration or Biopsy:

Diagnosis of diseases, including brucellosis, histoplasmosis, blastomycosis, tuberculosis, and leishmaniasis, can sometimes be made by detection of the organisms in the bone marrow. *Brucella* spp. can be isolated on culture, as can fungi, but parasitic agents must be visualized in smears or sections made from bone marrow material.

Many of the etiologic agents associated with disseminated infections in patients with human immunodeficiency virus (HIV) may be visualized or isolated from the bone marrow. Some of these organisms include cytomegalovirus, *Cryptococcus neoformans*, and *Mycobacterium avium* complex.

Bone Biopsy:

A small piece of infected bone is occasionally sent to the microbiology laboratory to identify the etiologic agent of osteomyelitis (infection of bone). Patients develop osteomyelitis from hematogenous spread of an infectious agent, invasion of bone tissue from an adjacent site (e.g., joint infection, dental infection), breakdown of tissue caused by trauma or surgery, or lack of adequate circulation followed by colonization of a skin ulceration with microorganisms. Once established, infections in bone may progress toward chronicity, particularly if blood supply is insufficient in the affected area.

Staphylococcus aureus, seeded during bacteremia, is the most common etiologic agent of osteomyelitis among patients of all age groups. The toxins and enzymes produced by this bacterium, as well as its ability to adhere to smooth surfaces and produce a protective glycocalyx coating, seem to contribute to the organism's pathogenicity. Osteomyelitis in younger patients is often associated with a single agent. Such infections are usually of hematogenous origin. Other organisms recovered from hematogenously acquired osteomyelitis include *Salmonella* spp., *Haemophilus* spp., Enterobacteriaceae, *Pseudomonas* spp., *Fusobacterium necrophorum*, and yeasts. *S. aureus* or *P. aeruginosa* is often recovered from cases in patients with drug addictions. Parasites or viruses are rarely, if ever, etiologic agents of osteomyelitis. Bone biopsies from infections that have spread to a bone from a contiguous source or that are associated with poor circulation, especially in patients with diabetes, are likely to yield multiple isolates. Gram-negative bacilli are increasingly common among hospitalized patients; a break in the skin (surgery or intravenous line) may precede establishment of gram-negative osteomyelitis.

Breaks in skin from other causes, such as a bite wound or trauma, also may be the initial event leading to underlying bone infection. For example, a human bite may lead to infection with *Eikenella corrodens*, whereas an animal bite may result in *Pasteurella multocida* osteomyelitis.

Poor oral hygiene may lead to osteomyelitis of the jaw with *Actinomyces* spp., *Capnocytophaga* spp., and other oral flora, particularly anaerobes. Pigmented *Prevotella* and *Porphyromonas*, *Fusobacterium*, and *Peptostreptococcus* spp. are often involved. Pelvic infection in the female may result in a mixed aerobic and

anaerobic osteomyelitis of the pubic bone. Patients with neuropathy (pathologic changes in the peripheral nervous system) in the extremities, notably patients with diabetes, who may have poor circulation, may experience an unrecognized or notable trauma.

They develop ulcers on the feet that do not heal, become infected, and may eventually progress to involve underlying bone. These infections are usually polymicrobial, involving anaerobic and aerobic bacteria. *Prevotella* or *Porphyromonas*, other gram-negative anaerobes, including the *Bacteroides fragilis* group, *Peptostreptococcus* spp., *Staphylococcus aureus*, and group A and other streptococci are frequently encountered.

Molecular testing, such as polymerase chain reaction, may be useful in determining the infectious organism associated with the patient's condition when the laboratory is unable to recover the organism by traditional culture.

Solid tissues:

Pieces of tissue are removed from patients during surgical or needle biopsy procedures or may be collected at autopsy. Any agent of infection may cause disease in tissue, and laboratory practices should be adequate to recover bacteria, fungi, and viruses and detect the presence of parasites. Fastidious organisms (e.g., *Brucella* spp.) and agents of chronic disease (e.g., systemic fungi and mycobacteria) may require special media and long incubation periods for isolation. Some agents requiring special supportive or selective media are listed in Table (5).

Table(5)Infectious Agents in Tissue Requiring Special Media:

<i>Actinomyces</i> spp.
<i>Brucella</i> spp.
<i>Legionella</i> spp.
<i>Bartonella</i> (Rochalimaea) <i>henselae</i> (cat-scratch disease bacilli)
Systemic fungi
<i>Mycoplasma</i> spp.
<i>Mycobacterium</i> spp.
Viruses

Laboratory diagnostic procedures:**Specimen collection and transport:**

Requirements for the collection and transport of specimens from sterile body sites vary because of the numerous types of specimens that can be collected and submitted to the laboratory for testing.

Fluids and Aspirates

Most specimens (pleural, peritoneal, pericardial, and synovial fluids) are collected by aspiration with a needle and syringe. Collecting pericardial fluid is not without risk to the patient because the sample is collected from the cavity immediately adjacent to the heart.

Collection is performed by needle aspiration with electrocardiographic monitoring or as a surgical procedure. Laboratory personnel should be alerted in advance of the procedure, ensuring that the appropriate media, tissue culture media, and stain procedures are available immediately. Body fluids from sterile sites should be transported to the laboratory in a sterile tube or airtight vial. From 1 to 5 mL of specimen is adequate for isolation of most bacteria, but the larger the specimen, the better, particularly for isolation of *M. tuberculosis* and fungi; at least 5 mL should be submitted for recovery of these organisms. Ten milliliters of fluid is recommended for the diagnosis of peritonitis. Anaerobic transport vials are available from several sources. These vials are prepared in an oxygenfree atmosphere and are sealed with a rubber septum or short stopper through which the fluid is injected. Transportation of fluid in a syringe capped with a sterile rubber stopper is not recommended. Most clinically significant anaerobic bacteria survive adequately in aerobic transport containers (e.g., sterile, screw-capped tubes) for short periods if the specimen is purulent and of adequate volume. However, collection in anaerobic transport media is recommended, and procedures vary in different laboratories. Specimens received in anaerobic transport vials should be inoculated to routine aerobic (an enriched broth, blood, chocolate, and sometimes MacConkey agar plates) and anaerobic media as quickly as possible.

Specimens for recovery of fungi or mycobacteria may be transported in sterile, screw-capped tubes. At least 5 to 10 mL of fluid are required for adequate recovery of small numbers of organisms. If gonococci or chlamydia are suspected, additional aliquots should be sent to the laboratory for smears and appropriate cultures. With respect to pericardial, pleural, synovial, and peritoneal fluids, the inoculation of blood culture broth bottles at the bedside or in the laboratory may be beneficial.

An additional specimen should be submitted to the laboratory for a Gram stain. The specimen in the blood culture bottle is processed as a blood culture, facilitating the recovery of small numbers of organisms and diluting out the effects of antibiotics. Citrate or sodium polyanetholsulfonate (SPS) may be used as an anticoagulant.

Specimens collected by percutaneous needle aspiration (paracentesis) or at the time of surgery should be inoculated into aerobic and anaerobic blood culture bottles immediately at the bedside. Fluid from CAPD patients can be submitted to the laboratory in a sterile tube, urine cup, or the original bag. The bag is entered with a sterile needle and syringe to withdraw fluid for culture. Fluid should be directly inoculated into blood culture bottles (at least 20 mL [10 mL in each of two culture bottles]). Numerous studies indicate that in addition to blood culture bottles, an adult Isolator tube is a sensitive and specific method for culture.

Bone:

Bone marrow is typically aspirated from the interstitium of the iliac crest. Usually, this material is not processed for routine bacteria, because blood cultures are equally useful, and false-positive cultures for skin bacteria (*Staphylococcus epidermidis*) are frequent. Some laboratories report good recovery from bone marrow material injected into a pediatric Isolator tube (ISOLATOR 1.5 mL, Alere, altham, MA) as a collection and transport device. The lytic agents within the Isolator tube are thought to lyse cellular components, presumably freeing intracellular bacteria for enhanced recovery. Bone removed at surgery or by percutaneous biopsy is sent to the laboratory in a sterile container.

Tissue:

Tissue specimens are obtained following careful preparation of the skin. It is critical that biopsy specimens be collected aseptically and submitted to the microbiology laboratory in a sterile container. A wide-mouthed, screwcapped bottle or plastic container is recommended.

Anaerobic organisms survive within infected tissue long enough to be recovered from culture. A small amount of sterile, nonbacteriostatic saline may be added to keep the specimen moist. Because homogenizing with a tissue grinder can destroy some organisms by the shearing forces generated during grinding, it is often best to use a sterile scissors and forceps to mince larger tissue specimens into small pieces suitable for culturing.

Note that *Legionella* spp. may be inhibited by saline; a section of lung should be submitted without saline for *Legionella* isolation.

If anaerobic organisms are of concern, a small amount of tissue can be placed into a loosely capped, wide mouthed plastic tube and sealed into an anaerobic pouch system, which also seals in moisture enough for survival of organisms in tissue until the specimen is plated. The surgeon should take responsibility for seeing that a second specimen is submitted to anatomic pathology for histologic studies. Formaldehyde-fixed tissue is not useful for recovery of viable microorganisms, although some organisms can be recovered after very short periods. Material from draining sinus tracts should include a portion of the tract's wall obtained by deep curettage. Tissue from infective endocarditis should contain a

portion of the valve and vegetation if the patient is undergoing valve replacement. In some instances, contaminated material may be submitted for microbiologic examination. Specimens, such as tonsils or autopsy tissue, may be surface cauterized with a heated spatula or blanched by immersing in boiling water for 5 to 10 seconds to reduce surface contamination.

The specimen may then be dissected with sterile instruments to permit culturing of the specimen's center, which will not be affected by the heating. Alternatively, larger tissues may be cut in half with sterile scissors or a blade and the interior portion cultured for microbes.

Because surgical specimens are obtained at great risk and expense to the patient, and because supplementary specimens cannot be obtained easily, it is important that the laboratory save a portion of the original tissue (if enough material is available) in a small amount of sterile broth in the refrigerator and at -70°C (or, if necessary, at -20°C) for at least 4 weeks in case additional studies are indicated. If the entire tissue must be ground up for culture, a small amount of the suspension should be placed into a sterile tube and refrigerated.

Specimen processing, direct examination, and culture:

Fluids and Aspirates: Techniques for laboratory processing of sterile body fluids are similar except for those previously discussed that are directly inoculated into blood culture bottles. Clear fluids may be concentrated by centrifugation or filtration, whereas purulent material can be inoculated directly to media. Anybody fluid received in the laboratory that is already clotted must be homogenized to release trapped bacteria and minced or cut to release fungal cells. Either processing such specimens in a motorized tissue homogenizer or grinding them manually in a mortar and pestle or glass tissue grinder allows better recovery of bacteria. Hand grinding is often preferred, because motorized grinding can generate considerable heat and thereby kill microorganisms in

the specimen. Grinding may lyse fungal elements; therefore, it is not recommended with specimens processed for fungi. Small amounts of whole material from a clot should be aseptically cut with a scalpel and placed directly onto media for isolation of fungi.

All fluids should be processed for direct microscopic examination. In general, if one organism is seen per oil immersion field, at least 10⁵ organisms per milliliter of specimen are present. In such cases, often only a few organisms are present in normally sterile body fluids. Therefore, organisms must be concentrated in body fluids. For microscopic examination, cytocentrifugation should be used to prepare Gram-stained

smears because organisms can be further concentrated up to 1000-fold. Body fluids should be concentrated by either filtration or high-speed centrifugation. Once the sample is concentrated, the supernatant is aseptically decanted or aspirated with a sterile pipette, leaving approximately 1 mL liquid in which to thoroughly mix the sediment. Vigorous vortexing or drawing the sediment up and down into a pipette several times is required to adequately suspend the sediment. This procedure should be done in a biologic safety cabinet. The suspension is used to inoculate media. Direct potassium hydroxide (KOH) or calcofluor white preparations for fungi and acid-fast stain for mycobacteria can also be performed.

Specimens for fungi should be examined by direct wet preparation or by preparing a separate smear for periodic acid-Schiff (PAS) staining in addition to Gram stain. Either 10% KOH or calcofluor white is recommended for visualization of fungal elements from a wet preparation.

In addition to hyphal forms, material from the thoracic cavity may contain spherules of *Coccidioides* or budding yeast cells.

Lysis of leukocytes before concentration of CAPD effluents can significantly enhance recovery of organisms. Filtration of CAPD fluid through a 0.45-mm pore membrane filter allows a greater volume of fluid to be processed and usually yields better results. Because the numbers of infecting organisms may be low (fewer than 1 organism per 10 mL of fluid), a large quantity of fluid must be processed. Sediment obtained from at least 50 mL of fluid has been recommended. If the specimen is filtered, the filter should be cut aseptically into three pieces, one of which is placed on chocolate agar for incubation in 5% carbon dioxide, one on MacConkey agar, and the other on a blood agar plate for anaerobic incubation. If fluids have been concentrated by centrifugation, the resulting sediment should be inoculated to an enrichment broth, blood, and chocolate agars. Because these specimens are from normally sterile sites, selective media are inadvisable because they may inhibit the isolation of anaerobes, mycobacteria, fungi, *Chlamydia* spp., and viruses should be used when such cultures are clinically indicated.

Bone: Clotted bone marrow aspirates or biopsies must be homogenized or ground to release trapped microorganisms. Specimens are inoculated to the same media as for other sterile body fluids. A special medium for enhancement of growth of *Brucella* spp. and incubation in 10% carbon dioxide may be needed. A portion of the specimen may be inoculated directly to fungal media. Sections are also made from biopsy material (bone) for fixation, staining, and examination (usually by anatomic pathologists) for the presence of mycobacterial, fungal, or parasitic agents. With respect to obtaining specimens from patients suspected of having osteomyelitis, cultures taken from open wound sites above infected bone or material taken from a draining sinus leading to an area of osteomyelitis may not reflect the actual etiologic agent of the underlying

osteomyelitis. Cultures of samples of bone obtained during wound debridement surgery appear to be more useful for directing antibiotic therapy for better clinical outcome.

Diagnosis of prosthetic (artificial) joint infections is often difficult. Unfortunately, there is no universally accepted definition for the diagnosis of infection in the absence of microbiologic evidence because clinical symptoms such as pain do not differentiate infection from mechanical joint failure. There is no standardized approach to the laboratory diagnosis of these infections, and published data are conflicting. Further complicating the diagnosis is that the most common bacteria causing prosthesis infections are common skin contaminants such as coagulase-negative staphylococci. Some studies have reported that culture is relatively insensitive, possibly because of the organisms residing in biofilms, whereas polymerase chain reaction (PCR) assays were able to detect a majority of prosthetic joint infections. Atkins

and colleagues recommended that five or six operative bone specimens be submitted for culture and that the cutoff for a definite diagnosis of infection be three or more of these specimens yielding the same organism.

However, a recent study using PCR and culture using multiple media types and prolonged incubation found that appropriate culture was adequate to exclude bacterial infection in hip prostheses and PCR did not enhance diagnostic sensitivity for infection.

Normal bone is difficult to break up; however, most infected bone is soft and necrotic. Therefore, grinding the specimen in a mortar and pestle may break off some pieces. Small shavings from the most necrotic-looking areas of the bone specimen may sometimes be scraped off aseptically and inoculated to media. Pieces should be placed directly into media for recovery of fungi. Small bits of bone can be ground with sterile broth to form a suspension for bacteriologic and mycobacterial cultures.

If anaerobes are to be recovered, all manipulations are best performed in an anaerobic chamber. If such an environment is unavailable, microbiologists should work quickly within a biosafety cabinet to inoculate prerduced anaerobic plates and broth with material from the bone.

Solid Tissue: Tissue should be manipulated in a laminar flow biological safety cabinet. Processing tissue within an anaerobic chamber is even better. The microbiologist should cut through the infected area (which is often discolored) with a sterile scalpel blade. Half of the specimen can be used for fungal cultures and the other half for bacterial cultures. Both types of microbial agents should be considered in all tissue specimens. Some samples should also be sent to surgical pathology for histologic examination.

Specimens should be cultured for viruses or acid-fast bacilli when requested. Material that is to be cultured for parasites should be finely minced or teased before inoculation into broth. Direct examination of stained tissue for parasites is often performed in the anatomic pathology lab. Imprint cultures of tissues may yield bacteriologic results identical to homogenates and may help differentiate microbial infection within the tissue's center from surface colonization (growth only at the edge).

Additional media can be inoculated for incubation at lower temperatures, which may facilitate recovery of certain systemic fungi and mycobacteria.

Tissue may also be inoculated to tissue culture cells for isolation of viruses. Brain, lung, spinal fluid, and blood are generally good specimens for viral isolation. Tissue may be examined by immunofluorescence for the presence of herpes simplex virus, varicella-zoster virus, cytomegalovirus, or rabies viral particles. Lung tissue should be examined by direct fluorescent antibody test for *Legionella* spp.

The tissues of all fetuses, premature infants, and babies who have died of an infectious process should be cultured for *Listeria*. Specimens of the brain, spinal fluid, blood, liver, and spleen are most likely to contain the organism.

Infections of the Lower Respiratory System:

The respiratory tract can be divided into two major areas: the upper respiratory tract consists of all structures above the larynx, whereas the lower respiratory tract follows airflow below the larynx through the trachea to the bronchi and bronchioles and then into the alveolar spaces where gas exchange occurs.

The respiratory and gastrointestinal tracts are the two major connections between the interior of the body and the outside environment. The respiratory tract is the pathway through which the body acquires fresh oxygen and removes unneeded carbon dioxide. It begins with the nasal and oral passages, which humidify inspired air, and extends past the nasopharynx and oropharynx to the trachea and then into the lungs.

The trachea divides into bronchi, which subdivide into bronchioles, the smallest branches that terminate in the alveoli. Some 300 million alveoli are estimated to be present in the lungs; these are the primary microscopic gas exchange structures of the respiratory tract.

Familiarization with the anatomic structure of the thoracic cavity ensures proper specimen collection from various sites in the lower respiratory tract for processing by the laboratory. The thoracic cavity, which contains the heart and lungs, has three partitions separated from one another by pleura.

The lungs occupy the right and left pleural cavities, whereas the mediastinum (space between the lungs) is occupied mainly by the esophagus, trachea, large blood vessels, and heart.

Pathogenesis of the respiratory tract:

Basic concepts

Microorganisms primarily cause disease by a limited number of pathogenic mechanisms. Because these mechanisms relate to respiratory tract infections. Encounters between the human body and microorganisms occur many times each day. However, establishment of infection after such contact tends to be the exception rather than the rule.

Whether an organism is successful in establishing an infection depends not only on the organism's ability to cause disease (pathogenicity) but also on the human host's ability to prevent the infection.

Host Factors The human host has several mechanisms that nonspecifically protect the respiratory tract from infection: the nasal hairs, convoluted passages, and the mucous lining of the nasal turbinates; secretory IgA and nonspecific antibacterial substances (lysozyme) in respiratory secretions; the cilia and mucous lining of the trachea; and reflexes such as coughing, sneezing, and swallowing. These mechanisms prevent foreign objects or organisms from entering the bronchi and gaining access to the

lungs, which remain sterile in the healthy host. Aspiration of minor amounts of oropharyngeal material, as occurs often during sleep, plays an important role in the pathogenesis of many types of pneumonia. Once particles escape the mucociliary sweeping activity and enter the alveoli, alveolar macrophages ingest them and carry them to the lymphatics. In addition to these nonspecific host defenses, normal flora of the nasopharynx and oropharynx help prevent colonization by pathogenic organisms of the upper respiratory tract.

Normal bacterial flora prevent the colonization by pathogens by competing for the same space and nutrients as well as production of bacteriocins and metabolic products that are toxic to invading organisms.

Some of the bacteria that can be isolated as part of the indigenous flora of healthy hosts, as well as many species that may cause disease under certain circumstances and are often isolated from the respiratory tracts of healthy persons, are listed in Table(1).

Under certain circumstances and for unknown reasons, these colonizing organisms can cause disease—perhaps because of previous damage by a viral infection, loss of some host immunity, or physical damage to the respiratory epithelium (e.g., from smoking).

Differentiation of normal flora of the respiratory tract is important for determining the importance of an isolate in the clinical laboratory. Colonization does not always represent an infection. It is important to differentiate colonization from infection based on the specimen source, number of organisms present, and presence or quantity of white blood cells.

(Organisms isolated from normally sterile sites in the respiratory tract by sterile methods that avoid contamination with normal flora should be definitively identified and reported to the clinician.)

Microorganism Factors:

Organisms possess traits or produce products that promote colonization and subsequent infection in the host. The virulence, or disease-producing capability of an organism, depends on several factors including adherence, production of toxins, amount of growth or proliferation, tissue damage, avoiding the host immune response, and ability to disseminate.

(Table 1) Organisms Present in the Nasopharynx and Oropharynx of Healthy Humans:

Possible Pathogens

Acinetobacter spp.

Viridans streptococci, including *Streptococcus anginosus* group

Beta-hemolytic streptococci

Streptococcus pneumoniae

Staphylococcus aureus

Neisseria meningitidis

Mycoplasma spp.

Haemophilus influenzae

Haemophilus parainfluenzae

Moraxella catarrhalis

Candida albicans

Herpes simplex virus

Enterobacteriaceae

Mycobacterium spp.

Pseudomonas spp.

Burkholderia cepacia

Filamentous fungi
Klebsiella ozaenae
Eikenella corrodens
Bacteroides spp.
Peptostreptococcus spp.
Actinomyces spp.
Capnocytophaga spp.
Actinobacillus spp., *A. actinomycetemcomitans*
Haemophilus aphrophilus
Entamoeba gingivalis
Trichomonas tenax
Rarely Pathogens
Nonhemolytic streptococci
Staphylococci
Micrococci
Corynebacterium spp.
Coagulase-negative staphylococci
Neisseria spp., other than *N. gonorrhoeae* and
N. meningitidis
Lactobacillus spp.
Veillonella spp.
Spirochetes
Rothia dentocariosa
Leptotrichia buccalis
Selenomonas
Wolinella
Stomatococcus mucilaginosus
Campylobacter spp.

Adherence. For any organism to cause disease, it must first gain a foothold within the respiratory tract to grow to sufficient numbers to produce symptoms. Therefore, most etiologic agents of respiratory tract disease must first adhere to the mucosa of the respiratory tract. The presence of normal flora and the overall state of the host affect the ability of microorganisms to adhere. Surviving or growing on host tissue without causing overt harmful effects is termed *colonization*. Except for those microorganisms inhaled directly into the lungs, all etiologic agents of disease must first colonize the respiratory tract before they can cause harm.

Streptococcus pyogenes possess specific adherence factors such as fimbriae comprised of molecules such as lipoteichoic acids and M proteins. These molecules appear as a thin layer of fuzz surrounding the bacteria. *Staphylococcus aureus* and certain viridans streptococci are other bacteria that possess these lipoteichoic acid adherence complexes. Many gram-negative bacteria (which do not have lipoteichoic acids), including

Enterobacteriaceae, *Legionella* spp., *Pseudomonas* spp., *Bordetella pertussis*, and *Haemophilus* spp., also adhere by means of proteinaceous finger-like surface fimbriae. Viruses possess either a hemagglutinin (influenza and parainfluenza viruses) or other proteins that mediate their epithelial attachment.

(Table2): Respiratory Tract Pathogens:

Definite Respiratory Tract Pathogens:

Corynebacterium diphtheriae (toxin producing)

Mycobacterium tuberculosis

Mycoplasma pneumoniae

Chlamydia trachomatis

Chlamydia pneumoniae

Bordetella pertussis

Legionella spp.

Pneumocystis jiroveci (*Pneumocystis carinii*)

Nocardia spp.

Histoplasma capsulatum

Coccidioides immitis

Cryptococcus neoformans (may also be recovered from patients without disease)

Blastomyces dermatitidis

Viruses (respiratory syncytial virus, human metapneumovirus, adenoviruses, enteroviruses, hantavirus, herpes simplex virus, influenza and parainfluenza virus, rhinoviruses, severe acute respiratory syndrome)

Rare Respiratory Tract Pathogens:

Francisella tularensis

Bacillus anthracis

Yersinia pestis

Burkholderia pseudomallei

Coxiella burnetii

Chlamydia psittaci

Brucella spp.

Salmonella spp.

Pasteurella multocida

Klebsiella rhinoscleromatis

Varicella-zoster virus (VZV)

Parasites

Toxins. Certain microorganisms are almost always considered to be etiologic agents of disease if they are present in any numbers in the respiratory tract because they possess virulence factors that are expressed in every host. These organisms are listed in Table 2.

The production of extracellular toxin was one of the first pathogenic mechanisms discovered among bacteria. *Corynebacterium diphtheriae* is a classic example of a bacterium that produces disease through the action of an extracellular toxin.

Once the organism colonizes the upper respiratory epithelium, it produces a toxin that is disseminated systemically, adhering preferentially to central nervous system cells and muscle cells of the heart. Systemic disease is characterized by myocarditis, peripheral neuritis, and local disease that can lead to respiratory distress. Growth of *C. diphtheriae* causes necrosis and sloughing of the epithelial mucosa, producing a “diphtheritic (pseudo) membrane,” which may extend from the anterior nasal mucosa to the bronchi or may be limited to any area between—most often the tonsillar and peritonsillar areas. The membrane may cause sore throat and interfere with respiration and swallowing. Although nontoxic strains of *C. diphtheriae* can cause local disease, it is much milder than disease associated with toxigenic strains.

Some strains of *Pseudomonas aeruginosa* produce a toxin similar to diphtheria toxin. Whether this toxin actually contributes to the pathogenesis of respiratory tract infection with *P. aeruginosa* has not been established.

Bordetella pertussis, the agent of whooping cough, also produces toxins. The role of these toxins in production of disease is not clear. They may act to inhibit the activity of phagocytic cells or to damage cells of the respiratory tract. *Staphylococcus aureus* and beta-hemolytic streptococci

produce extracellular enzymes capable of damaging host cells or tissues. Extracellular products of staphylococci aid in the production of tissue necrosis and the destruction of phagocytic cells and contribute to the abscess formation associated with infection caused by this organism. Although *S. aureus* can be recovered from throat specimens, it has not been proved to cause pharyngitis.

Enzymes of streptococci, including hyaluronidase, allow rapid dissemination of the bacteria.

Microorganism Growth. In addition to adherence and toxin production, pathogens cause disease by merely growing in host tissue, interfering with normal tissue function, and attracting host immune effectors, such as neutrophils and macrophages. Once these cells begin to attack the invading pathogens and repair the damaged host tissue, an expanding reaction ensues with more nonspecific and immunologic factors being attracted to the area, increasing the amount of host tissue damage. Respiratory viral infections usually progress in this manner, as do many types of pneumonias, such as those caused by *Streptococcus pneumoniae*, *S. pyogenes*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Mycobacterium tuberculosis*, and most gram-negative bacilli.

Avoiding the Host Response. Another virulence mechanism present in various respiratory tract pathogens is the ability to evade host defense mechanisms. *S. pneumoniae*, *N. meningitidis*, *H. influenzae*, *Klebsiella pneumoniae*, mucoid *P. aeruginosa*, *Cryptococcus neoformans*, and others possess polysaccharide capsules that serve both to prevent engulfment by phagocytic host cells and to protect somatic antigens from being exposed to host immunoglobulins. The capsular material is produced in such abundance by certain bacteria, such as pneumococci, that soluble polysaccharide antigen particles can bind host antibodies, blocking them from serving as opsonins.

Vaccine consisting of capsular antigens provides host protection to infection, indicating that the capsular polysaccharide is a major virulence mechanism of *H. influenzae*, *S. pneumoniae*, and *N. meningitidis*.

Some respiratory pathogens evade the host immune system by multiplying within host cells. *Chlamydia trachomatis*, *Chlamydia psittaci*, *Chlamydia pneumoniae*, and all viruses replicate within host cells. They have evolved methods for being taken in by the “nonprofessional” phagocytic cells of the host to where they thrive within the intracellular environment. Once within these cells, the organism is protected from host humoral immune factors and other phagocytic cells. This protection lasts until the host cell becomes sufficiently damaged that the organism is then recognized as foreign by the host and is attacked. A second group of organisms that cause respiratory tract disease comprises organisms capable of survival within phagocytic host cells (usually macrophages).

Once inside the phagocytic cell, these respiratory tract pathogens are able to multiply. *Legionella*, *Pneumocystis jiroveci* (*Pneumocystis carinii*), and *Histoplasma capsulatum* are some of the more common intracellular pathogens.

Mycobacterium tuberculosis is the classic representative of an intracellular pathogen. In primary tuberculosis, the organism is carried to an alveolus in a droplet nucleus, a tiny aerosol particle containing tubercle bacilli. Once phagocytized by alveolar macrophages, organisms are carried to the nearest lymph node, usually in the hilar or other mediastinal chains. In the lymph node, the organisms slowly multiply within macrophages. Ultimately, *M. tuberculosis* destroys the macrophage and is subsequently taken up by other phagocytic cells. Tubercle bacilli multiply to a critical mass within the protected environment of the macrophages, which are prevented from accomplishing phagosome-lysosome fusion capable of destroying the bacteria. Having reached a critical mass, the organisms spill out of the destroyed macrophages,

through the lymphatics, and into the bloodstream, producing mycobacteremia and carrying tubercle bacilli to many parts of the body. In most cases, the host immune system reacts sufficiently at this point to kill the bacilli; however, a small reservoir of live bacteria may be left in areas of normally high oxygen concentration, such as the apical (top) portion of the lung. These bacilli are walled off, and years later, an insult to the host, either immunologic or physical, may cause breakdown of the focus of latent tubercle bacilli, allowing active multiplication and disease (secondary tuberculosis). In certain patients with primary immune defects, the initial bacteremia seeds bacteria throughout a compromised host, leading to disseminated or miliary tuberculosis. Growth of the bacteria within host macrophages and histiocytes in the lung causes an influx of more effector cells, including lymphocytes, neutrophils, and histiocytes, eventually resulting in granuloma formation, then tissue destruction and cavity formation. The lesion consists of a semisolid, amorphous tissue mass resembling semisoft cheese, from which it received the name caseating necrosis (death of cells or tissues). The infection can extend into bronchioles and bronchi from which bacteria are disseminated via respiratory secretions and coughing. Aerosolized droplets are produced by coughing and contain organisms that are inhaled by the next susceptible host. Other portions of the patient’s lungs may become infected as well through aspiration (inhalation of a fluid or solid).

Diseases of the lower respiratory tract:

Bronchitis acute:

Acute bronchitis is characterized by acute inflammation of the tracheobronchial tree. This condition may be part of, or preceded by, an upper respiratory tract infection such as influenza (the “flu”) or the common cold. Most infections occur during the winter when acute respiratory tract infections are common.

The pathogenesis of acute bronchitis has no specific documented etiology but appears to be a mixture of viral cytopathic events and a response by the host immune system. Regardless of the cause, the protective functions of the bronchial epithelium are disturbed and excessive fluid accumulates in the bronchi. Depending on the etiology, destruction of the bronchial epithelium may be either extensive (e.g., influenza virus) or minimal (e.g., rhinovirus colds).

Clinically, bronchitis is characterized by cough, variable fever, and sputum production. Sputum (pus from the lungs) is often clear at the onset but may become purulent as the illness persists. Bronchitis may manifest as croup (a clinical condition marked by a barking cough or hoarseness).

The value of microbiologic studies to determine the cause of acute bronchitis in otherwise healthy individuals has not been established. Acute bronchitis is caused by viral agents, such as influenza and respiratory syncytial virus (RSV). The bacterium *Bordetella pertussis* is often associated with bronchitis in infants and preschool children (Table 3).

Chronic versus Acute:

Chronic bronchitis is a common condition affecting about 10% to 25% of adults. This disease is defined by clinical symptoms in which excessive mucus production leads to coughing up sputum on most days during at least 3 consecutive months for more than 2 successive years.

(Table3): Major Causes of Acute Bronchitis:

Bacteria	Viruses
<i>Bordetella pertussis</i> , <i>B. parapertussis</i> , <i>Mycoplasma pneumoniae</i> , <i>Chlamydia pneumoniae</i>	Influenza virus, adenovirus, rhinovirus, coronavirus (other less common viruses: respiratory syncytial virus, human metapneumovirus, coxsackie A21 virus)

(Table 3): Viral Agents That Cause Bronchiolitis:

Respiratory syncytial virus Parainfluenza viruses, types 1-3 Rhinoviruses Adenoviruses Influenza viruses Enteroviruses Human metapneumovirus

Patients with chronic bronchitis can suffer from acute flare-ups of infection, but determination of the cause of the infection is difficult. Potentially pathogenic bacteria, such as nonencapsulated strains of *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, are frequently cultured from the bronchi of these patients.

Because of chronic colonization, it is difficult to incriminate one of these organisms as the specific cause of an acute infection in patients with chronic bronchitis.

BRONCHIOLITIS:

Bronchiolitis, the inflammation of the smaller diameter bronchiolar epithelial surfaces, is an acute viral lower respiratory tract infection that primarily occurs during the first 2 years of life.

The disease is primarily caused by viruses including a recently discovered virus, human metapneumovirus. RSV accounts for 40% to 80% of cases of bronchiolitis and demonstrates a marked seasonality; the etiologic agents of bronchiolitis are listed in (Table 4).

Initially, the virus replicates in the epithelium of the upper respiratory tract, but in the infant it rapidly spreads to the lower tract airways. Early inflammation of the bronchial epithelium progresses to necrosis. Symptoms such as wheezing may be related to the type of inflammatory response to the virus as well as other host factors.

For the most part, patients are managed based on clinical parameters, with the laboratory having a role in cases that require hospitalization; a specific viral etiology can be identified in a large number of infants by viral isolation from respiratory secretions, preferably from a nasal wash.

Pneumonia:

Pneumonia (inflammation of the lower respiratory tract) is a major cause of illness and death. There are two major categories of pneumonias: those considered community-acquired pneumonia (patients are believed to have acquired their infection outside the hospital setting) and those including hospital- or ventilator-associated (patients are believed to have acquired their infection within the hospital setting, usually at least 2 days following admission) or health care-associated pneumonia (affects only patients hospitalized in an acute care hospital for 2 or more days within 90 days of infection from a long-term care facility, or patients who have received recent intravenous antibiotic therapy, chemotherapy, or wound care within 30 days of the current infection, or who have attended a hospital or hemodialysis clinic). Nevertheless, once a microorganism has successfully invaded the lung, disease can follow affecting the alveolar spaces and their supporting structure, the interstitium, and the terminal bronchioles.

Pathogenesis

Organisms can cause infection of the lung by four possible routes: by upper airway colonization or infection that subsequently extends into the lung, by aspiration of organisms (thereby avoiding the upper airway defenses), by inhalation of airborne droplets containing the organism, or by seeding of the lung via the blood from a distant site of infection.

Viruses cause primary infections of the respiratory tract, as well as inhibit host defenses that, in turn, can lead to a secondary bacterial infection. For example, viruses may destroy respiratory epithelium and disrupt normal

ciliary activity. Presumably, the growth of viruses in host cells disrupts the function of the latter and encourages the influx of nonspecific immune effector cells exacerbating the damage. Damage to host epithelial tissue by virus infection is known to predispose patients to secondary bacterial infection.

Clinical Manifestations

The symptoms suggestive of pneumonia include fever, chills, chest pain, and cough. In the past, pneumonias were classified into two major groups: (1) typical or acute pneumonias (e.g., *Streptococcus pneumoniae*) and (2) atypical pneumonias, based on whether the cough was productive or nonproductive of mucoid sputum. However, analysis of symptoms of pneumonia caused by the atypical pneumonia pathogens (*Mycoplasma pneumoniae*, *Legionella pneumophila*, and *Chlamydophila pneumoniae*) has revealed no significant differences from those symptoms of patients with typical bacterial pneumonias. Because of this overlap in symptoms, it is important to consider all possible etiologies associated with the patient's clinical presentation.

Chronic Lower Respiratory Tract Infections:

Mycobacterium tuberculosis is the most likely etiologic agent of chronic lower respiratory tract infection, but fungal infection and anaerobic pleuropulmonary infection may also run a subacute or chronic course. Mycobacteria other than *M. tuberculosis* may also cause such disease, particularly *M. avium* complex and *M. kansasii*. Although possible causes of acute, community-acquired lower respiratory tract infections, fungi and parasites are more commonly isolated from patients with chronic disease. *Actinomyces* and *Nocardia* may also be associated with gradual onset of symptoms. *Actinomyces* is usually associated with an infection of the pleura or chest wall, and *Nocardia* may be isolated along with an infection caused by *M. tuberculosis*.

The pathogenesis of many of the infections caused by agents of chronic lower respiratory tract disease is characterized by the requirement for breakdown of cell-mediated immunity in the host or the ability of these agents to avoid being destroyed by host cell-mediated immune mechanisms. This may be caused by an effect on macrophages, the ability to mask foreign antigens, sheer size, or some other factor, allowing microbes to grow within host tissues without eliciting an overwhelming local immune reaction.

Cystic fibrosis (CF) is a genetic disorder that leads to persistent bacterial infection in the lung, causing airway wall damage and chronic obstructive lung disease. Eventually, a combination of airway secretions and damage leads to poor gas exchange in the lungs, cardiac malfunction, and subsequent death. Patients with CF may present as young adults with chronic respiratory tract disease or, more commonly, as children with gastrointestinal problems and stunted growth. *Staphylococcus aureus* is the most prevalent opportunistic bacterial pathogen infecting 55% of children 0–9 years of age with CF, with *Pseudomonas aeruginosa* the most prevalent (81%) in older children. A very mucoid *Pseudomonas*, characterized by production of copious amounts of extracellular capsular polysaccharide, can be isolated from the sputum of almost all patients with CF who are older than 18 years of age, becoming more prevalent with increasing age after 5 years. Even if CF has not been diagnosed, isolation of a mucoid *Pseudomonas aeruginosa* from sputum should alert the clinician to the possibility of underlying disease.

Microbiologists should always report this unusual morphologic feature. In addition to mucoid *Pseudomonas* and *Staphylococcus aureus*, important pathogens in patients with CF are likely to harbor *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Ralstonia* spp., *Cupriavidus* spp.,

Pandora spp., *Escherichia coli*, strains of *Burkholderia cepacia* complex, fast growing mycobacteria, RSV, influenza and fungi including *Aspergillus*, *Scedosporium* spp., and *Exophiala dermatidis*. In addition, due to the viscous mucous plugs associated with CF, several anaerobic organisms have been detected in the lungs of CF patients including *Prevotella*, *Bifidobacterium*, *Veillonella*, *Peptostreptococcus* and *Fusobacterium*.

Using advanced diagnostic molecular methods, additional organisms have also been identified in chronic polymicrobial CF infections including viridans streptococci, *Streptococcus constellatus*, *Streptococcus intermedius* and *Streptococcus anginosus*.

Lung abscess is usually a complication of acute or chronic pneumonia. In these circumstances, organisms infecting the lung cause localized destruction of the lung parenchyma (functional elements of the lung). Symptoms associated with lung abscess are similar to those of acute and chronic pneumonia, except symptoms fail to resolve with treatment.

Immunocompromised Patients: Patients with Neoplasms. Patients with cancer are at high risk to become infected because of either granulocytopenia or other defects in phagocytic defenses, cellular or humoral immune dysfunction, damage to mucosal surfaces and the skin, and various medical procedures such as blood product transfusion.

Transplant Recipients. For successful organ transplantation, the recipient's immune system must be suppressed. As a result, these patients are predisposed to infection. Regardless of the type of organ transplant (heart, renal, bone marrow, heart/lung, liver, pancreas), most infections occur within 4 months following transplantation. Major infections can occur within the first month but are usually associated with infections carried over from the pretransplant period. Pulmonary infections are of great importance in this patient population. Some of the most common causes of pneumonia include *S. aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pneumocystis jiroveci*, and cytomegalovirus. In addition, other organisms such as *Cryptococcus neoformans*, *Aspergillus* spp., *Candida* spp., *Nocardia* sp. and over, can cause life-threatening pulmonary infection.

HIV-Infected Patients. Patients who are infected with human immunodeficiency virus (HIV) are at high risk for developing pneumonia. opportunistic infections as a result of severe immunodeficiency are a major cause of illness and death among these patients.

In the United States, the most common opportunistic infection among patients with acquired immunodeficiency syndrome is *Pneumocystis jiroveci* pneumonia. Although *P. jiroveci* remains a major pulmonary pathogen, other organisms must be considered in this patient population, including *Mycobacterium tuberculosis* and *Mycobacterium avium* complex, as well as common bacterial pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. In addition to these common pathogens, many other organisms can cause lower

respiratory tract infections, including *Nocardia* spp., *Rhodococcus equi* (a gram-positive, aerobic, pleomorphic organism), and *Legionella* spp.

Pleural infections

As a result of an organism infecting the lung and subsequently gaining access to the pleural space via an abnormal passage (fistula), the patient may develop an empyema (pus in a body cavity such as the pleural cavity).

(Table 5): Examples of Infectious Agents Frequently Associated with Certain Malignancies:

Malignancy (site and type of infections)	Pathogen
Acute nonlymphocytic leukemia (pneumonia, oral lesions, cutaneous lesions, urinary tract infections, hepatitis, most often sepsis without obvious focus)	Enterobacteriaceae <i>Pseudomonas</i> Staphylococci <i>Corynebacterium jeikeium</i> <i>Candida</i> <i>Aspergillus</i> Mucor Hepatitis C and other non-A, non-B
Acute lymphocytic leukemia (pneumonia, cutaneous lesions, pharyngitis, disseminated disease)	Streptococci (all types) <i>Pneumocystis jiroveci</i> (<i>P. carinii</i>) Herpes simplex virus Cytomegalovirus Varicella zoster vi
Lymphoma (disseminated disease, pneumonia, urinary tract infections, sepsis, cutaneous lesions)	<i>Brucella</i> <i>Candida</i> (mucocutaneous) <i>Cryptococcus neoformans</i> Herpes simplex virus (cutaneous) Varicella zoster virus Cytomegalovirus <i>Pneumocystis jiroveci</i> (<i>P. carinii</i>) <i>Toxoplasma gondii</i> <i>Listeria monocytogenes</i> Mycobacteria <i>Nocardia</i> <i>Salmonella</i> Staphylococci Enterobacteriaceae <i>Pseudomonas</i> <i>Strongyloides stercoralis</i>
Multiple myeloma	<i>Haemophilus influenza</i>

(pneumonia, cutaneous lesions, sepsis	<i>Streptococcus pneumoniae</i> <i>Neisseria meningitides</i> Enterobacteriaceae <i>Pseudomonas</i> Varicella zoster virus <i>Candida</i> <i>Aspergillus</i>

Symptoms in these patients are insidious because early in the course of disease they are related to the primary infection in the lung. Once enough purulent exudate is formed, typical physical and radiographic findings indicative of an empyema are produced.

Laboratory diagnosis of lower Respiratory tract infections:

Specimen collection and transport:

Although rapid determination of the etiologic agent is of paramount importance in managing pneumonia, the responsible pathogen is not identified in as many as 50% of patients, despite extensive diagnostic testing.

Sputum

Expectorated. The examination of expectorated sputum has been the primary means of determining the causes of bacterial pneumonia. However, lower respiratory tract secretions will be contaminated with upper respiratory tract secretions, especially saliva, unless they are collected using an invasive technique. For this reason, sputum is among the least clinically relevant specimens received for culture in microbiology laboratories, even though it is one of the most numerous and time-consuming specimens.

Good sputum samples: depend on thorough health care worker education and patient understanding throughout all phases of the collection process. Food should not have been ingested for 1 to 2 hours before expectoration and the mouth should be rinsed with saline or water just before expectoration. Patients should be instructed to provide a deep-coughed specimen.

The material should be expelled into a sterile container, with an attempt to minimize contamination by saliva. Specimens should be transported to the laboratory immediately. Even a moderate amount of time at room temperature can result in the loss of viable infectious agents and the recovery of pathogens.

Induced. Patients unable to produce sputum may be assisted by respiratory therapists, who use postural drainage and thoracic percussion to stimulate production of acceptable sputum. Before specimen collection, patients should brush the buccal mucosa, tongue, and gums with a wet toothbrush. As an alternative, an aerosol-induced specimen may be collected for the isolation of mycobacterial or fungal agents. Induced sputum is also recognized for its high diagnostic yield in cases of *Pneumocystis jiroveci* pneumonia. Aerosol-induced specimens are collected by allowing the patient to breathe aerosolized droplets, using an ultrasonic nebulizer containing 10% 0.85% NaCl or until a strong cough reflex is initiated.

Lower respiratory secretions obtained in this way appear watery, resembling saliva, although they often contain material directly from alveolar spaces. These specimens are usually adequate for culture and should be accepted

in the laboratory without prescreening. Obtaining such a specimen may obviate the need for a more invasive procedure, such as bronchoscopy or needle aspiration.

The gastric aspirate is used exclusively for isolation of acid-fast bacilli and may be collected from patients who are unable to produce sputum, particularly young children. Before the patient wakes up in the morning, a nasogastric tube is inserted into the stomach and contents are withdrawn (on the assumption that acid-fast bacilli from the respiratory tract were swallowed during the night and will be present in the stomach). The relative resistance of mycobacteria to acidity allows them to remain viable for a short period. Gastric aspirate specimens must be delivered to the laboratory immediately so that the acidity can be neutralized. Specimens can be neutralized and then transported if immediate delivery is not possible.

Endotracheal or Tracheostomy Suction Specimens:

Patients with tracheostomies are unable to produce sputum in the normal fashion, but lower respiratory tract secretions can easily be collected in a Lukens trap. Tracheostomy aspirates or tracheostomy suction specimens should be treated as sputum by the laboratory.

Patients with tracheostomies rapidly become colonized with gram-negative bacilli and other nosocomial pathogens. Such colonization per se is not clinically relevant, but these organisms may be aspirated into the lungs and cause pneumonia. Culture results should be correlated with clinical signs and symptoms.

Bronchoscopy. Bronchoscopy specimens include bronchoalveolar

lavage (BAL), bronchial washing, bronchial brushing, and transbronchial biopsies. The diagnosis of pneumonia, particularly in HIV-infected and other immunocompromised patients, often necessitates the use of more invasive procedures. Fiberoptic bronchoscopy has dramatically affected the evaluation and management of these infections. With this method, the

bronchial mucosa can be directly visualized and collected for biopsy, and the lung tissue can be sent for transbronchial biopsy for the evaluation of lung cancer and other lung diseases. Although transbronchial biopsy is important, the procedure is often associated with significant complications such as bleeding.

The sample should be transported in sterile 0.85% saline. During bronchoscopy, physicians obtain bronchial washings or aspirates, bronchoalveolar lavage (BAL) samples, protected bronchial brush samples, or specimens for transbronchial biopsy. Bronchial washings or

aspirates are collected using a small amount of sterile physiologic saline inserted into the bronchial tree and withdrawing the fluid. These specimens will be contaminated with upper respiratory tract flora such as viridians streptococci and *Neisseria* spp. Recovery of potentially pathogenic organisms from bronchial washings should be attempted.

A deep sampling of desquamated host cells and secretions can be collected through bronchoscopy and BAL. Lavages are especially suitable for detecting *Pneumocystis* cysts and fungal elements. During this procedure, a high volume of saline (100 to 300 mL) is infused into a lung segment through the bronchoscope to obtain cells and protein of the pulmonary interstitium and alveolar spaces. It is estimated that more than 1 million alveoli are sampled during this process. The value of this technique in conjunction with quantitative culture for the diagnosis of most major respiratory tract pathogens, including bacterial pneumonia, has been documented. Scientists have found significant correlation between acute bacterial pneumonia and greater than 10³ to 10⁴

bacterial colonies per milliliter of BAL fluid. BAL has been shown to be a safe and practical method for diagnosing opportunistic pulmonary infections in immunosuppressed patients. At bedside, nonbronchoscopic “mini BAL” using a Metras catheter has been introduced; typically 20 mL or less of saline is instilled.

Another type of respiratory specimen is obtained via a protected catheter bronchial brush as part of a bronchoscopy examination. Specimens obtained by this moderately invasive collection procedure are suited for microbiologic studies, particularly in aspiration pneumonia. Protected specimen brush bristles collect from 0.001 to 0.01 mL of material. Upon receipt, contents of the bronchial brush may be suspended in 1 mL of broth solution with vigorous vortexing and inoculated onto culture media using a 0.01-mL calibrated inoculating loop. Some researchers have indicated that specimens obtained via double-lumen–protected catheters are suitable for both anaerobic and aerobic cultures.

Colony counts of greater than or equal to 1000 organisms per milliliter in the broth diluent (or 10⁶/mL in the original specimen) have been considered to correlate with infection.

Transtracheal Aspirates. Percutaneous transtracheal aspirates (TTAs) are obtained by inserting a small plastic catheter into the trachea via a needle previously inserted through the skin and cricothyroid membrane. This invasive procedure, although somewhat uncomfortable for the patient and not suitable for all patients (it cannot be used in uncooperative patients, in patients with bleeding tendency, or in patients with poor oxygenation), reduces the likelihood that a specimen will be contaminated by upper respiratory tract flora and diluted by added fluids, provided care is taken to keep the catheter from being coughed back up into the pharynx. Although this technique is rarely used, anaerobes, such as *Actinomyces* and those associated with aspiration pneumonia, can be isolated from TTA specimens.

Other Invasive Procedures. When pleural empyema is present, thoracentesis may be used to obtain infected fluid for direct examination and culture. This constitutes an excellent specimen that accurately reflects the bacteriology of an associated pneumonia.

Blood cultures, of course, should always be obtained from patients with pneumonia. For patients with pneumonia, a thin needle aspiration of material from the involved area of the lung may be performed percutaneously. If no material is withdrawn into the syringe after the first try, approximately 3 mL of sterile saline can be injected and then withdrawn into the syringe. Patients with emphysema, uremia, thrombocytopenia, or pulmonary hypertension may be at increased risk of complication (primarily pneumothorax [air in the pleural space] or bleeding) from this procedure.

The specimens obtained are very small in volume, and protection from aeration is usually impossible. This technique is more frequently used in children than in adults.

The most invasive procedure for obtaining respiratory tract specimens is the open lung biopsy. Performed by surgeons, this method is used to procure a wedge of lung tissue. Biopsy specimens are extremely helpful for diagnosing severe viral infections, such as herpes simplex pneumonia, for rapid diagnosis of *Pneumocystis* pneumonia, and for other hard-to-diagnose or life-threatening pneumonias.

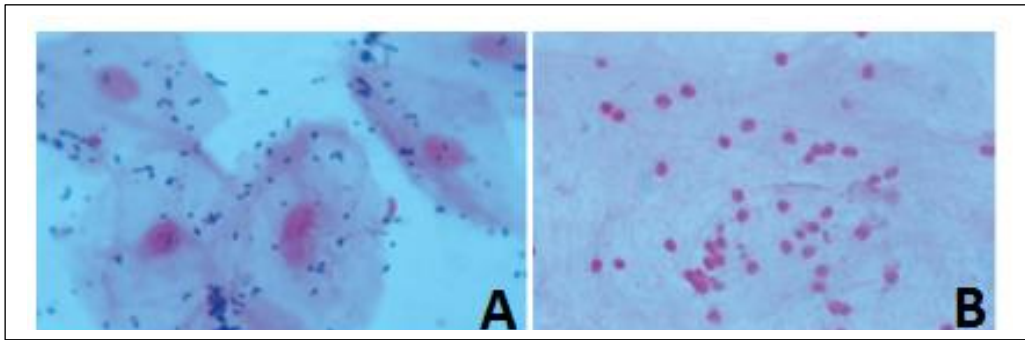


Figure 1 Gram stain of sputum specimens. **A**, This specimen contains numerous polymorphonuclear leukocytes and no visible squamous epithelial cells, indicating that the specimen is acceptable for routine bacteriologic culture. **B**, This specimen contains numerous squamous epithelial cells and rare polymorphonuclear leukocytes, indicating an inadequate specimen for routine sputum culture.

Specimen processing:

Direct Visual Examination:

Lower respiratory tract specimens can be examined by direct wet preparation for parasites and special procedures for *Pneumocystis*. Fungal elements can be visualized under phase microscopy with 10% potassium hydroxide, under ultraviolet light with calcofluor white, or using periodic acid-Schiff–stained smears.

For most other evaluations, the specimen must be fixed and stained. Bacteria and yeasts can be recognized on Gram stain. One of the most important uses of the Gram stain, however, is to evaluate the quality of expectorated sputum received for routine bacteriologic culture.

A portion of the specimen consisting of purulent material is chosen for the stain. The smear can be evaluated adequately even before it is stained, thus negating the need for Gram stain of specimens later judged unacceptable.

An acceptable specimen yields fewer than 10 squamous epithelial cells per low-power field (100×). The number of white blood cells may not be relevant, because many patients are severely neutropenic and specimens from these patients will not show white blood cells on Gram stain examination. On the other hand, the presence of 25 or more polymorphonuclear leukocytes per 100× field, together with few squamous epithelial cells, implies an excellent specimen.

Samples that contain predominantly upper respiratory tract material should be rejected. Previously, only expectorated sputa were suitable for rejection based on microscopic screening. However, endotracheal aspirates (ETAs) from mechanically ventilated adult patients can be screened by Gram stain. Criteria used to reject ETAs from adult patients include greater than 10 squamous epithelial cells per low-power field or no organisms seen under oil immersion (1000×). In *Legionella* pneumonia, sputum may be scant and watery, with few or no host cells. Such specimens may be positive by direct fluorescent antibody stain and culture, and they should not be subjected to screening procedures. Conversely, sputum from patients with CF should be screened. A throat swab is an acceptable specimen from patients with CF in selected clinical settings and should be processed in a

similar manner as CF sputum. Staining of respiratory samples is useful and should be compared to culture results to reveal errors in procedures, specimen collection, and transport or specimen identification.

Respiratory secretions may need to be concentrated before staining. The cytocentrifuge instrument has been used successfully for this purpose, concentrating the cellular material in an easily examined monolayer on a glass slide. As an alternative, specimens are centrifuged, and the sediment is used for visual examinations and cultures.

For screening purposes, the presence of ciliated columnar bronchial epithelial cells, goblet cells, or pulmonary macrophages in specimens obtained by bronchoscopy or BAL indicates a specimen from the lower respiratory tract.

In addition to the Gram stain, respiratory specimens may be stained for acid-fast bacilli with either the classic Ziehl-Neelsen or the Kinyoun carbolfuchsin stain. Auramine or auramine-rhodamine is also used to detect acidfast organisms. Because they are fluorescent, these stains fluorescent superior already here comment only are more sensitive than the carbolfuchsin formulas and are preferable for rapid screening. Slides may be restained with the classic stains directly over the fluorochrome stains as long as all of the immersion oil has been removed carefully with xylene. All of the acid-fast stains will reveal *Cryptosporidium* spp. if they are present in the respiratory tract, as may occur in immunosuppressed patients. These patients are often at risk of infection with *P. jiroveci*.

Although the modified Gomori methenamine silver stain has been used traditionally to recognize *Nocardia*, *Actinomyces*, fungi, and parasites, it takes approximately 1 hour of the technologist's time to perform, is technically demanding, and is not suitable as an emergency procedure.

A fairly rapid stain, toluidine blue O, has been used in many laboratories with some success. Toluidine blue O stains *Pneumocystis*, *Nocardia asteroides*, and some fungi.

A monoclonal antibody stain is the optimum stain for *Pneumocystis* for less invasive specimens such as BAL and induced sputa.

Direct fluorescent antibody (DFA) staining has been used to detect *Legionella* spp. in lower respiratory tract specimens. Sputum, pleural fluid, aspirated material, and tissues are all suitable specimens. Because there are so many different serotypes of legionellae, polyclonal antibody reagents and a monoclonal antibody directed against all serotypes of *Legionella pneumophila* are used. Because of low sensitivity (50% to 75%), DFA results should not be relied on in lieu of culture. Rather, *Legionella* culture, DFA or urinary antigen, and serology should be performed for optimum sensitivity.

Commercially available DFA reagents are also used to detect antigens of numerous viruses, including herpes simplex, cytomegalovirus, adenovirus, influenza viruses, and RSV. Commercial suppliers of reagents provide procedure information for each of these tests. Monoclonal and polyclonal fluorescent stains for *Chlamydia trachomatis* are available and may be useful for staining respiratory secretions of infants with pneumonia.

A number of molecular amplification techniques for the direct detection of respiratory pathogens have been described; however, the sensitivity and specificity of these assays vary greatly from one study to another. Amplification assays are also available for the direct detection of *Mycobacterium tuberculosis* on smearpositive specimens.

Rapid direct detection from respiratory samples is now available using nucleic acid-based methods. The xTAG Respiratory Viral Panel (RVP) , can be used for the simultaneous detection of influenza (four types), RSV, human metapneumovirus,

and adenovirus from nasopharyngeal swabs. In addition, the FilmArray Respiratory Panel is capable of detecting upper respiratory tract infections associated with coronavirus (four types) , adenovirus, influenza (five types), rhinovirus, parainfluenza virus (four types), enterovirus, human metapneumovirus, RSV, *Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* in approximately 1 hour directly from patient samples. Smaller molecular panels are also available such as the real-time multiplex amplification kit for influenza A, B, and RSV. All of the previously mentioned methods are FDA-approved.

In addition to these, there are a variety of research-useonly and other molecular respiratory panels in clinical validation studies. It is important when considering the use of a molecular assay that the laboratory consider their patient population including severity of illness, immune status, and transplant histories.

Routine Culture:

Most of the commonly sought etiologic agents of lower respiratory tract infection are isolated on routine media: 5% sheep blood agar, MacConkey agar for the isolation and differentiation of gram-negative bacilli, and chocolate agar for *Haemophilus* and *Neisseria* spp. Because of contaminating oral flora, sputum specimens, specimens obtained by bronchial washing and lavage, tracheal aspirates, and tracheostomy or endotracheal tube aspirates are not inoculated to enrichment broth or incubated anaerobically. Only specimens obtained by percutaneous aspiration (including transtracheal aspiration) and protected bronchial brush are suitable for anaerobic culture; the latter must be done quantitatively for proper interpretation. Transtracheal and percutaneous lung aspiration material may be inoculated to enriched thioglycollate as well as to solid media. For suspected cases of Legionnaires' disease, buffered charcoal-yeast extract (BCYE) agar and selective BCYE should be inoculated. Plates should be streaked in four quadrants to provide a basis for objective semiquantitation to define the amount of growth. After 24 to 48 hours of incubation, the numbers and types of colonies are recorded. For *Legionella* cultures, colonies form on the selective agar after 3 to 5 days at 35° C.

Sputum specimens from patients known to have CF should be inoculated to selective agar, such as specific chromogenic agar, for recovery of *S. aureus* and selective horse blood–bacitracin, incubated anaerobically and aerobically, for recovery of *H. influenzae* that may be obscured by the mucoid *Pseudomonas* on routine media.

The use of a selective medium for *B. cepacia*, such as PC

or OFPBL agars, is also necessary. For interpretation of culture results on those specimens contaminated by normal oropharyngeal flora (e.g., expectorated and induced sputum, bronchial washings), growth of the predominant aerobic and facultative anaerobic bacteria is reported. To ensure optimum culture reporting, conditions must be well defined in terms of an objective grading system for streaked plates. Finally, the clinical significance of culture findings depends not only on standardized and appropriate laboratory methods but also on how specimens are collected and transported, other laboratory data, and the patient's clinical presentation.

Numerous bacterial agents that cause lower respiratory tract infections are not detected by routine bacteriologic culture. *Mycobacteria*, *Chlamydia*, *Nocardia*, *Bordetella pertussis*, *Legionella*, and *Mycoplasma pneumoniae* require special procedures for detection; this also applies

to viruses and fungi. Optimal recovery for *Mycobacterium tuberculosis* requires multiple specimens for acid-fast staining culture, and at least one sample for molecular testing as recommended by the Centers for Disease Control.

Refer to the appropriate chapter section for more information regarding these organisms. Finally, one must keep in mind those potential agents for bioterrorist attack, such as *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*, that might be recovered from respiratory Specimens.

Upper Respiratory Tract Infections and other Infections of the Oral Cavity and Neck:

The upper respiratory tract includes all the structures down to the larynx: the sinuses, throat, nasal cavity, epiglottis, and larynx; the throat is also called the pharynx.

The pharynx is a tubelike structure that extends from the base of the skull to the esophagus. Made of muscle, this structure is divided into three parts:

- Nasopharynx (portion of the pharynx above the soft palate)
- Oropharynx (portion of the pharynx between the soft palate and epiglottis)
- Laryngopharynx (portion of the pharynx below the epiglottis that opens into the larynx)

The oropharynx and nasopharynx are lined with stratified squamous epithelial cells that are teeming with microbial flora. The tonsils are contained within the oropharynx; the larynx is located between the root of the tongue and the upper end of the trachea.

Pathogenesis

It is important to keep in mind that upper respiratory tract infections may spread and become more serious because the mucosa (mucous membrane) of the upper tract is continuous with the mucosal lining of the sinuses, eustachian tube, middle ear, and lower respiratory tract.

Diseases of the upper respiratory tract

Diseases of the upper respiratory tract are named according to the anatomic sites involved. Most of these infections are self-limiting, and the majority of infections are of viral origin.

Laryngitis: Acute laryngitis is usually associated with the common cold or influenza syndromes. Characteristically, patients complain of hoarseness and lowering or deepening of the voice. Acute laryngitis is generally a benign illness.

Acute laryngitis is almost exclusively associated with viral infection. Although numerous viruses can cause laryngitis, adenoviruses, coronavirus, and human metapneumovirus are the most common etiologic agents.

If examination of the larynx reveals an exudate or membrane on the pharyngeal or laryngeal mucosa, streptococcal infection, mononucleosis, or diphtheria should be suspected. Chronic laryngitis, although less frequently associated with infectious agents, may be caused by bacteria or fungal isolates.

Infections have been identified that are associated with methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida* spp.

Laryngotracheobronchitis

Another clinical syndrome closely related to laryngitis is acute laryngotracheobronchitis, or croup. Croup is a relatively common illness in young children, primarily those younger than 3 years of age. Of significance, croup can represent a potentially more serious disease if the infection extends downward from the larynx to involve the trachea or even the bronchi. Illness is characterized by variable fever, inspiratory stridor (difficulty in moving enough air through the larynx), hoarseness, and a harsh, barking, nonproductive cough. These symptoms last for 3 to 4 days, although the cough may persist for a longer period. In young infants, severe respiratory distress and fever are common symptoms. Similar to the etiologic agents of laryngitis, viruses are a primary cause of croup; parainfluenza viruses are the major etiologic agents. In addition to parainfluenza viruses, influenza viruses, respiratory syncytial virus, and adenoviruses can also cause croup.

Also capable of causing croup, though not as frequently, are *Mycoplasma pneumoniae*, rhinoviruses, and enteroviruses.

Epiglottitis: Epiglottitis is an infection of the epiglottis and other soft tissues above the vocal cords. Infection of the epiglottis can lead to significant edema (swelling) and inflammation. Most commonly, children between the ages of 2 and 6 years of age are infected. These children typically present with fever, difficulty in swallowing because of pain, drooling, and respiratory obstruction with inspiratory stridor. Epiglottitis is a potentially life-threatening disease because the patient's airway can become completely obstructed (blocked) if not treated.

In contrast to laryngitis, epiglottitis is usually associated with bacterial infections. In the past, 2- to 4-year-old children were typically infected with *Haemophilus influenza* type b as the primary cause of epiglottitis. However, due to the common use of *Haemophilus influenzae* type b conjugated vaccine, the typical patient is an adult with a sore throat. Other organisms occasionally implicated are streptococci and staphylococci. Diagnosis is established on clinical grounds, including the visualization of the epiglottis, which appears swollen and bright red in color.

Bacteriologic culture of the epiglottis is contraindicated because swabbing of the epiglottis may lead to respiratory obstruction. Of importance, *H. influenzae* bacteremia usually occurs in children with epiglottitis caused by this organism.

Pharyngitis and Tonsillitis: Pharyngitis (sore throat) and tonsillitis are common upper respiratory tract infections affecting both children and adults. Acute pharyngitis is an illness that frequently causes people to seek medical care.

Clinical Manifestations. Infection of the pharynx is associated with pharyngeal pain. Visualization of the pharynx reveals erythematous (red) and swollen tissue.

Depending on the causative microorganism, either inflammatory exudate (fluid with protein, inflammatory cells, and cellular debris), vesicles (small blister-like sacs containing liquid) and mucosal ulceration, or nasopharyngeal lymphoid hyperplasia (swollen lymph nodes) may be observed.

Pathogenesis. Pathogenic mechanisms differ and depend on the organism causing the pharyngitis. For example, some organisms directly invade the pharyngeal mucosa (e.g., *Arcanobacterium haemolyticum*), others

elaborate toxins and other virulence factors at the site (e.g., *Corynebacterium diphtheriae*), and still others invade the pharyngeal mucosa and elaborate toxins and other virulence factors (e.g., group A streptococci [*Streptococcus pyogenes*]).

Epidemiology/Etiologic Agents. Most cases of pharyngitis occur during the colder months and often accompany other infections, primarily those caused by viruses. Patients with respiratory tract infections caused by influenza types A and B, parainfluenza, coxsackie A, rhinoviruses, or coronaviruses frequently complain of a sore throat. Pharyngitis, often with ulceration, is also commonly found in patients with infectious mononucleosis caused by either Epstein-Barr virus or cytomegalovirus.

Although less common, pharyngitis caused by adenovirus or herpes simplex virus is clinically severe. Finally, acute retroviral syndrome caused by human immunodeficiency virus 1 (HIV-1) is associated with acute pharyngitis.

Although different bacteria can cause pharyngitis or tonsillitis, the primary cause of bacterial pharyngitis is *Streptococcus pyogenes* (or group A beta-hemolytic streptococci).

Viral pharyngitis or other causes of pharyngitis/ tonsillitis must be differentiated from that caused by *S. pyogenes*, because pharyngitis resulting from *S. pyogenes* is treatable with penicillin and a variety of other antimicrobials, whereas viral infections are not. In addition, treatment is of particular importance because infection with *S. pyogenes* can lead to complications such as acute rheumatic fever and glomerulonephritis. These complications are referred to as poststreptococcal sequelae (diseases that follow a streptococcal infection) and are primarily immunologically mediated.

S. pyogenes may also cause pyogenic infections (suppurations) of the tonsils, sinuses, and middle ear, or cellulitis as secondary pyogenic sequelae after an episode of pharyngitis. Accordingly, streptococcal pharyngitis is usually treated to prevent both the suppurative and nonsuppurative sequelae, as well as to decrease morbidity. Although bacteria other than group A streptococci may cause pharyngitis, this occurs less often. Large colony isolates of groups C and G streptococci (classified as *Streptococcus dysgalactiae* subsp. *equisimilis*) are pyogenic streptococci with similar virulence traits as *S. pyogenes*; symptoms of pharyngitis caused by these agents are also similar to *S. pyogenes*. In contrast to *S. pyogenes*, these agents are rarely associated with poststreptococcal sequelae, namely glomerulonephritis and possibly rheumatic fever.

Recent studies have demonstrated that these streptococci can exchange genetic information with *S. pyogenes* and thus potentially obtain virulence factors usually associated with *S. pyogenes* such as M proteins, streptolysin O, and superantigen genes. *Arcanobacterium haemolyticum* is also a cause of pharyngitis among adolescents. Examples of agents that can cause pharyngitis or tonsillitis are listed in (Table 1).

Table 1: Examples of bacteria that can cause acute pharyngitis and/or tonsillitis:

Organism	Disease	RelativeFrequency
<i>Streptococcus Pyogenes</i>	Pharyngitis/tonsillitis/ rheumatic fever/ scarlet fever	15% to 35%
Group C and G beta-hemolytic streptococci	Pharyngitis/tonsillitis	<3% to 11%

<i>Arcanobacterium</i> (<i>Corynebacterium</i>) <i>Haemolyticum</i>	Pharyngitis/tonsillitis/ Rash	<1% to 10%
<i>Neisseria</i> <i>Gonorrhoeae</i>	Pharyngitis/ disseminated Disease	Rare*
<i>Corynebacterium</i> <i>Ulcerans</i>	Pharyngitis	Rare
<i>Mycoplasma</i> <i>Pneumoniae</i>	Pneumonia/bronchitis/ Pharyngitis	Rare
<i>Yersinia</i> <i>Enterocolitica</i>	Pharyngitis/enterocolitis	Rare
Human immunodeficiency virus-1	Pharyngitis/acute retroviral disease	Rare

Although *H. influenzae*, *S. aureus*, and *S. pneumoniae* are frequently isolated from nasopharyngeal and throat cultures, they have not been shown to cause pharyngitis. Carriage of any of these organisms, as well as *Neisseria meningitidis*, may have clinical importance for some patients. Cultures of specimens obtained from the anterior often yield *S. aureus*. The carriage rate for this organism is especially high among health care workers, and 10%-30% of the general population can be colonized with this microbe, depending on the population characteristics.

Vincent's angina, also called acute necrotizing ulcerative gingivitis, or trench mouth, is a mixed bacteriaspirochetal infection of the gingival edge. The infection is relatively rare today, but it is considered a serious disease because it is often complicated by septic jugular thrombophlebitis, bacteremia, and widespread metastatic infection.

Adults are more often affected than children; poor oral hygiene is a predisposing factor. Multiple anaerobes, especially *Fusobacterium necrophorum*, are implicated in this syndrome. Although Gram stain of a throat specimen is usually not predictive, in those patients with symptoms suggestive of Vincent's angina, Gram stain reveals numerous fusiform, gram-negative bacilli, and spirochetes.

Peritonsillar Abscesses: Peritonsillar abscesses are generally considered a complication of tonsillitis. This infection is most common in children older than 5 years of age and in young adults. It is important to treat these infections because they can spread to adjacent tissues, as well as erode into the carotid artery to cause an acute hemorrhage. The predominant organisms isolated in peritonsillar abscesses include non-spore-forming anaerobes, such as *Fusobacterium* (especially *F. necrophorum*), *Bacteroides* (including the *B. fragilis* group), and anaerobic cocci. *Streptococcus pyogenes* and viridans streptococci may also be involved.

Rhinitis: Rhinitis (common cold) is an inflammation of the nasal mucous membrane or lining. Depending on the host response and the etiologic agent, rhinitis is characterized by variable fever, increased mucous secretions, inflammatory edema of the nasal mucosa, sneezing, and watery eyes. With rare exceptions, rhinitis is typically associated with viral infections (20%-25%); some of these agents are listed in (Table 1). Rhinitis is

common because of the large number of different causative viruses, and reinfections may occur. Bacterial agents associated with rhinitis (10%-15%) include *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and Group A streptococci.

(Table 1): Viral Agents That Can Cause Rhinitis:

Rhinoviruses
Coronaviruses
Adenoviruses
Parainfluenza and influenza viruses
Respiratory syncytial virus
Enterovirus

Miscellaneous Infections Caused by Other Agents:

***Corynebacterium diphtheriae*.** Pharyngitis caused by *Corynebacterium diphtheriae* is less common than streptococcal pharyngitis. After an incubation period of 2 to 4 days, diphtheria usually presents as pharyngitis or tonsillitis.

Patients are often febrile and complain of sore throat and malaise (body discomfort). The hallmark for diphtheria is the presence of an exudate or membrane that is usually on the tonsils or pharyngeal wall. The graywhite

membrane is a result of the action of diphtheria toxin on the epithelium at the site of infection. Complications occur frequently with diphtheria and are usually seen during the last stage of the disease (paroxysmal stage). The most feared complications are those involving the central nervous system such as seizures, coma, or blindness.

***Bordetella pertussis*.** Although mass immunization programs have greatly reduced the incidence of pertussis, enough cases (because of outbreaks and regional epidemics) still occur. In 2010, the CDC reported 27,500 cases of pertussis. This increased number of identifiable cases may be due to improved awareness and improved diagnostic methods, such as nucleic acid-based testing.

Klebsiella spp. Rhinoscleroma is a rare form of chronic, granulomatous infection of the nasal passages, including the sinuses and occasionally the pharynx and larynx. Associated with *Klebsiella rhinoscleromatis* and *Klebsiella ozaenae*, the disease is characterized by nasal obstruction appearing over a long period, caused by tumor-like growth with local extension. *K. ozaenae* may contribute to another infrequent condition called ozena, characterized by a chronic, mucopurulent nasal discharge that is often foul smelling. It is caused by secondary, low-grade anaerobic infection.

ORAL CAVITY: Stomatitis is an inflammation of the mucous membranes of the oral cavity. Herpes simplex virus is the primary agent of this disease, in which multiple ulcerative lesions are seen on the oral mucosa. These lesions are painful and can be found in the mouth and in the oropharynx. Herpetic infections of the oral cavity are prevalent among immunosuppressed patients.

Thrush: *Candida* spp. can also invade the oral mucosa. Immunosuppressed patients, including very young infants, may develop oral candidiasis, called thrush. Oral thrush can extend to produce pharyngitis or esophagitis, a common finding in patients with acquired immunodeficiency syndrome and in other immunosuppressed patients. Thrush is suspected if whitish patches of exudate on an area of inflammation are observed on the buccal (cheek) mucosa, tongue, or oropharynx. Oral mucositis or pharyngitis in the granulocytopenic patient may be caused by Enterobacteriaceae, *S. aureus*, or *Candida* spp. and is manifested by erythema, sore throat, and possibly exudates or ulceration.

Diagnosis of upper respiratory tract infections:

Collection and transport of specimens:

Sterile swabs are suitable for collecting most upper respiratory tract microorganisms. Swabs for detection of group A streptococci (*Streptococcus pyogenes*) are the only exception. Throat swabs are also adequate for recovery of adenoviruses and herpes viruses, *Corynebacterium diphtheriae*, *Mycoplasma*, *Chlamydia*, and *Candida* spp. Recovery of *C. diphtheriae* is enhanced by culturing both the throat and nasopharynx.

Nasopharyngeal swabs are better suited for recovery of *Bordetella pertussis*, *Neisseria* spp., along with several viruses including respiratory syncytial virus, parainfluenza virus, and the other viruses causing rhinitis.

nasopharyngeal secretions collected by either aspiration or washing

Direct visual examination or detection

A Gram stain of material obtained from upper respiratory secretions or lesions may not improve diagnosis.

Yeast-like cells can be identified, which are helpful in identifying thrush, and the characteristic pattern of fusiform and spirochetes of Vincent's angina may be visualized. Gram's crystal violet (allowed to remain on the slide for 1 minute before rinsing with tap water) and the Gram stain can be used to identify the spirilla and fusiform bacilli of Vincent's angina.

However, if crystal violet is used, the smear should be very thin because everything will be intensely Gram positive, making a thick smear difficult to read. Additionally, spirilla and bacilli may be stained using a dilute solution of carbol fuchsin.

For causes of pharyngitis, Gram stains are unreliable. Direct smears of exudate from membrane-like lesions used to differentiate diphtheria from other causes are also not reliable or recommended.

Fungal elements, including yeast cells and pseudohyphae, may be visualized with a 10% potassium hydroxide (KOH) preparation, calcofluor white fluorescent stain, or periodic acid-Schiff (PAS) stain.

Direct examination of material obtained from the nasopharynx of suspected cases of whooping cough using a fluorescent antibody stain has been shown to yield some early positive results for detection of *B. pertussis*.

Various methods, including fluorescent antibody stain reagents, enzyme immunoassays, and nucleic acid amplification methods are also commercially available to detect numerous viral agents.

Culture of *Streptococcus pyogenes* (Beta-Hemolytic Group A Streptococci) are usually beta-hemolytic, with less than 1% being nonhemolytic. Three variables must be taken into consideration regarding successful culture

of group A streptococci from pharyngeal specimens: medium, atmosphere, and duration of incubation. Kellogg recommended four combinations of media and atmosphere of incubation for throat specimens.

Regardless of the medium and atmosphere of incubation employed, culture plates should be incubated for at least 48 hours before reporting as negative for group A streptococci. In addition, the incubation of sheep blood agar in 5% to 10% CO₂ was strongly discouraged.

Drawbacks to culture include an extended incubation time of 24 to 48 hours for visible colony formation with additional manipulations of the beta-hemolytic organisms for definitive identification. If sufficient numbers of pure colonies are not available for identification, a subculture requiring additional incubation is necessary. By placing a 0.04-unit differential bacitracin filter paper disk, available commercially directly on the area of initial inoculation, presumptive identification of *S. pyogenes* can be made after overnight incubation (all of group A and a very small percentage of group B streptococci are susceptible). However, use of the bacitracin disk in the primary area of inoculation reduces the sensitivity and specificity of culture and identification of *S. pyogenes*.

Sometimes growth of too few beta-hemolytic colonies or overgrowth of other organisms makes interpretation difficult. Therefore, using the bacitracin disk as the only method of identification of *S. pyogenes* is not recommended.

New selective agars, such as streptococcal selective agar, have been developed that suppress the growth of almost all normal flora and beta-hemolytic streptococci except for groups A and B and *Arcanobacterium haemolyticum*. Direct antigen or nucleic detection tests or the PYR test can also be carried out on isolated beta-hemolytic colonies.

Corynebacterium diphtheriae

If diphtheria is suspected, the physician must communicate this information to the clinical laboratory. Because streptococcal pharyngitis is included in the differential diagnosis of diphtheria and because dual infections do occur, cultures for *Corynebacterium diphtheriae* should be plated onto sheep blood agar or streptococcal selective agar, as well as onto special media for recovery of this agent. These special media include a Loeffler's agar slant and a cystine-tellurite agar plate.

Recovery of this organism is improved when culturing specimens from the throat and nasopharynx of potentially infected patients. In addition to culture, rapid toxigenicity assays, including immunoassays and polymerase chain reaction, may be used to assist in the diagnosis. Caution should be used when interpreting molecular assays, because positive results have been associated with related species of *Corynebacteria*.

Bordetella pertussis

Freshly prepared Bordet-Gengou agar was the first medium developed for isolation of *Bordetella pertussis*. Today, Regan-Lowe or charcoal horse blood agar is recommended for use in diagnostic laboratories.

Neisseria

Specimens received in the laboratory for isolation of *Neisseria meningitidis* (for detection of carriers) or *N. gonorrhoeae* should be plated to a selective medium, either modified Thayer-Martin or Martin-Lewis agar. After 24 to 48 hours of incubation in 5% to 10% carbon dioxide.

Epiglottitis:

Clinical specimens from cases of epiglottitis (swab obtained by a physician) should be plated to sheep blood agar, chocolate agar (for recovery of *Haemophilus* spp.), and a streptococcal selective medium. *Staphylococcus aureus*, *Streptococcus pneumoniae*, and beta-hemolytic streptococci are all potential etiologic agents of this disease.

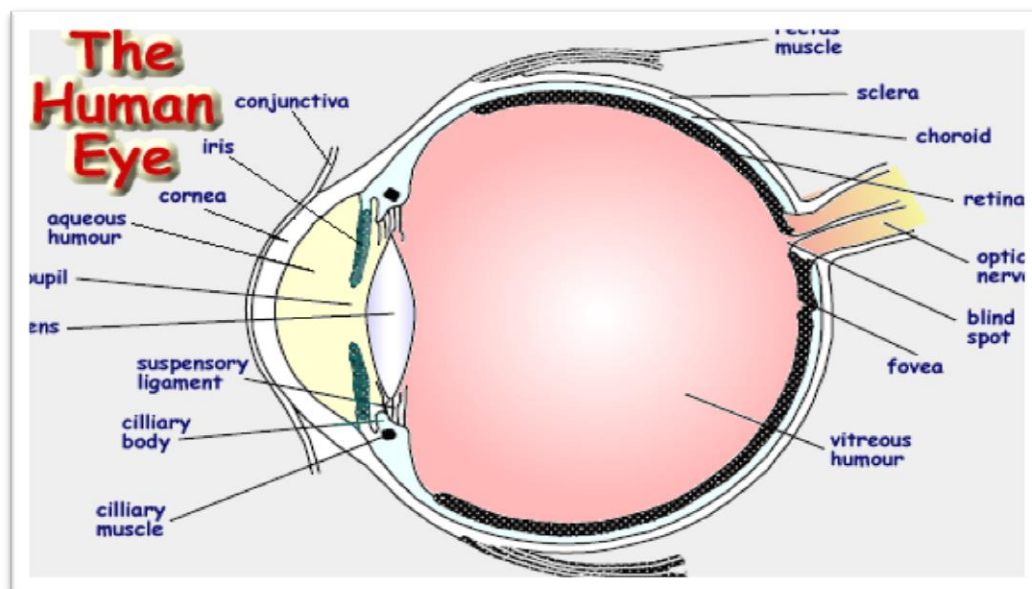
Eye Diagnostic Microbiology

When we reject the specimens

1. Inappropriate specimen transport device
2. mislabeled specimen
3. Unlabeled specimen
4. specimen received after prolonged delay (usually more than two hour)
5. specimen received in expired transport media

Eye pathogens and commensals

Common pathogens	commensals
<i>Streptococcus pyogenes</i>	<i>Staphylococcus epidermidis</i>
<i>Pseudomonas aeruginosa</i>	<i>Lactobacillus spp</i>
<i>Chlamydia trachomatis</i>	<i>Propionibacterium spp</i>
<i>Streptococcus pneumoniae</i>	<i>Staphylococcus aureus</i>
<i>Haemophilus influenzae</i>	Various Enterobacteriaceae
<i>Haemophilus aegyptius</i>	Various streptococcus spp



Specimen collection

1. Pull down the lower eyelid so that the lower conjunctival fornix is exposed.
2. Swab the fornix without touching the rim of the eyelid with the sterile cotton swab.

3. Place the swab immediately in a bacterial transport medium or, if the specimen is brought to the laboratory immediately, in a sterile test tube with 0.5 mL of buffered saline (pH 7)

***Time relapse before processing the sample***

Eye specimen should be processed immediately because tears contains lysosomes which may kill the organism

Media used in eye swab culture

- A. Blood Agar plate
- B. Chocolate Agar plate
- C. MacConkey's Agar plate

Result reporting

- 1) Report Gram stain finding as an initial report.
- 2) Report the isolated pathogen and its sensitivity pattern as a final report.

Turnaround time

- 1) Gram stain results should be available 1 hour after specimen receipt.
- 2) Isolation of a possible pathogen can be expected after 2-3 days.
- 3) Negative culture will be reported out 1-2 days after the receipt of the specimen.

Notes

- a. All bacteria isolated in fair amounts and not resembling contaminants will be identified and tested for antibiotic susceptibility, including susceptibility to chloramphenicol.
- b. If trachoma is suspected, conjunctival scraping should be smeared onto a microscopic slide, air-dried and fixed in absolute methanol. Chlamydia antigen detection systems are available for this purpose
- c. *Chlamydia trachomatis*, an exclusively human pathogen, is a non-motile, Gram negative intracellular bacterium having a unique developmental cycle in the infected cell. Metabolically inactive elementary body (EB) which is infectious enters the host cell (columnar epithelial cell) and develops into a reticulate body (RB) which divides rapidly by binary fission leading to a release of infectious

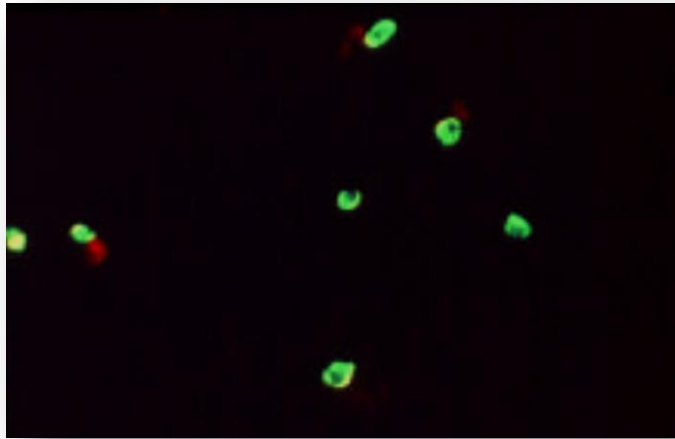


Figure shows immunofluorescence staining showing the reticulate bodies of Chlamydia trachomatis grown on McCoy cell line culture

Ear Diagnostic Microbiology

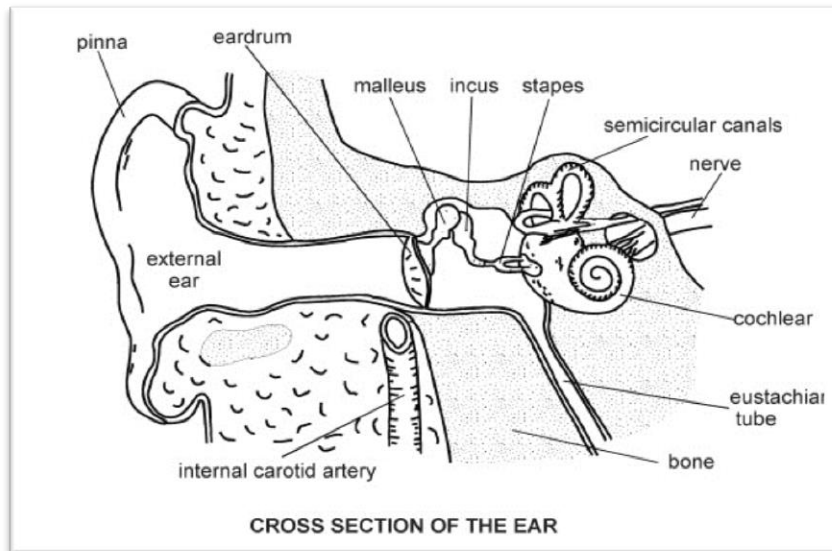
Etiological diagnosis of external or media otitis by aerobic and anaerobic culture with identification and susceptibility test of the isolated organism(s).

Types of specimen

Pus from the external or middle ear.

Criteria of specimen rejection

1. Inappropriate specimen transport device
2. Mislabelled specimen
3. Unlabelled specimen
4. Specimen received after prolonged delay (usually more than two hour)
5. Specimen received in expired transport media



<i>Common pathogens</i>	<i>Commensal flora at external canal</i>
<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
<i>Streptococcus pyogenes</i>	<i>Lactobacillus spp.</i>
<i>Pseudomonas aeruginosa</i>	<i>Propionibacterium spp.</i>
<i>Other Gram negative bacilli</i>	<i>Staphylococcus aureus</i>
<i>Streptococcus pneumoniae</i>	<i>Various Enterobacteriaceae</i>
<i>Haemophilus influenzae</i>	<i>Various Streptococcus spp</i>
<i>Anaerobic bacteria</i>	<i>Candida spp. other than albicans</i>
<i>Proteus spp.</i>	<i>Occasion Pseudomonas aeruginosa</i>

Specimen collection

1. Collect a specimen of the discharge on a thin, sterile cotton wool or Dacron swab.
2. Place the swab in a container with the transport medium, breaking off the swab stick to allow the stopper to be replaced tightly.
3. Label the specimen and send it to the laboratory.

Time relapse before processing the sample

Not more than 2 hours

Media used in eye swab culture

- 1) Blood Agar plate
- 2) Chocolate Agar plate
- 3) MacConkey Agar plate

Interfering factors

- ✚ Patient on antibiotic therapy.
- ✚ Improper sample collection.

Result reporting

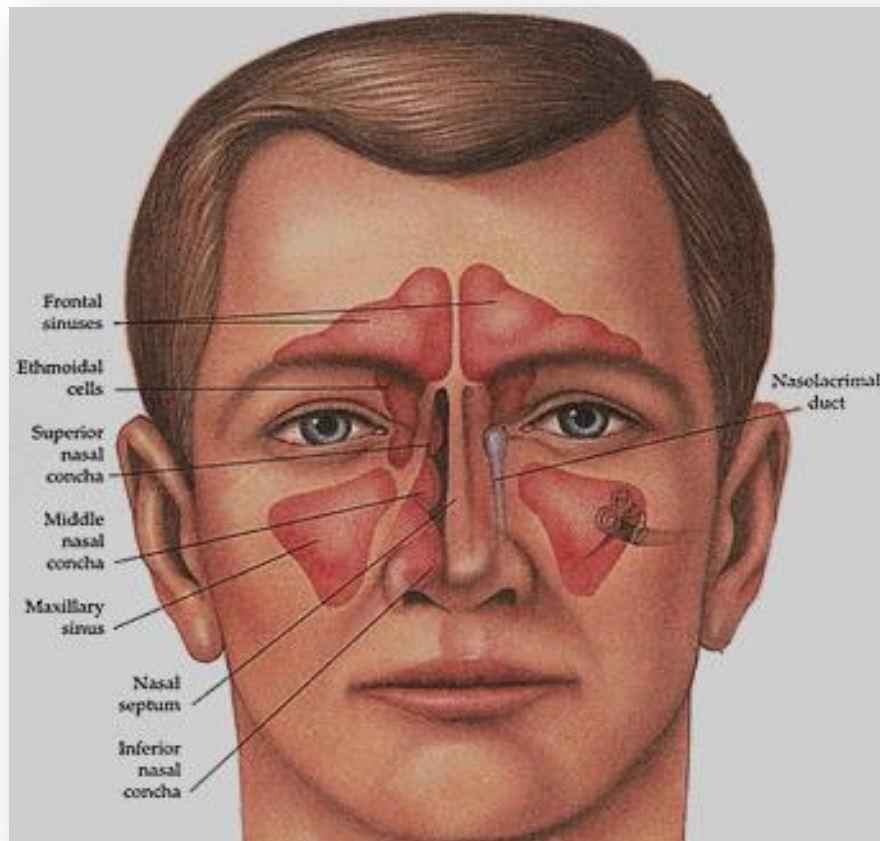
- ✚ Report Gram stain finding as an initial report.
- ✚ Report the isolated pathogen and its sensitivity pattern as a final report.

For external ear infections only *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Aspergillus* will be looked for and reported.

For middle ear infections only *Pneumococcus*, *Streptococcus pyogenes*, *Haemophilus influenzae* and *Staphylococcus aureus* will be reported with a susceptibility test.

For the chronic discharging ear, *Bacteroides* species and fungi will also be reported in addition to the organisms reported for middle ear infections.

Para nasal Sinus Diagnostic Microbiology



<i>Pathogens infect the sinuses</i>
<i>Viruses</i>
<i>Rhinovirus</i>
<i>Adenovirus</i>
<i>Influenzae virus</i>
<i>Parainfluenzae virus</i>
<i>Bacteria</i>
<i>Streptococcus pneumoniae</i>
<i>Haemophilus influenzae</i>
<i>Moraxella catarrhalis</i>
<i>Staphylococcus aureus</i>
<i>Streptococcus pyogenes</i>

Most sinus infections are viral, and only a small proportion develops a secondary bacterial infection. Rhinoviruses, influenza viruses, and Parainfluenzae viruses are the most common causes of sinusitis.

The most common bacteria isolated from pediatric and adult patients with community-acquired acute purulent sinusitis are Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pyogenes. Staphylococcus aureus and anaerobic bacteria (Prevotella and Porphyromonas, Fusobacterium and Peptostreptococcus spp.) are the main isolates in chronic sinusitis.

Pseudomonas aeruginosa and other aerobic and facultative gram-negative rods are commonly isolated from patients with nosocomial sinusitis, the immunocompromised host, those with HIV infection, and in cystic fibrosis.

Fungi and Pseudomonas aeruginosa are the most common isolates in neutropenic patients. The microbiology of sinusitis is influenced by the previous antimicrobial therapy, vaccinations, and the presence of normal flora capable of interfering with the growth of pathogens.

The nasal cavity must be disinfected with Povidone solution / chlorhexidine solution, swabs were taken from the infected sinuses. Two swabs were taken, one for aerobic and fungus, and another for anaerobic microorganisms.

Swabs were inoculated in MacConkey agar and Chocolate agar. The specimens were incubated at 35° C in a 5% carbon dioxide environment. The plates were evaluated daily for at least two days for any microbial growth. For anaerobic culture, swabs were transported via thioglycolate broth and later inoculated in blood agar plates. These were incubated anaerobically at 35° C, and evaluated for any microbial growth daily for at least five days. Fungal analysis was done by KOH mount and culture on Sabouraud Chloroamphenicol agar plate.

Skin (wound, abscess, burns) Diagnostic Microbiology

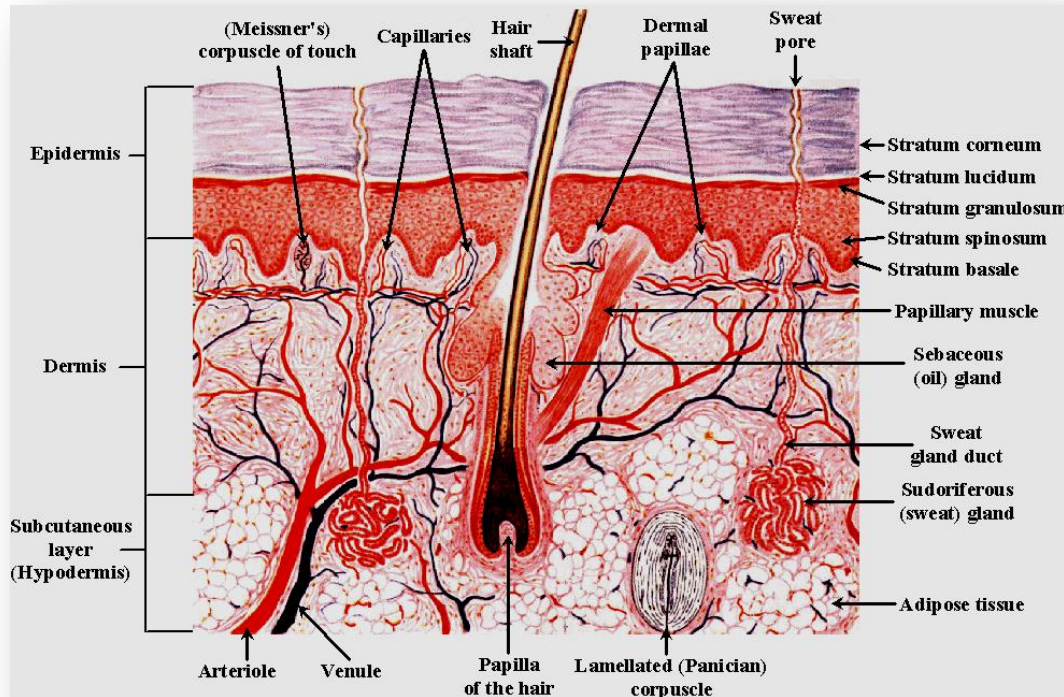
Types of specimen

- ✚ Swabs from the infected area or aspiration from deep wounds
- ✚ Swabs in anaerobic transport media for the isolation of anaerobes

Criteria of specimen rejection

- 1) Inappropriate specimen transport device
- 2) Mislabelled specimen
- 3) Unlabelled specimen dried samples and specimen received after prolonged delay
(Usually more than 72 hours)
- 4) specimen received in expired transport media

<i>Pathogenic bacteria</i>	<i>Commensals bacteria</i>
<i>Pseudomonas aeruginosa</i>	<i>Alpha haemolytic streptococci</i>
<i>Proteus spp</i>	<i>Corynebacterium spp.</i>
<i>E. coli</i>	<i>Coagulase negative Staph.</i>
<i>Klebsiella spp</i>	<i>Propionibacterium spp.</i>
<i>Morganella</i>	<i>Bacillus spp.</i>
<i>Providencia</i>	
<i>Streptococcus pyogenes</i>	
<i>Staphylococcus aureus</i>	
<i>Enterococcus spp.</i>	
<i>Clostridium perfringens</i>	
<i>Fusobacterium spp</i>	
<i>Peptostreptococcus spp</i>	
<i>Mycobacterium tuberculosis</i>	
<i>Nocardia spp.</i>	
<i>Actinomyces israelii</i>	



Specimen collection

Pus from abscess is best to be collected at the time; the abscess is incised and drained. Using sterile technique, aspirate or collect from drainage tube up to 5ml of pus, transfer to sterile container. If pus is not being discharged use sterile cotton wool swab to sample from the infected site, extend the swab deeply into the depth of the lesion. Immerse the swab in container of transport medium, label it and send to the laboratory as soon as possible.

Time relapse before processing the sample

30 minutes

Storage

Maintain specimen swab at room temperature. Do not refrigerate

Media used in culture

1. Blood Agar,
2. Chocolate Agar,
3. MacConkey Agar
4. Thioglycollate broth

Culturing procedure

Streak one blood agar plates, one chocolate, MacConkey and inoculate thioglycollate broth tube. Gram stain to check the presence or absence and if present the type or types and the predominant organisms.

Interfering factors

- ❖ Patient on antibiotic therapy
- ❖ Improper sample collection

Result reporting

- ✚ Report Gram stain finding as an initial report
- ✚ Report the isolated pathogen/s and its sensitivity pattern as a final report

Turnaround time

Gram stain results should be available 1 hour after specimen receipt. Isolation of a possible pathogen can be expected after 2-3 days. Negative culture will be reported out 1-2 days after the receipt of the specimen.

Note below

Contamination of the specimen with normal flora is one of the major obstacles in obtaining good results. Care should be taken to avoid contaminating the specimen with normal flora. This could be accomplished by swabbing superficial infected wounds with 70% alcohol before we take our swab.

Semen analysis

The availability of semen renders possible direct examination of male germ cells, giving precious data that are not accessible for female germ cells.

Semen analysis includes the examination of :

- ❖ Spermatozoa
- ❖ Other cells present in semen
- ❖ Seminal fluid

Semen analysis includes:

- Spermatozoa analysis: number, vitality, motility, morphology
- Immunological analysis : anti-spermatozoa antibodies detection
- Seminal fluid analysis : biochemical markers of accessory glands secretions

Semen analysis is subdivided in :

- Macroscopic analysis : volume, pH, liquéfaction time
- Microscopic analysis: concentration, motility and vitality
- Immunological analysis: IgG and IgA anti-sperm antibodies detection
- Bacteriological analysis: Detection of infection, in addition to colonisation
- Evaluation of spermatozoa morphology : detailed exam of the morphology of 100 to 200 spermatozoa
- Biochemical analysis of seminal plasma : markers of accessory glands of the reproductive tract

Some parameters measured in semen analysis are well established and their reference values have remained stable over time such as :

- volume
- pH
- minimal concentration of spermatozoa and minimum

absolute number of spermatozoa per ejaculate

- minimal motility
- maximal leucocyte concentration tolerance

- In contrast, the minimum percentage of spermatozoa with normal morphology has considerably varied over time, and the definition of normal morphology as well.

Reference values of semen variables;

- ❖ Volume: 2.0 ml or more
- ❖ pH: 7.2 or more
- ❖ Sperm concentration: 20×10^6 spermatozoa/ml or more
- ❖ Total sperm count: 40×10^6 spermatozoa or more
- ❖ Motility: 50% or more motile (grade a+b) or 25% or more with progressive motility (grade a) within 60 min after collection.
- ❖ Vitality: 75% or more live
- ❖ White blood cells: Fewer than 1×10^6 /ml

Semen bacteriology:

- ❖ The general semen bacteriology is always positive but pathogen germs are rarely present:
 - Culture and antibiogram
 - Aerobic Culture: Gram + germs or Gram - germs
 - Mix germs
 - Anaerobic Culture

Cultures on enriched media: *Mycoplasma hominis*, *Ureaplasma urealyticum*: pathogenicity threshold 10^4 CFU/ml

- ❖ PCR in urine: *Chlamydia trachomatis*: treatment with tetracyclin, no known resistance.

Micro-organisms capable of causing infection of seminal tract

Classical germs	Aerobic, gram -	Aerobic, Gram +	Anaerobic
Chlamydia Trachomatis	E. Coli	Gardnerella Vaginalis	Bacteroides
Nisseria Gonorrhoeae	Enterobacter	Streptococcus Faecalis	Bifidobacterium
Treponema Vaginalis	Klebsiella	Staphylococcus Aureus	Fusobacterium
Mycoplasma	Proteus	Staphylococcus	Lactobacille

		Epidermidis	
Ureaplasma	Pseudomonas	Streptococcus	Peptococcus
Ureaplasma		Agalactiae	
Corynebacterium		Streptococcus	Propionibacter
		Saprophyte	Peptostreptococcus

Biochemical markers of seminal plasma:

Semen volume is constituted by the sequential secretion of Cowper's glands (small volume), prostate (40%), testicle and epididymis (10%) and seminal vesicles (50%).

Specific biochemical markers allow to test the secretions of these different compartments.

Markers currently used in our laboratory :

- ❖ Seminal vesicles : Fructose
- ❖ Prostate : Zinc
- ❖ Epididymis : Carnitine

Certain morphological defects can compromise the fertilizing capacity of spermatozoa

- Defects of the midpiece or of the flagella can interfere with motility
- An incomplete or absent acrosome can prevent penetration of the oocyte zona pellucida
- Excessive head size is a sign of incomplete DNA condensation For many other defects of the head it is impossible to predict if they have an impact on the spermatozoa fertilizing ability.

The relationship between sperm morphology and fertility is dependent from evaluation at two distinct levels :

1- Each spermatozoa is individually examined and classified as normal or abnormal according to a definition of the normal morphology of spermatozoa

2- The percentage of spermatozoa with normal morphology in semen is calculated and used to predict fertility according to threshold values under which fertility decreases

Semen analysis brings valuable informations about the male reproductive function It allows :

- to assess spermatogenesis and accessory glands function
- to detect immunological, inflammatory or infectious problems

General structure and classification of viruses

General properties of viruses:

1. Viruses are smaller than bacteria, they range in size between 20-300 nanometer (nm) (Table 2-1).
2. Viruses contain only one type of nucleic acid, either DNA or RNA, but never both.
3. Viruses consist of nucleic acid surrounded by a protein coat. Some viruses have additional lipoprotein envelope.
4. Viruses lack cellular organelles, such as mitochondria and ribosomes.
5. Viruses are obligate cellular parasites. They replicate only inside living cells.
6. Viruses replicate through replication of their nucleic acid and synthesis of the viral protein.
7. Viruses do not multiply in chemically defined media.
8. Viruses do not undergo binary fission.

Virus is a broad general term for any aspect of the infectious agent and includes:

- the infectious or inactivated virus particle
- viral nucleic acid and protein in the infected cell

Virion is the physical particle in the extra-cellular phase which is able to spread to new host cells; complete intact virus particle is able to spread to new host cells; complete intact virus particle.

Table (2-1) : Comparison between viruses and bacteria

Comparison between viruses and bacteria

No.	Property	Viruses	Bacteria
1	Size	20-300 nm	1000 nm
2	Genome (type of nucleic acid)	DNA or RNA but not both	DNA and RNA
3	Cell wall	Envelope present in some viruses	Cell wall
4	Ribosomes	No ribosomes	Ribosomes
5	Multiplication by binary fission	–	+
6	Sensitivity to antibiotics	–	+
7	Growth in culture media	Grow only in living host cell	Grow in culture media

The structure of viruses:

1. Viral nucleic acid:

The viral nucleic acid is located internally and can be either single- or double- stranded RNA or DNA. The nucleic acid can be either linear or circular. The DNA is always a single molecule, the RNA can exist either as a single molecule or in several pieces (segmented).

- Some RNA viruses are positive polarity and others are negative polarity.
- Positive polarity is defined as an RNA with same base sequence as the mRNA. (positive strand RNA)
- Negative polarity has a base sequence that is complementary to the mRNA. (Negative strand RNA) (Figure 2-1)

2. Capsid:

The protein shell, or coat, that encloses the nucleic acid genome and mediates the attachment of the virus to specific receptors on the host cell surface.

3. Capsomeres:

Morphologic units seen in electron microscope. Each capsomere, consisting of one or several proteins.

Naked viruses are composed of nucleic acid + capsid (nucleocapsid)

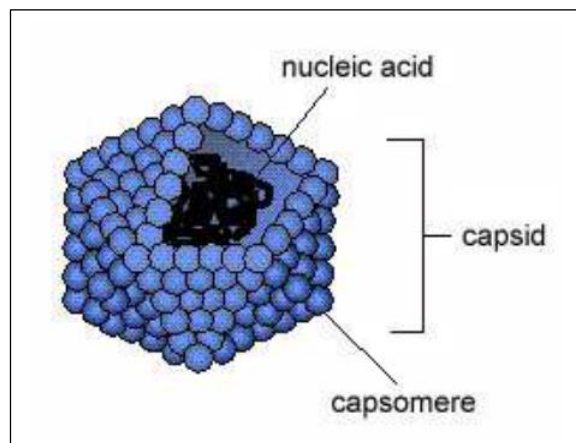


Figure 2-1 Naked virus composition

4. Viral envelope :

The envelope is a lipoprotein membrane composed of lipid derived from the host cell membrane and protein that is virus- specific.

Furthermore, there are frequently **glycoproteins** in form of spike-like projections on the surface, which attach to host cell receptors.

Matrix protein mediates the interaction between the capsid proteins and envelope.

The presence of an envelope confers instability on the virus Enveloped viruses NA
+ capsid + envelope →

The whole virus particle is called **virion**. (Figure 2-2)

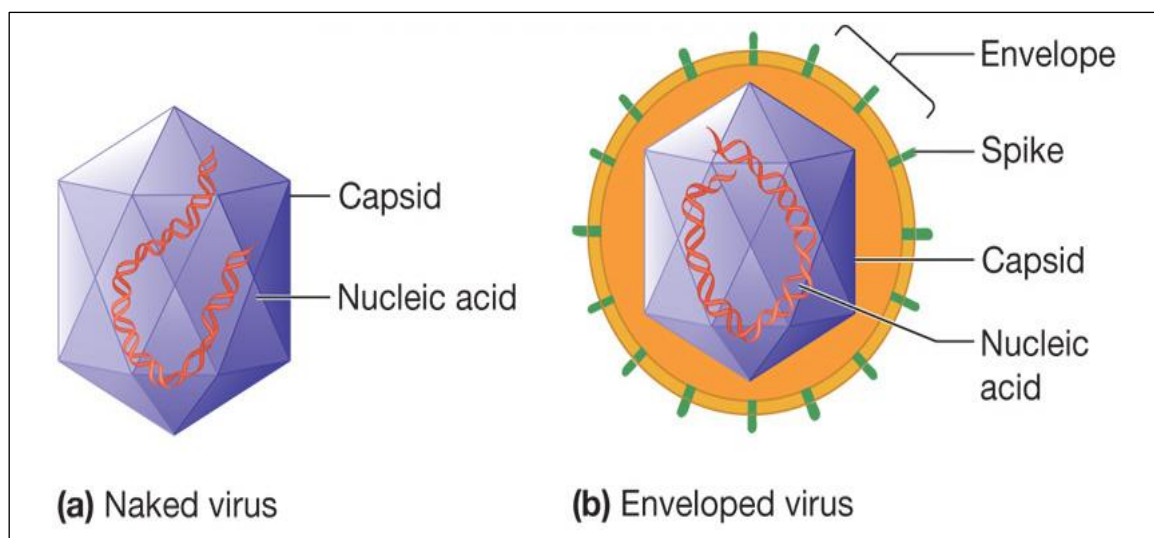


Figure 2-2 illustrate the difference between enveloped virus and naked virus

Types of symmetry of virus particles: (Figure 2-3)

Viruses are divided into three groups, based on the morphology of the nucleocapsid and the arrangement of capsomeres.

1. Icosahedral symmetry

Composed of 12 vertices, has 20 faces (each an equilateral triangle) with the approximate outline of a sphere.

e.g. Virus that cause yellow fever and Poliovirus

2. Helical symmetry

The virus particle is elongated or pleomorphic (not spherical), and the nucleic acid is spiral.

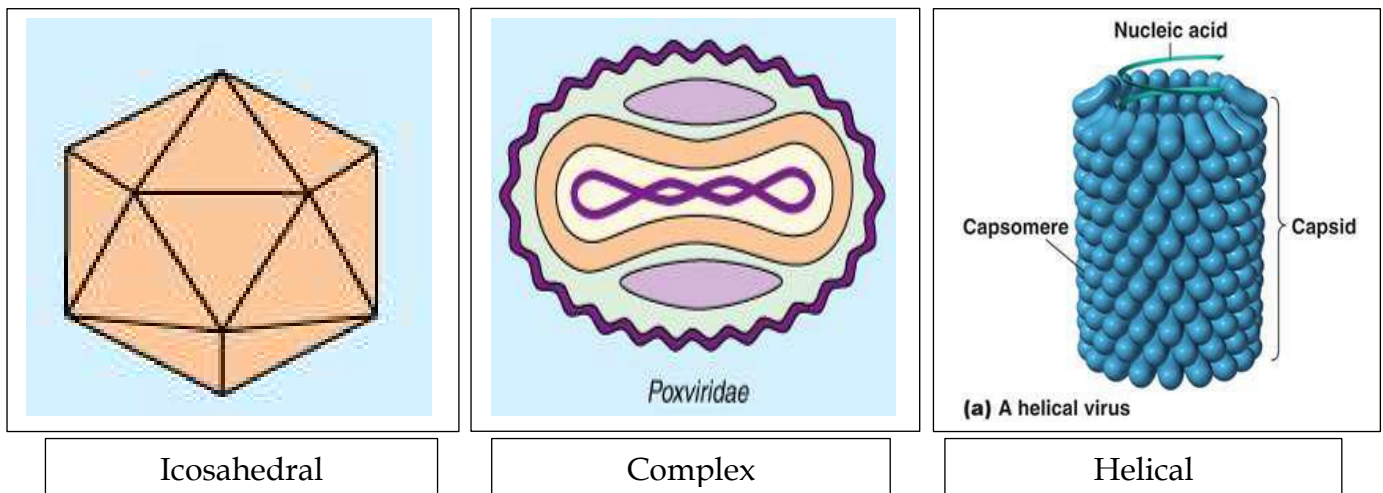
Capsomeres are arranged round the nucleic acid.

e.g. Rabies virus

3. Complex structures

The virus particle does not confirm either cubic or helical symmetry

e.g. Poxviruses



Figure(2-3) Types of symmetry of virus particles

Reaction to physical and chemical agents:

1. Heat and cold:

Viral infectivity is generally destroyed by heating at 50-60 C⁰ for 30 mint., Viruses can be preserved at -90 C⁰ or -196 C⁰ (liquid nitrogens).

2. PH:

Viruses can be preserved at physiological PH (7.3).

3. Ether susceptibility :

Ether susceptibility can be used to distinguish viruses that possess an envelope from those that do not.

4. Detergents:

Nonionic detergents solubilize lipid constituents of viral membranes. The viral proteins in the envelope are released.

Anionic detergents also solubilize viral envelopes; in addition, they disrupt capsids into separated polypeptides.

5. Salts

Many viruses can be stabilized by salt in concentrations of 1 mol/L.

e.g. MgCl₂, MgSO₄, Na₂SO₄.

6. Radiation

Ultraviolet, X-ray, and high-energy particles inactivate viruses.

7. Formaldehyde

Destroys viral infectivity by reacting with nucleic acid.

8. Antibiotics

Antibacterial antibiotics have no effect on viruses.

Classification of viruses:

1. Virion morphology, including size, shape, type of symmetry, presence or absence of envelope.
2. Virus genome properties, including type of nucleic acid (DNA or RNA), size of genome, strandedness (single or double), whether linear or circular, positive or negative sense (polarity), segments (number, size).
3. Physicochemical properties of the virion, including PH stability, thermal stability, and susceptibility to physical and chemical agents, especially ether and detergents.
4. Virus protein properties, including number, size and functional activities of structural and non-structural proteins, amino acid sequences, and special functional activities (transcriptase, reverse transcriptase, neuraminidase, fusion activities).
5. Genome organization and replication, including gene order, strategy of replication (patterns of transcription, translation), and cellular sites (accumulation of proteins, virion assembly, virion release).
6. Antigenic properties
7. Biological properties, including natural host range, mode of transmission, vector relationships, pathogenicity, tissue tropisms, and pathology.

Universal system of virus taxonomy:

Families – on the basis of virion morphology, genome structure and strategies of replication.

Virus family names have the suffix – **viridae**.

Genera – based on physicochemical or serological differences.

Genus names carry the suffix – **virus**.

Survey of DNA-containing

- Parvoviruses: human parvovirus B19
- Papovaviruses: papillomaviruses
- Adenoviruses: 47 types infect humans
- Herpesviruses: human herpesvirus 1-8
- Poxviruses: smallpox; vaccinia
- Hepadnaviruses: HBV

Survey of RNA-containing

- Picornaviruses
- Astroviruses
- Caliciviruses
- Reoviruses
- Arboviruses
- Togaviruses
- Flaviviruses
- Arenaviruses
- ronaviruses: SARS
- Retroviruses
- Bunyaviruses
- Othomyxoviruses
- Paramyxoviruses:
- Rhabdoviruses:rabies virus
- Bornaviruses: BDV
- Filoviruses
- Other viruses
- Viroids

Viral Replication : (Figure 2-4)

Steps in Viral Replication:

A. Attachment:

This is the first step in viral replication. Surface proteins of the virus interact with specific receptors on the target cell surface.

B. Penetration:

Enveloped with the viruses (e.g., HIV, influenza virus) penetrate cells through fusion of the viral envelope host cell membrane. Non-enveloped viruses penetrate cells by translocation of the virion across the host cell membrane or receptor mediated endocytosis .

C. Uncoating:

This process makes the nucleic acid available for transcription to permit multiplication of the virus.

D. Transcription and Translation:

The fact that viruses must use host cellular machinery to replicate and make functional and structural proteins.

Assembly and Release. The process of virion assembly involves bringing together newly formed viral nucleic acid and the structural proteins to form the nucleocapsid of the virus

E. Virus Shedding:

This is a necessary step to maintain a viral infection in populations of hosts. Shedding usually occurs from the body surfaces involved in viral entry. Shedding occurs at different stages of disease depending on the particular agent involved .

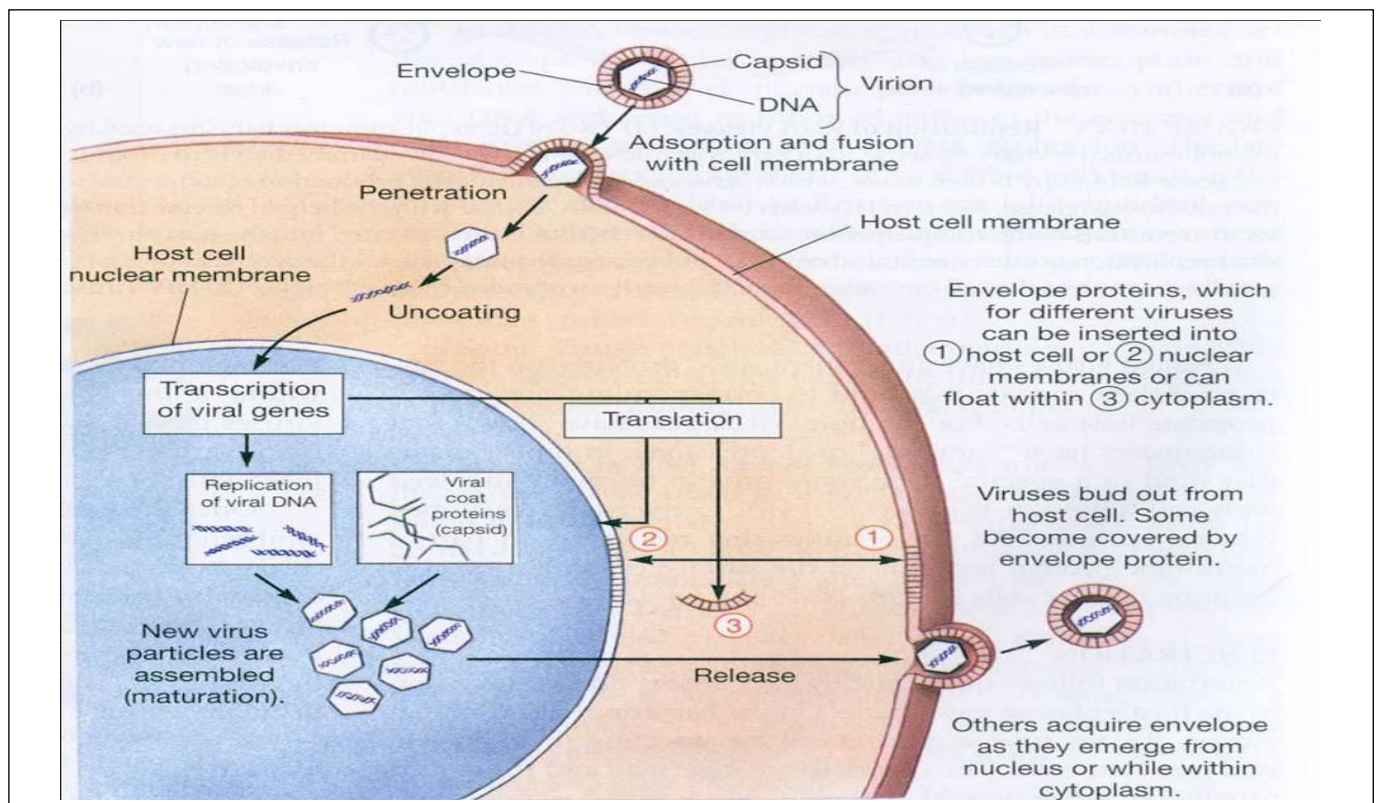


Figure (2-4) steps of viral replication

Patterns of viral infection

- **Viral infections can be:**
 - Acute (rapid and self-limiting)
 - Persistent (long term)
 - Latent (extreme versions of persistent infections)
 - Slow or transforming (complicated types of persistent infections)

Viral Pathogenesis :

Viral pathogenesis is the process by which a viral infection leads to disease.

A virus must first attach to and enter cells of one of the body surfaces: skin, respiratory tract, gastrointestinal tract, urogenital tract, or conjunctiva.

Major exceptions are those viruses that are introduced directly into the bloodstream by needles (hepatitis B, human immunodeficiency virus [HIV]), by blood transfusions, or by insect vectors (arboviruses)

Viruses usually replicate at the primary site of entry. Some, such as common cold viruses and rotaviruses produce disease at the portal of entry and have no necessity for further systemic spread.

Viral Spread and Cell Tropism

Many viruses produce disease at sites distant from their point of entry. Spread within the host .

Mechanisms of viral spread vary, but the most common route is via the bloodstream or lymphatics. The presence of virus in the blood is called viremia

Routes of infection

- Inhalation ;e.g influenza viruses
- Ingestion : polio viruses
- Parenteral : AIDs
- Transplacental, cytomegalovirus ,rubella.

Following infection the virus is transmitted by blood, cells, along nerves and become localized in certain tissue which it prefer (tropism) e.g. polio, rabies etc.

Mechanisms of viral injury

1. Inhibits host cell DNA, RNA or protein synthesis e.g. Poliovirus.
2. Direct cell killing by damaging host cell membrane e.g. Rhinoviruses.
3. Induce Immune reaction e.g hypersensitivity reaction in respiratory cyncytial viruses.

4. Damage host defence mechanism e.g. respiratory epithelium predisposes to the pneumonia
5. Induce cell proliferation & transformation result in neoplasia e.g. HBV, EBV.

Chemotherapy of Viral Infections :

Anti-bacterial drugs such as the penicillin antibiotics have proved very successful since they act against a bacterial structure, the cell wall that is not present in eukaryotic cells.

In contrast, most anti-viral agents have proved of little use therapeutically since the virus uses host-cell metabolic reactions and thus, for the most part, anti-viral agents will also be anti-cell agents.

A successful anti-viral drug should:

(i) interfere with a virus-specific function.

or

(ii) interfere with a cellular function so that the virus cannot replicate. To be specific, the anti-viral drug must only kill virus-infected cells

An ideal drug should be:

- Water-soluble
- Stable in the blood stream
- Easily taken up by cells

An ideal drug should NOT be:

- Toxic
- Carcinogenic
- Allergenic
- Mutagenic

Toxicity of an anti-viral drug may be acceptable if there is no alternative: such as, for example, in symptomatic rabies or hemorrhagic fever.

Basic Mechanisms:

Antiviral drugs specifically inhibit one or more steps of virus replication without causing unacceptable side effects. ([Figure 2-5](#))

Because of the close interaction between virus replication and normal cellular metabolism, it was originally thought too difficult to interrupt the virus replicative cycle without adversely affecting the host cell metabolism.

The life cycle of a virus comprises several stages such as binding to the cell surface, replication, protein synthesis etc. and all of these stages may be the target of anti-viral drugs.

The mechanism of action vary among antiviral :

1. Nucleoside and nucleotide Analogs:

The majority of available antiviral agents are nucleoside analogs. They inhibit nucleic acid by inhibition of polymerases essential for nucleic acid replication. In addition, some analogs can be incorporated into the nucleic acid and block further synthesis or alter its function .

Example for nucleoside analogs include acyclovir , lamivudine ,ribavirin and zidovudine : AZT Acyclovir (Zovirax) represents a major breakthrough in the treatment of herpes virus infections.

The main indications for its use are primary genital herpes

Nucleotide analogs differ from nucleoside analogs in having an attached phosphate group, their ability to persist in cells for long periods of time increases their potency Cidofovir is an example .

2. Reverse transcriptase inhibitor :

It acts by binding directly to reverse transcriptase and disrupting the enzyme's catalytic site, for example Nevirapine.

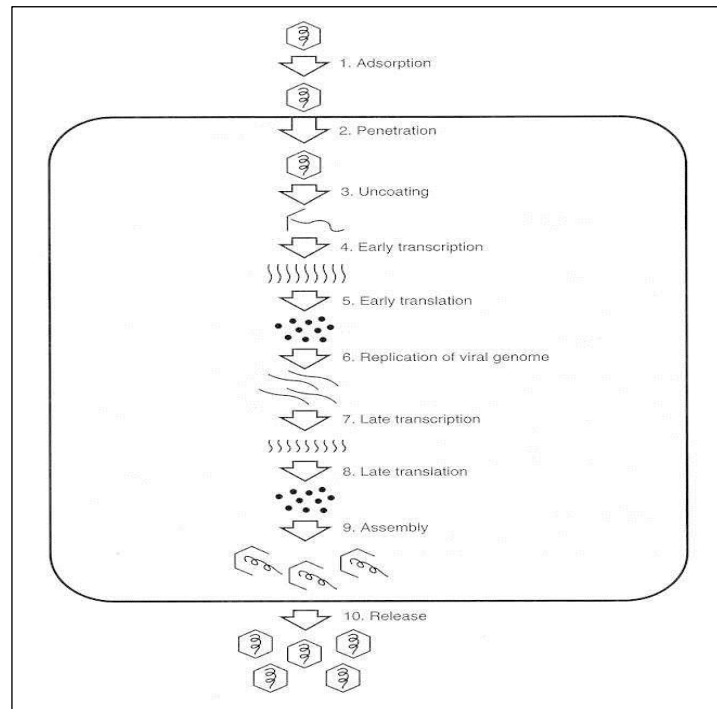
3. Protease inhibitor :

Saquinavir was the first protease inhibitor to be approved for treatment of HIV infection , which inhibit viral protease that is required for the last stage of replicative cycle . Inhibition of the protease yields noninfectious virus particles.

Other types of Antivirus agent

Fuzen Blocks the virus and cellular membrane fusion step involved in entry HIV into cells.

Amantadine and Rimantadine: specifically inhibit Influenza A virus by blocking viral uncoating.



(Figure 2-5) Virus replication cycle

Interferon:

Interferon are small proteins released by macrophages, lymphocytes, and tissue cells infected with a virus.

When a tissue cell is infected by a virus it release Interferon, Interferon will diffuse to surrounded cells. When it binds to a receptor on the surface of these adjacent cell they begun the production of proteins that prevents the spread of the virus through the body .

Three types of interferon: alpha, beta and gamma

Vaccine:

A vaccine is a biological preparation that provides active acquired immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe,

Type of viral vaccine:

1. Attenuated live viral vaccines

These attenuated viruses can infect and replicate in the recipient and produce a protective immune response without causing disease. Live attenuated viral vaccines can often confer lifelong immunity after one immunization series (e.g. : Measles vaccine ,Rubella vaccine Oral poliomyelitis vaccine (OPV)

2. Killed (inactivated) viral vaccines

Killed viral vaccines contain either whole virus particles, inactivated by chemical or physical means, or some component(s) of the virus. They do not generally produce lifelong immunity following one immunization series (e.g.: Rabies vaccine, Injectable poliomyelitis vaccine (IPV))

3. Recombinant-produced antigens

Application of a recombinant DNA strategy to develop new vaccines. This approach has made possible a safe and effective recombinant vaccine against hepatitis B virus, which has replaced the vaccine derived from the plasma of hepatitis B virus-infected individuals.

Virological Tests

Diagnostic Methods in Virology

1. Direct Examination
2. Indirect Examination (Virus Isolation)
3. Serology

Direct Examination

1. Antigen Detection immunofluorescence, ELISA etc.
2. Electron Microscopy morphology of virus particles
immune electron microscopy
3. Light Microscopy histological appearance
inclusion bodies
4. Viral Genome Detection hybridization with specific
nucleic acid probes polymerase chain reaction (PCR)

Indirect Examination

1. Cell Culture cytopathic effect (CPE)
haemabsorption
immunofluorescence
2. Eggs pocks on CAM
haemagglutination
inclusion bodies
3. Animals disease or death

Methods for Cultivation of Virus: (Figure 2-6)

Generally three methods are employed for the virus cultivation

1. Inoculation of virus into animals
2. Inoculation of virus into embryonated eggs (Figure 2-7)
3. Tissue culture

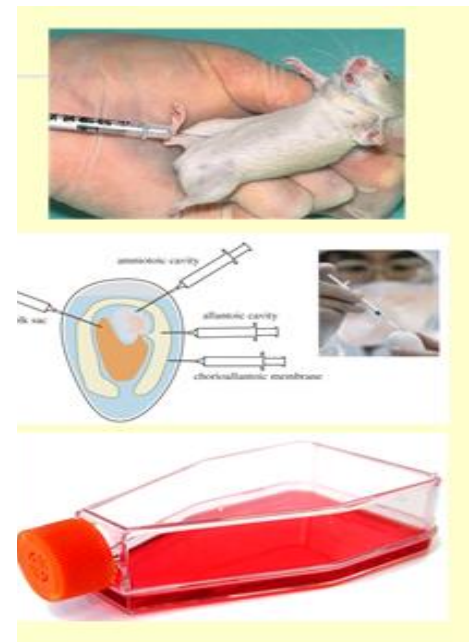


Figure 2-6 Method for cultivation of virus

Laminar Flow Hoods:

- Virologist's facility
- **Laminar vertical** flow hoods Contains HEPA filter Removes 99.97% of particles of 0.3 μ m or higher

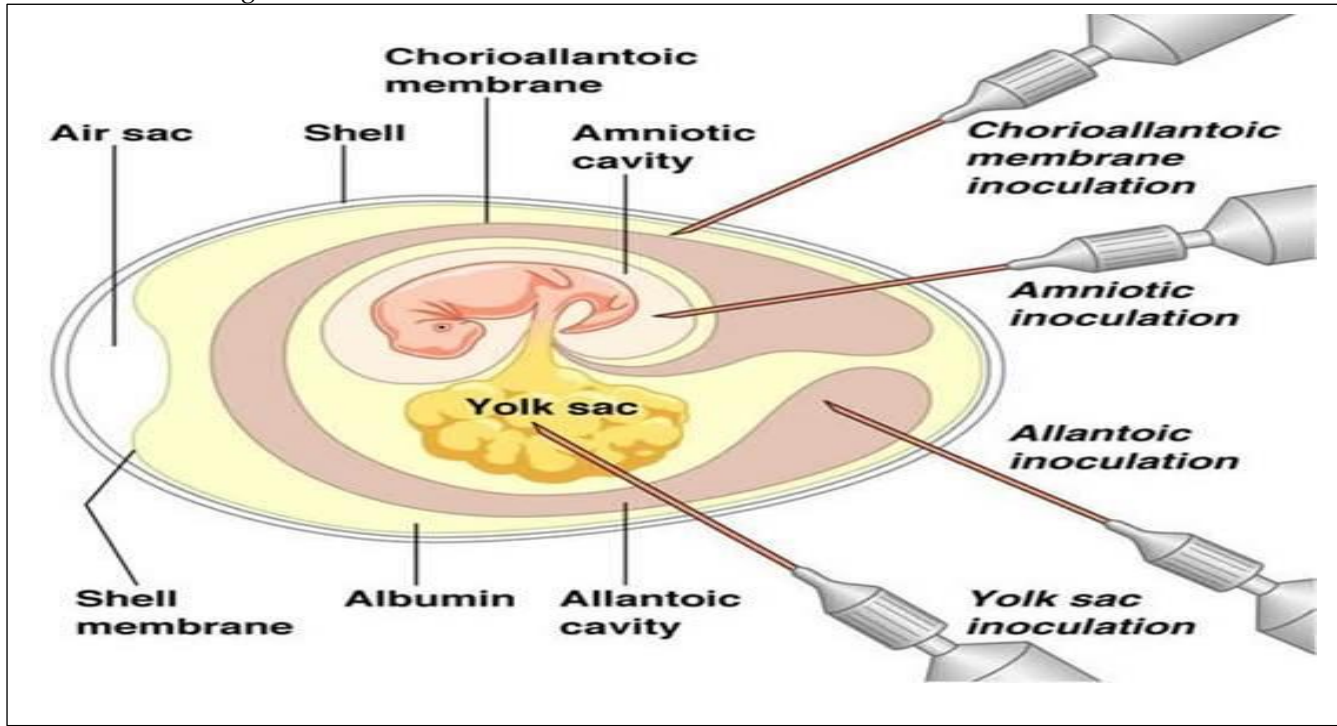


Figure 2-7 Inoculation of virus into embryonated eggs

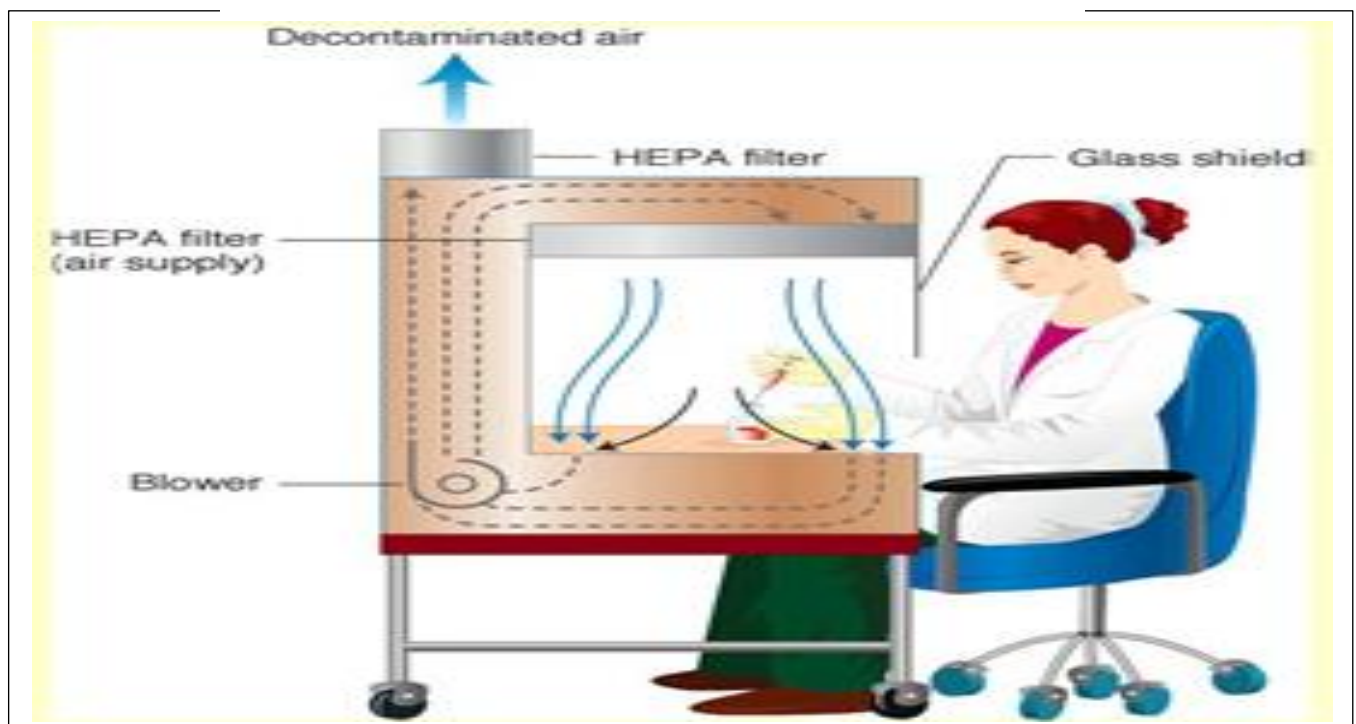


Figure2-8 laminar vertical flow hoods

Arranged by Sarah Mohssen

Quantitative assays

- Plaque assays
- TCID₅₀
- Haemagglutination assays
- Transformation assays

Virus Isolation :

Cell Cultures are most widely used for virus isolation, there are 3 types of cell cultures:

1. Primary cells - Monkey Kidney
2. Semi-continuous cells - Human embryonic kidney and skin fibroblasts
3. Continuous cells - HeLa, Vero, Hep2, LLC-MK2, MDCK

Primary cell culture are widely acknowledged as the best cell culture systems available since they support the widest range of viruses. However, they are very expensive and it is often difficult to obtain a reliable supply. Continuous cells are the most easy to handle but the range of viruses supported is often limited.

Cell culture Cytopathic Effect :

- Some viruses kill the cells in which they replicate, and infected cells may eventually detach from the cell culture plate.
- As more cells are infected, the changes become visible and are called cytopathic effects.(Figure 2-9)

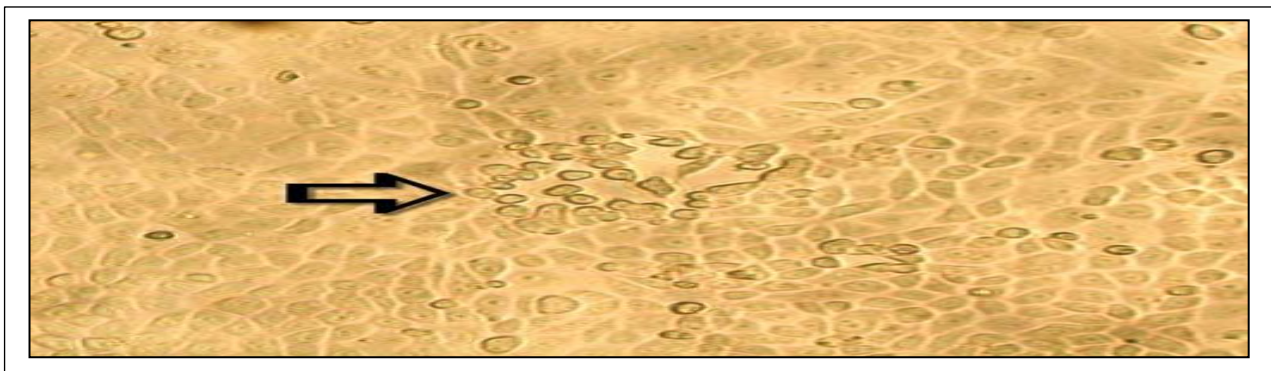


Figure 2-9 cell culture cytopathic effects

Examples of Cytopathic Effects of Viral Infection

- Nuclear shrinking (pyknosis)
- Proliferation of nuclear membrane
- Vacuoles in cytoplasm
- Syncytia (cell fusion)
- Margination and breaking of chromosomes
- Rounding up and detachment of cultured cells
- Inclusion bodies

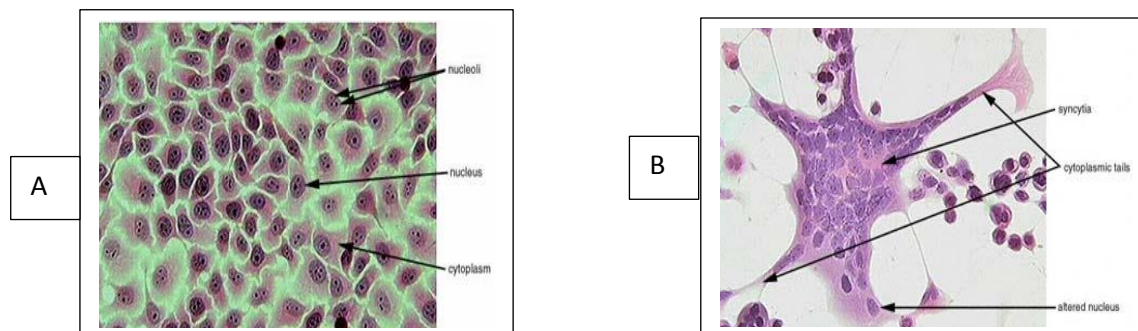


Figure 2-10 example at cytopathic effect A-Inclusion bodies , B-

Quantitative Assays-TCID50

Tissue Culture Infectious Dose: TCID50 Measure cytopathic effects other than lysis
Concentration of virus it takes to produce cytopathic effect (CPE) in 50% of the dishes of cells infected with virus (Table 2-2)(Figure 2-11)

Table(2-2) Example of endpoint dilution data

Virus Dilution		CPE Results								
10^{-2}	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	-	+	+	+	+	+
10^{-4}	-	+	-	+	+	-	-	+	-	+
10^{-5}	-	-	-	-	+	-	-	-	-	-
10^{-6}	-	-	-	-	-	-	-	-	-	-
10^{-7}	-	-	-	-	-	-	-	-	-	-

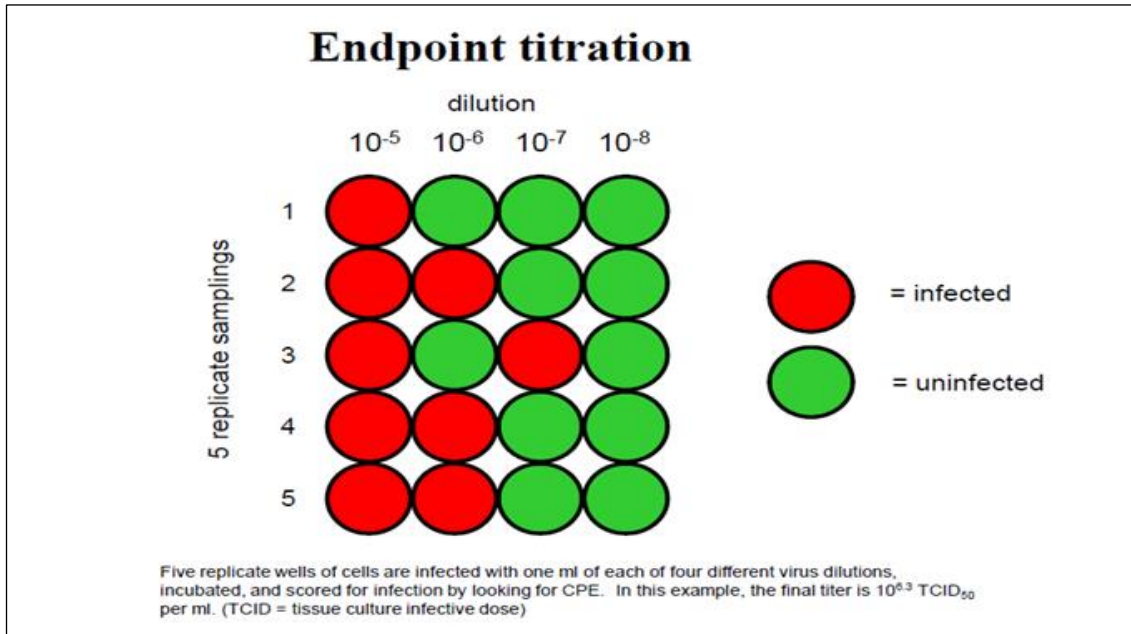


Figure (2-11)
Endpoint
titration of
cytopathic effect
by tissue culture
assay

Plaque assays Lytic viruses only

- Steps Serial dilution of virion-containing solution
- Create tissue culture plates
- Spread diluted virus
- Overlay with agar – prevents diffusion
- Count number of plaques
- Each plaque represents 1 PFU (Plaque Forming Unit)(Figure 2-12)

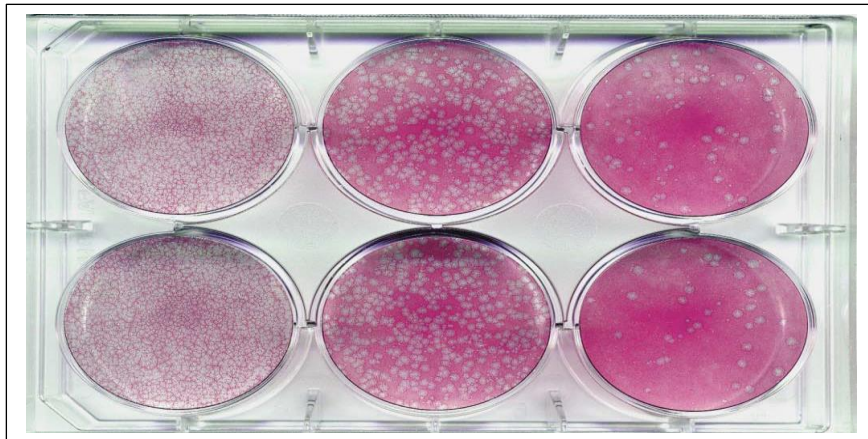


Figure 2-12 plaque neutralization assay

Plaque forming Units

- A single virus infective dose can cause an area of cell destruction
- Movement of virus within cell is restricted by an agar overlay

- This causes areas of localized destruction
- Plaques are enumerated under a microscope to determine the plaque forming units per ml (PFU/ml)
- This allows comparison of different viruses in the same unit

Calculation of PFU/mL(Figure 2-14)

Plaques are enumerated

Plaque Counts are averaged over wells

The average is then divided by the dilution times the volume

$$\frac{(43 + 40 + 38)/3}{(10 - 4 \times 0.1)} = 3,730,000 \text{ pfu/ml}$$



43	4	1	0
40	3	0	0
38	6	2	0
Plaques formed per well			

Figure (2-12)Plaque forming units calculation

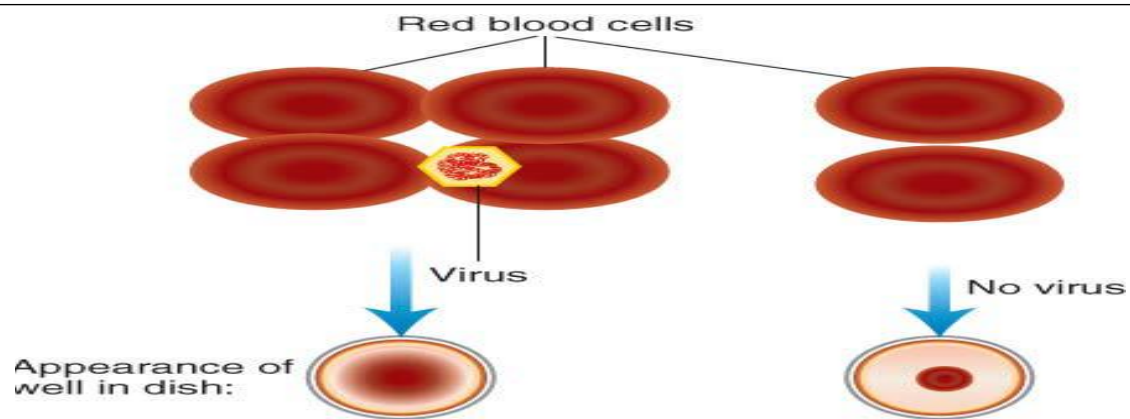
Problems with cell culture

- Long period (up to 4 weeks) required for result.
- Often very poor sensitivity, sensitivity depends on a large extent on the condition of the specimen.
- Susceptible to bacterial contamination.
- Susceptible to toxic substances which may be present in the specimen.
- Many viruses will not grow in cell culture e.g. Hepatitis B, Diarrhoeal viruses, parvovirus, papillomavirus.

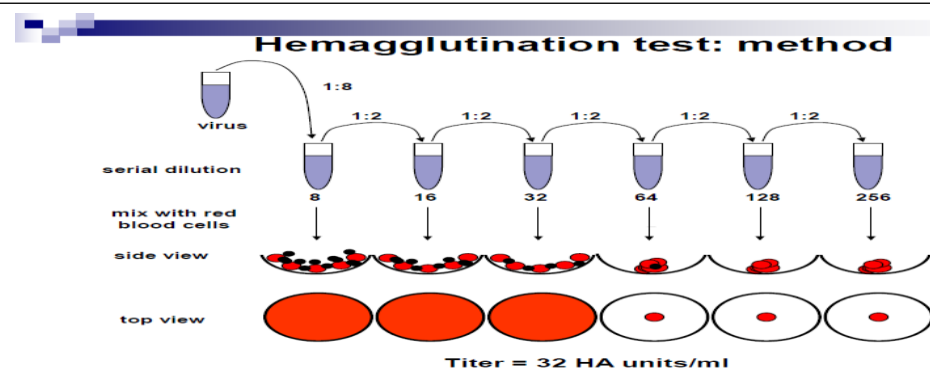
Hemadsorption Test

- Some viruses agglutinate RBCs Mumps, measles, influenza
- Haemagglutination Clumps RBCs (Figure 2-13)

A



B



C

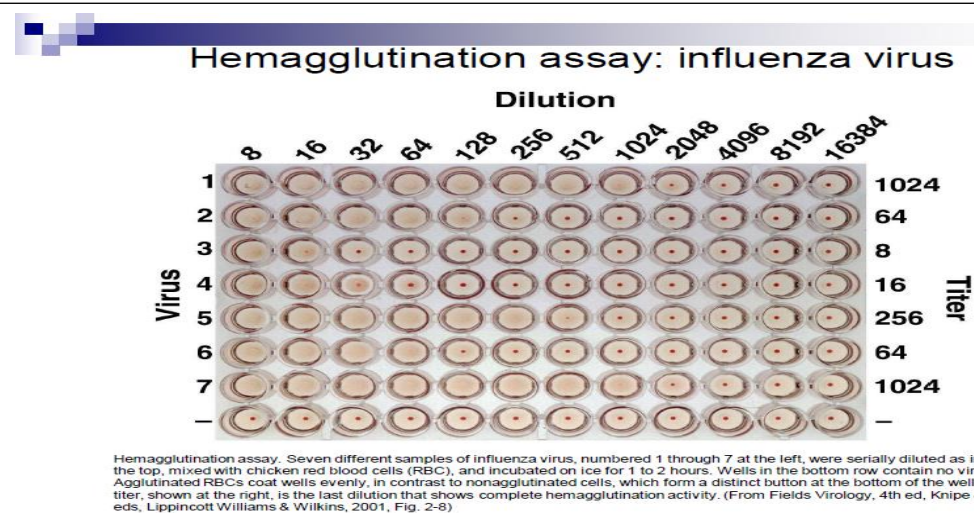
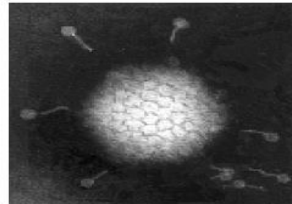


Figure (2-13)A-Haemagglutination assay (H) Principle of test B-Endpoint of Haemagglutination test C- Haemagglutination of culture virus

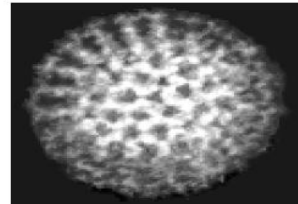
Arranged by Sarah Mohssen

A

Electronmicrographs



Adenovirus

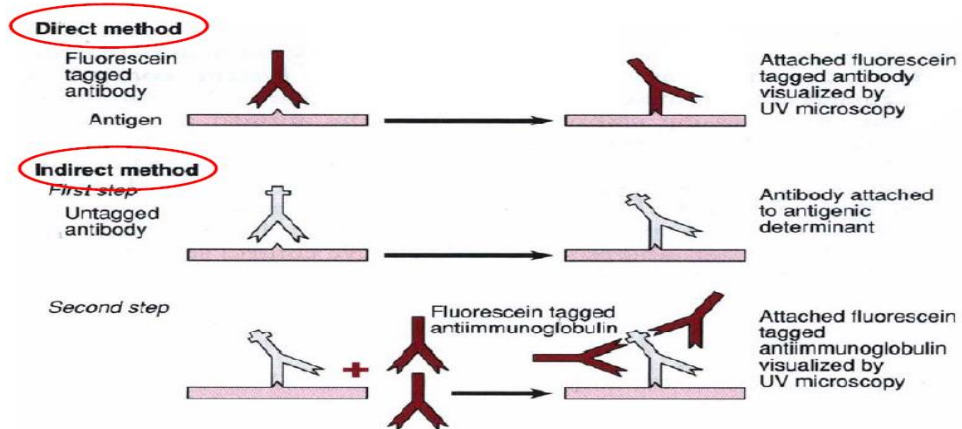


Rotavirus

(courtesy of Linda Stannard, University of Cape Town, S.A.)

B

Immunofluorescence



C

Immunofluorescence

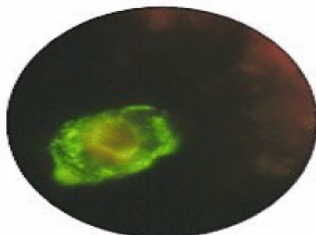
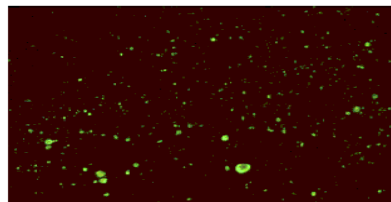


Fig. 3. HSV-infected epithelial cell from skin lesion (DFA)

(Virology Laboratory, Yale-New Haven Hospital)



Positive immunofluorescence test for rabies virus antigen. (Source: CDC)

Rapid Diagnosis Based on the Detection of Viral Antigens

Nasopharyngeal Aspirate	RSV Influenza A and B Parainfluenza Adenovirus
Faeces	Rotaviruses Adenoviruses Astrovirus
Skin	HSV VZV
Blood	CMV (pp65 antigenaemia test)

Methods for Rapid Diagnosis of Viral genome

1. Polymerase Chain Reaction (PCR);

2. Molecular hybridization (Southern or Northern Blot);

Polymerase Chain Reaction

▪ Advantages of PCR:

- Extremely high sensitivity, may detect down to one viral genome (Figure 2-15) per sample volume
- Easy to set up
- Fast turnaround time

▪ Disadvantages of PCR

- Extremely liable to contamination
- High degree of operator skill required
- Not easy to set up a quantitative assay.

- A positive result may be difficult to interpret, especially with latent viruses such as CMV, where any seropositive person will have virus present in their blood irrespective whether they have disease or not.
- These problems are being addressed by the arrival of commercial closed systems such as the Roche Cobas Amplicor which requires minimum handling. The use of synthetic internal competitive targets in these commercial assays has facilitated the accurate quantification of results. However, these assays are very expensive.

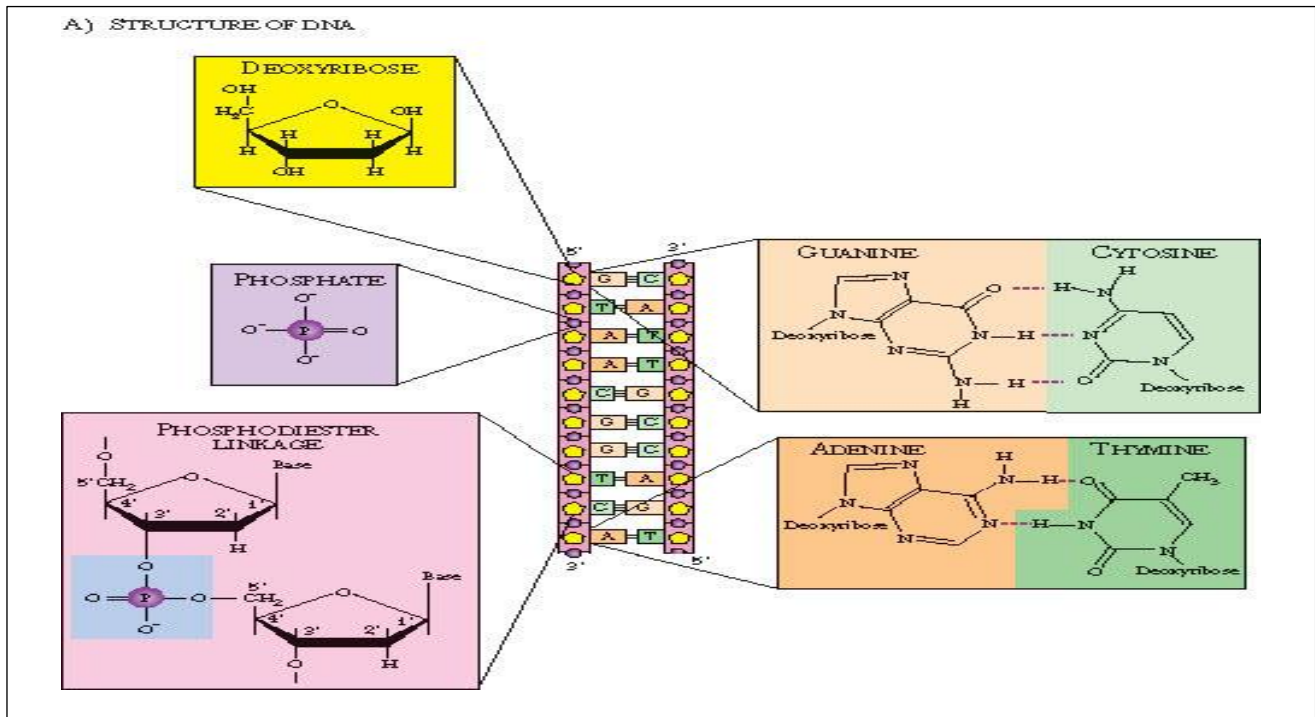


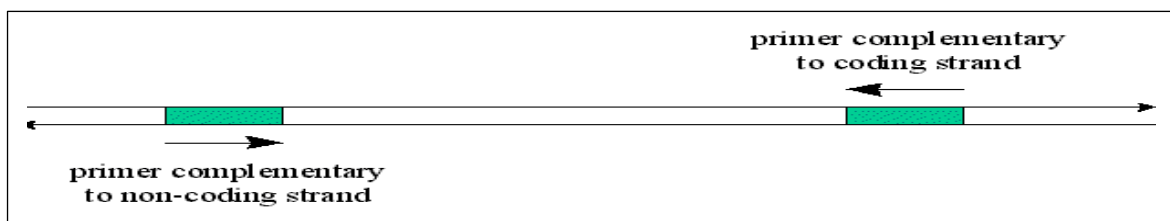
Figure (2-15) Structure of DNA

PCR - Polymerase Chain Reaction

PCR is an in vitro technique for the amplification of a region of DNA which lies between two regions of known sequence. (Figure 2-16)

PCR amplification is achieved by using oligonucleotide primers.

These are typically short, single stranded oligonucleotides which are complementary to the outer regions of known sequence.



Figure(2-16)
Two
amplification
regions of the
DNA

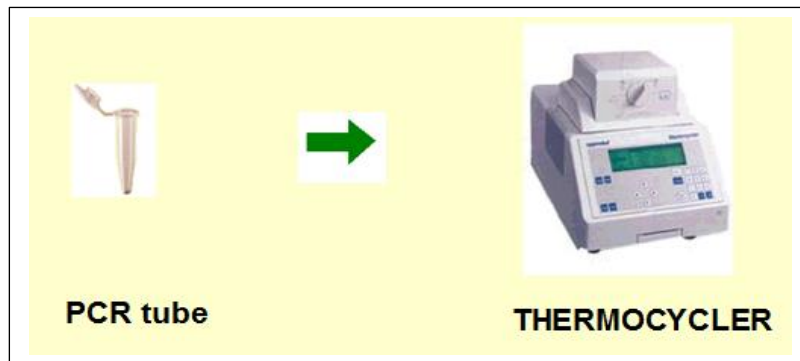
- The oligonucleotides serve as primers for DNA polymerase and the denatured strands of the large DNA fragment serves as the template.
- This results in the synthesis of new DNA strands which are complementary to the parent template strands.
- These new strands have defined 5' ends (the 5' ends of the oligonucleotide primers), whereas the 3' ends are potentially ambiguous in length.

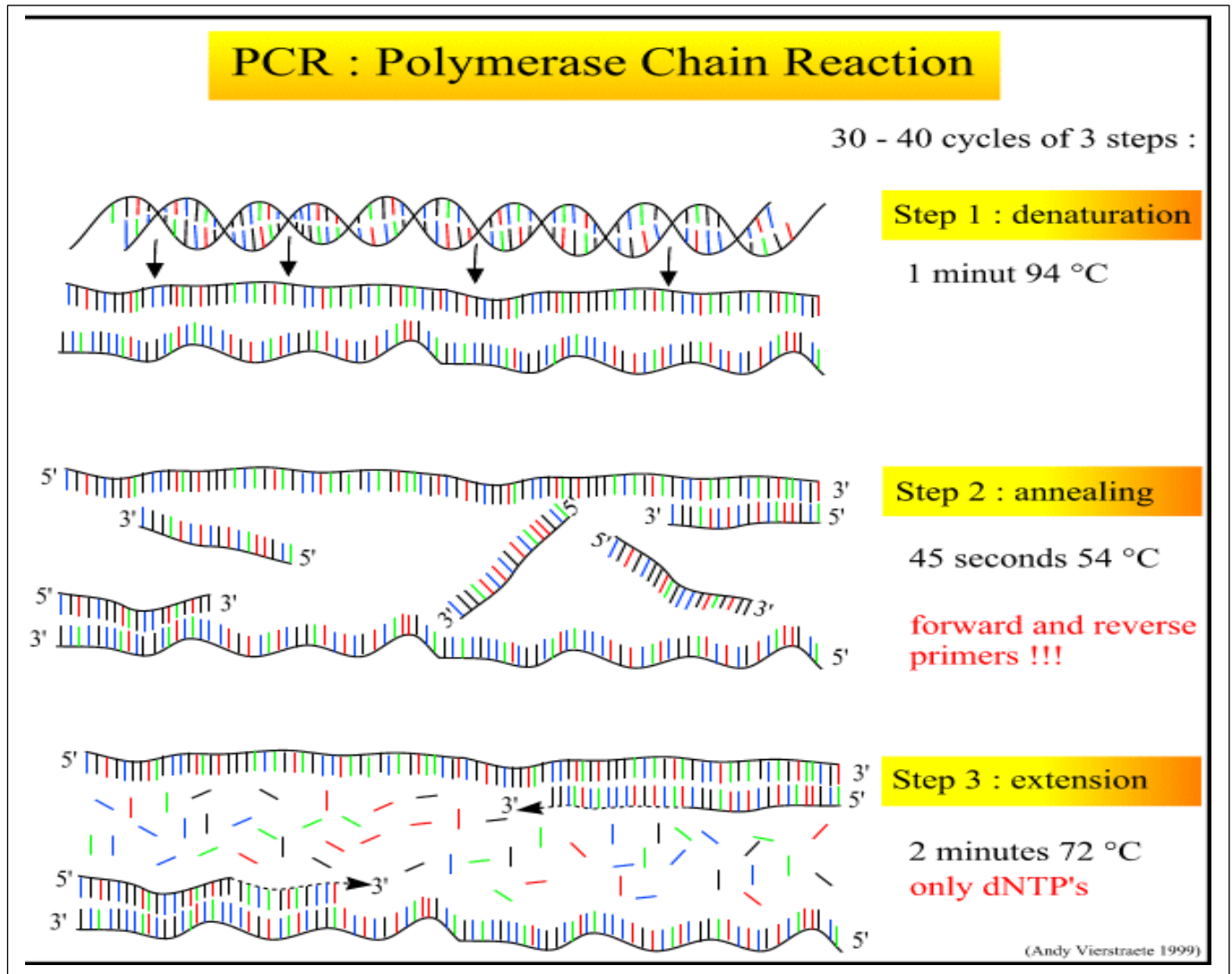
What is PCR? : (Figure2-17)

The "Reaction" Components

1. Target DNA - contains the sequence to be amplified
2. Pair of Primers - oligonucleotides that define the sequence to be amplified.
3. dNTPs - deoxynucleotidetriphosphates: DNA building blocks.
4. Thermostable DNA Polymerase - enzyme that catalyzes the reaction
5. Mg⁺⁺ ions - cofactor of the enzyme
6. Buffer solution - maintains pH and ionic strength of the reaction solution suitable for the activity of the enzyme

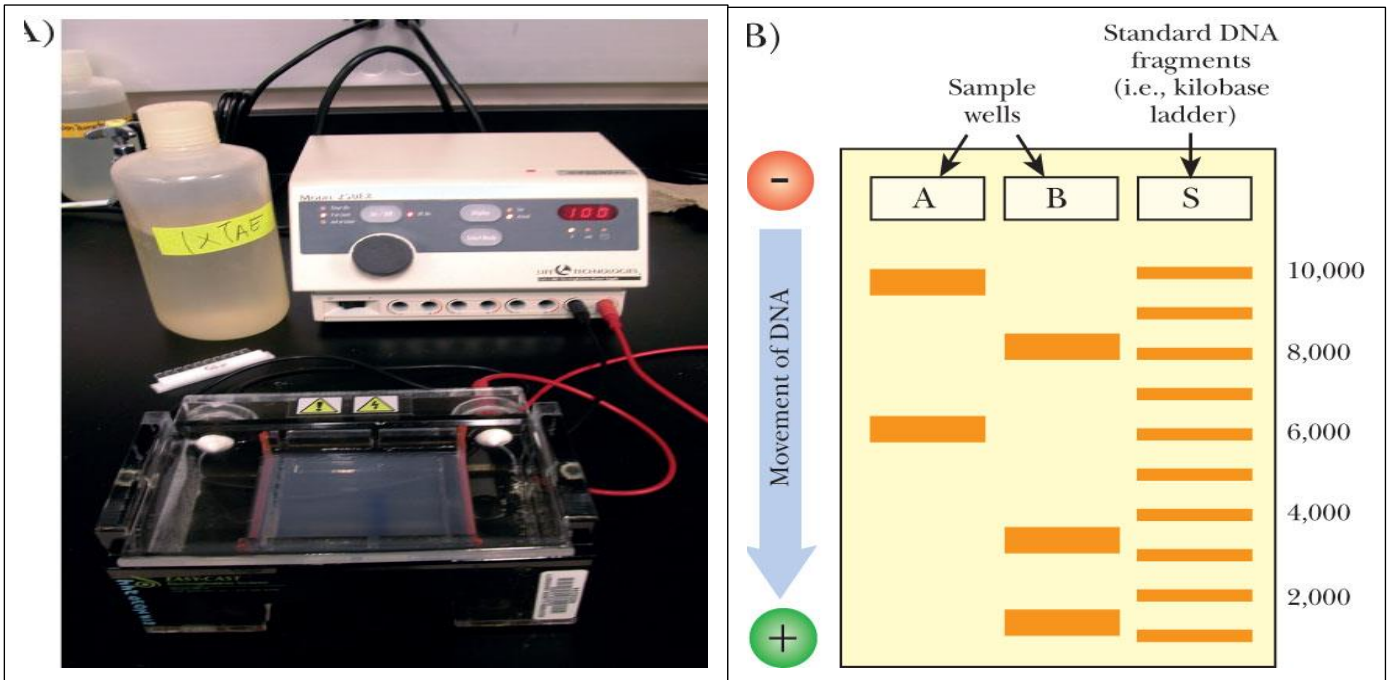
The Reaction:





Figure(2-17)A-PCR equipment (B)Polymerase chain reaction steps

How Gel Electrophoresis of DNA Works



Figure(2-18)DNA gel electrophoresis A-Equipment B-Principle assay

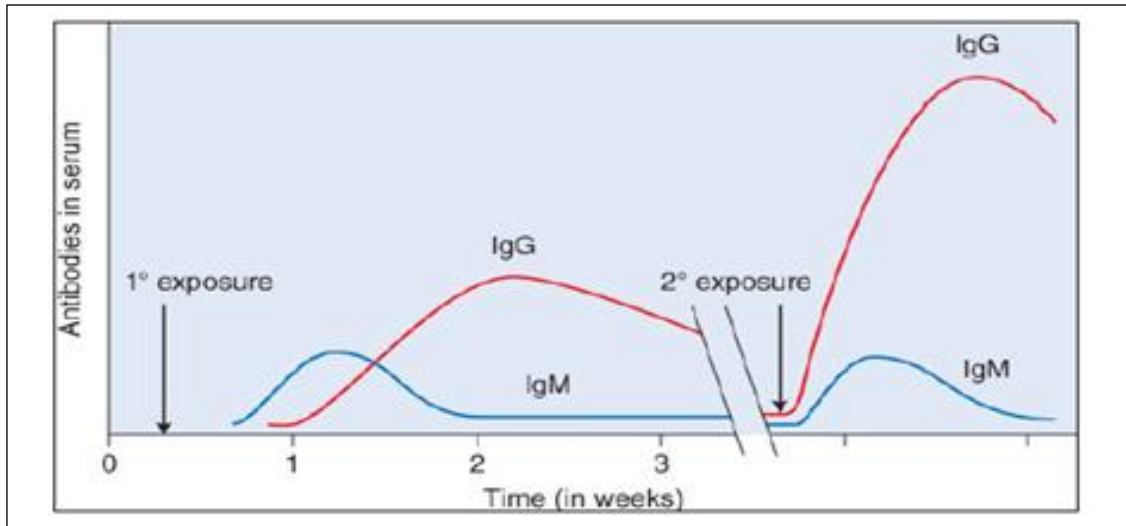
Serology

Viral infection induce immune response the detection of rising titers of antibody between acute and convalescent stages of infection, or detection of IgM in primary infection .(Figure 2-20)

Classical Techniques	Newer Techniques
1. Complement fixation tests (CFT)	1. Radioimmunoassay (RIA)
2. Haemagglutination inhibition tests	2. Enzyme linked immunosorbent assay (EIA)
3. Immunofluorescence techniques (IF)	3. Particle agglutination
4. Neutralization tests	4. Western Blot (WB)
5. Counter-immunoelectrophoresis	5. RIBA, Line immunoassay

Viral serology :

- ✓ Indirect
 - Primary and secondary responses to viral infection
 - IgM (1st exposure)
 - IgG (2nd exposure)



Figure(2-19)Primary and secondary antibody responses toward a viral pathogen

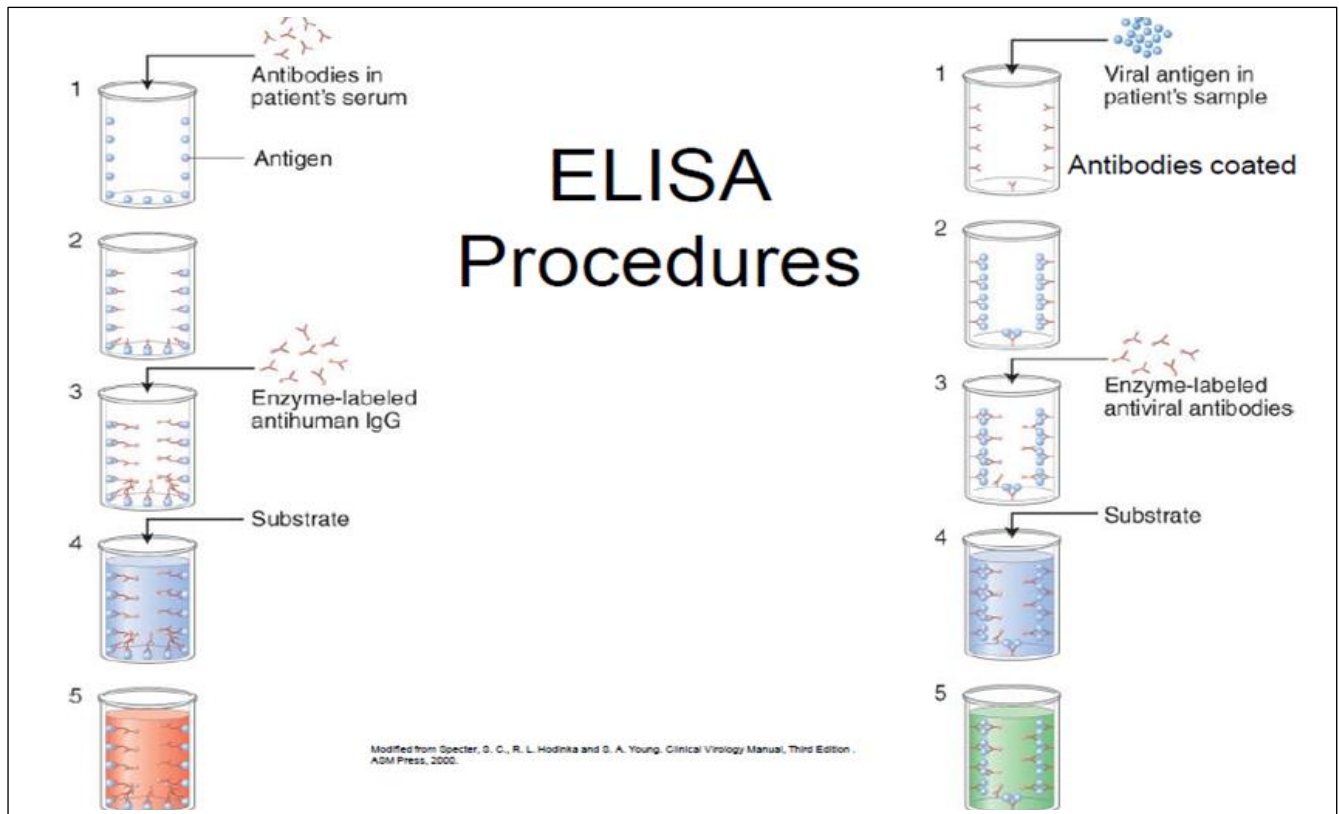


Figure (2-20)Principle of ELISA assay

How useful a serological result is depends on the individual virus.

- For example, for viruses such as rubella and hepatitis A, the onset of clinical symptoms coincide with the development of antibodies. The detection of IgM or rising titres of IgG in the serum of the patient would indicate active disease.

- However, many viruses often produce clinical disease before the appearance of antibodies such as respiratory and diarrhoeal viruses. So in this case, any serological diagnosis would be retrospective and therefore will not be that useful.
- There are also viruses which produce clinical disease months or years after seroconversion e.g. HIV and rabies. In the case of these viruses, the mere presence of antibody is sufficient to make a definitive diagnosis.(Figure 2-21)

ELISA for HIV antibody



Figure(2-21)Microplate ELISA for HIV antibody: Coloured wells indicate reactivity

Problems with Serology:

- Long period of time required for diagnosis for paired acute and convalescent sera.
- Mild local infections such as HSV genitals may not produce a detectable humoral immune response.
- Extensive antigenic cross-reactivity between related viruses e.g. HSV and VZV, Japanese B encephalitis and Dengue, may lead to false positive results.
- immunocompromised patients often give a reduced or absent humoral immune response.
- Patients with infectious mononucleosis and those with connective tissue diseases such as SLE may react non-specifically giving a false positive result.

- Patients given blood or blood products may give a false positive result due to the transfer of antibody.

CSF antibodies :

- Used mainly for the diagnosis of herpes simplex and VZV encephalitis
- CSF normally contain little or no antibodies
- presence of antibodies suggest meningitis or meningoencephalitis

$$\frac{\text{CSF antibody titre}}{\text{Serum antibody titre}} > \frac{1}{100} \text{ is indicative of meningitis}$$

- Diagnosis depends on the presence of an intact blood-brain barrier

What is blotting?

- Blots are techniques for transferring DNA , RNA and proteins onto a carrier so they can be separated, and often follows the use of a gel electrophoresis. The Southern blot is used for transferring DNA, the Northern blot for RNA and the western blot for PROTEIN.(Figure 2-22)

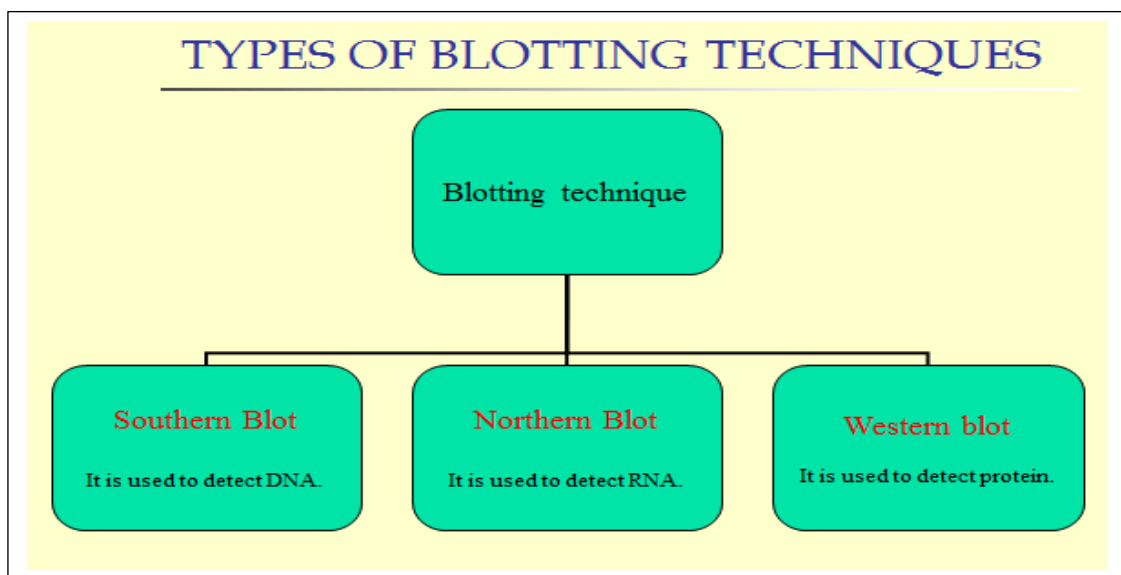


Figure (2-22)The three types of blotting techniques

Southern blotting

Professor Sir Edwin Southern, Professor of Biochemistry and Fellow of Trinity developed this method in 1975.

Southern won the Lasker Award for Clinical Medical Research prize for the method of finding specific DNA sequences he developed this procedure at Edinburgh University more than 30 years ago. The technique is known as DNA transfer or 'Southern blotting'



Figure (2-23) Professor Sir Edwin Southern

Southern Steps(Figure 2-24)(Figure 2-25)

1. DNA to be analyzed is digested to completion with a restriction endonuclease.
2. Electrophoresis to maximally separate restriction fragments in the expected size range. A set of standards of known size is run in one lane of the gel.
3. Blot fragments onto a nitrocellulose membrane.
4. Hybridize with the ^{32}P probe.
5. Autoradiography.

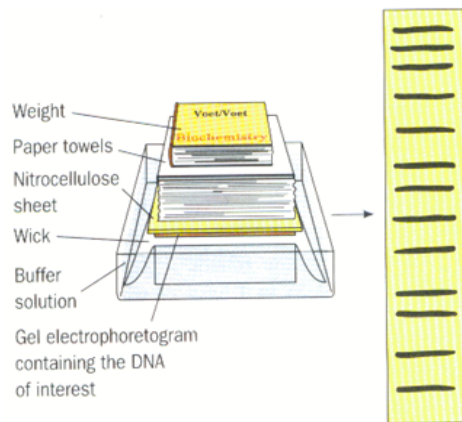
Step 2
Gel electrophoresis
Separates DNA fragments.

Soak gel in 0.5 M NaOH
Converts dsDNA to ssDNA



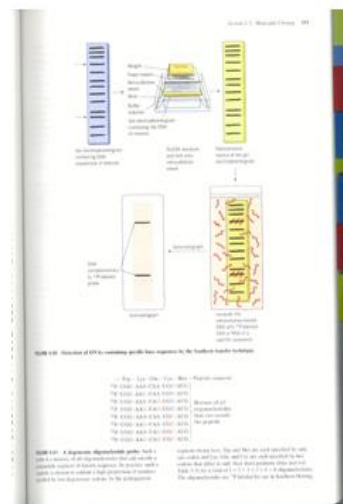
Step 3. Nitrocellulose Blot

- Cover gel with nitrocellulose paper...then...
- Cover nitrocellulose paper with thick layer of paper towels.
- Compress apparatus with heavy weight.
- ssDNA binds to nitrocellulose at same position it had on the gel.
- Vacuum dry nitrocellulose at 80°C to permanently fix DNA in place or cross link (via covalent bonds) the DNA to the mem



Step 4. Hybridization

- Incubate nitrocellulose sheet with a minimal quantity of solution containing ^{32}P -labeled ssDNA probe.
- Probe sequence is complementary to the DNA of interest.
- Incubate for several hours at suitable renaturation temperature that will permit probe to anneal to its target sequence(s).
- Wash & dry nitrocellulose sheet.



Step 5. Autoradiography

- Place nitrocellulose sheet over X-ray film.
- X-ray film darkens where the fragments are complementary to the radioactive probes.

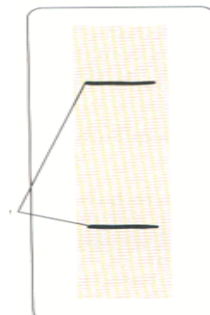
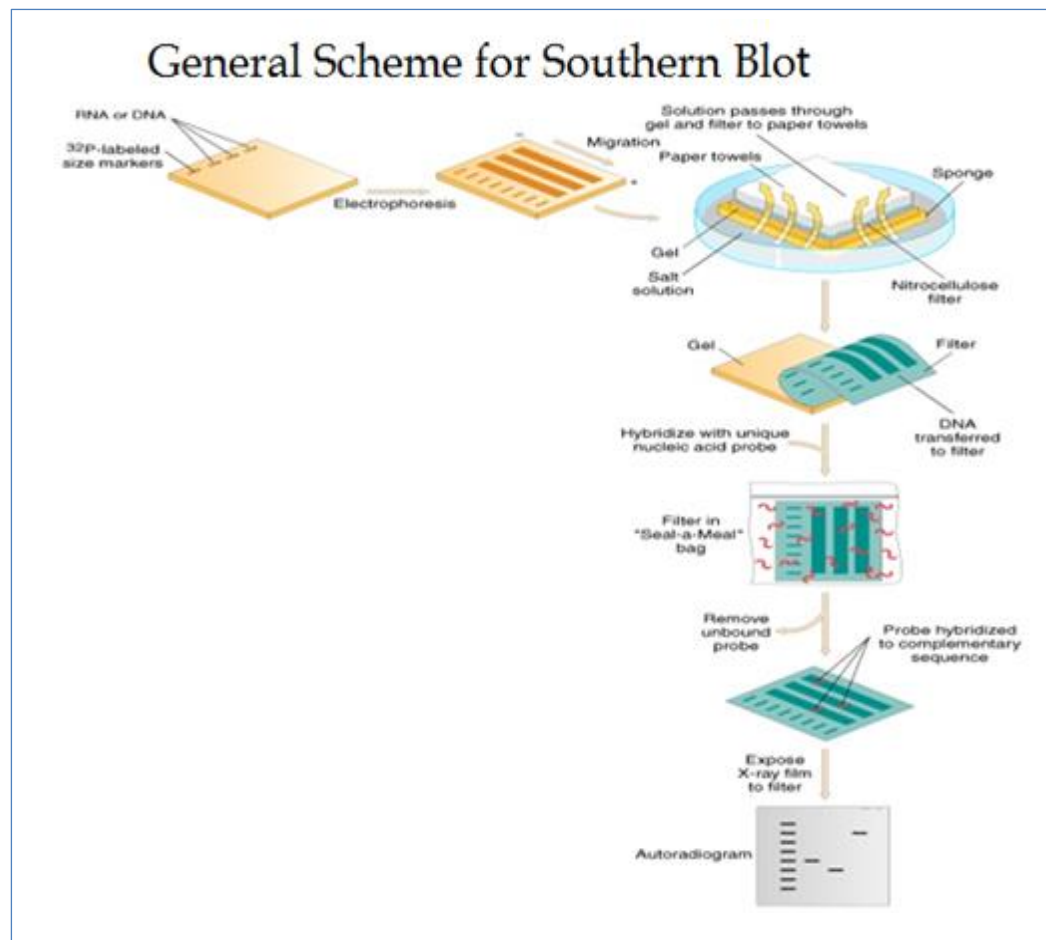


Figure (2-24)Steps of southern blot technique



Figure(2-25)General scheme for southern blot

Herpesviruses

Properties of herpesviruses

- Enveloped double stranded DNA viruses.
- Genome consists of long and short fragments which may be orientated in either direction, giving a total of 4 isomers.
- Three subfamilies:
 - A. Alphaherpesviruses - HSV-1, HSV-2, VZV
 - B. Betaherpesviruses - CMV, HHV-6, HHV-7
 - C. Gammaherpesviruses - EBV, HHV-8
- Set up latent or persistent infection following primary infection
- Reactivation are more likely to take place during periods of immunosuppression
- Both primary infection and reactivation are likely to be more serious in immunocompromised patients.

Herpesvirus Particle

HSV-2 virus particle. Note that all herpesviruses have identical morphology and cannot be distinguished from each other under electron microscopy.(Figure2-26)

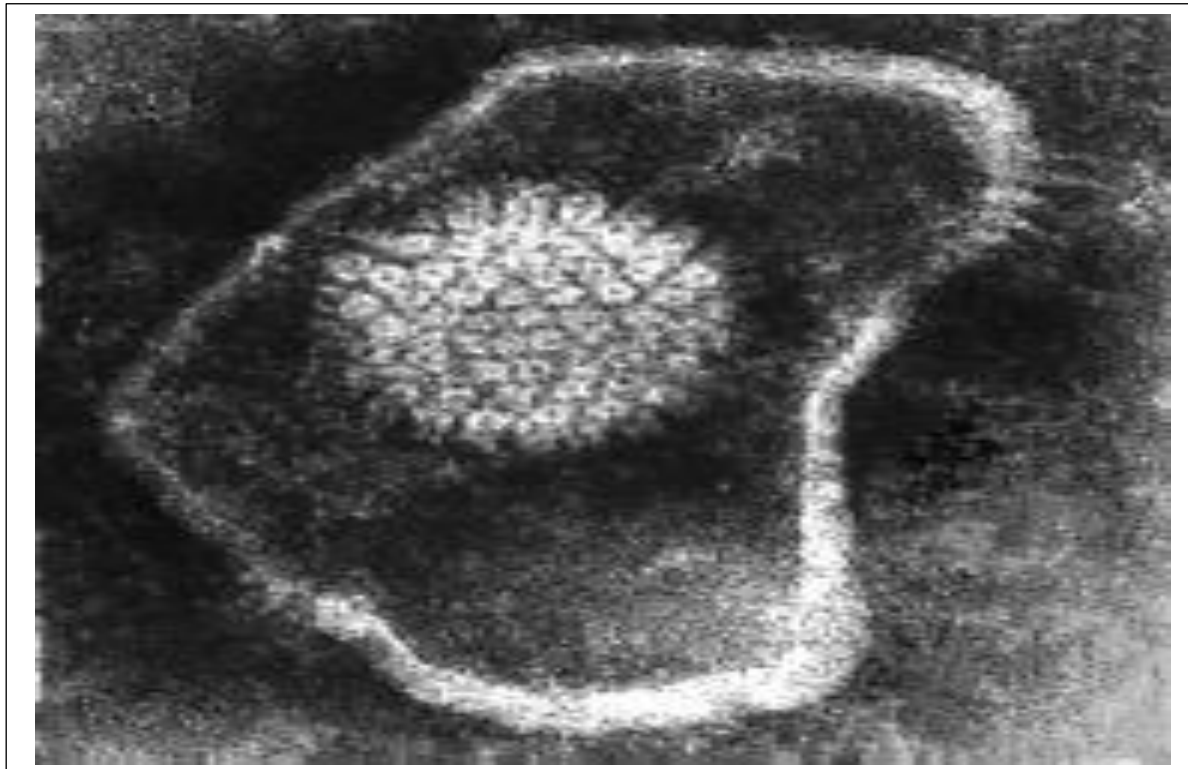


Figure (2-26) Herpesvirus Particle

Herpes Simplex Viruses

- Properties
- Belong to the alpha herpesvirus subfamily of herpesviruses
- Double stranded DNA enveloped virus with a genome of around 150 kb
- The genome of HSV-1 and HSV-2 share 50 - 70% homology.
- They also share several cross-reactive epitopes with each other. There is also antigenic cross-reaction with VZV.
- Man is the only natural host for HSV.

Epidemiology (1)

- HSV is spread by contact, as the virus is shed in saliva, tears, genital and other secretions.
- By far the most common form of infection results from a kiss given to a child or adult from a person shedding the virus.
- Primary infection is usually subclinical in most individuals. It is a disease mainly of very young children ie. those below 5 years.
- There are 2 peaks of incidence, the first at 0 - 5 years and the second in the late teens, when sexual activity commences.
- About 10% of the population acquires HSV infection through the genital route and the risk is concentrated in young adulthood.

Pathogenesis

- During the primary infection, HSV spreads locally and a short-lived viraemia occurs, whereby the virus is disseminated in the body. Spread to the craniospinal ganglia occurs.
- The virus then establishes latency in the craniospinal ganglia.
- The exact mechanism of latency is not known, it may be true latency where there is no viral replication or viral persistence where there is a low level of viral replication.
- **Reactivation** - It is well known that many triggers can provoke a recurrence. These include physical or psychological stress, infection; especially pneumococcal and meningococcal, fever, irradiation; including sunlight, and menstruation.

Clinical Manifestations

HSV is involved in a variety of clinical manifestations which includes :-

1. Acute gingivostomatitis
2. Herpes Labialis (cold sore)

3. Ocular Herpes
4. Herpes Genitalis
5. Other forms of cutaneous herpes
7. Meningitis
8. Encephalitis
9. Neonatal herpes

Oral-facial Herpes

Acute Gingivostomatitis

- Acute gingivostomatitis is the commonest manifestation of primary herpetic infection. (Figure 2-27)
- The patient experiences pain and bleeding of the gums. 1 - 8 mm ulcers with necrotic bases are present. Neck glands are commonly enlarged accompanied by fever.
- Usually a self-limiting disease which lasts around 13 days.

Herpes labialis (cold sore)

- Following primary infection, 45% of orally infected individuals will experience reactivation. The actual frequency of recurrences varies widely between individuals.
- Herpes labialis (cold sore) is a recurrence of oral HSV.
- A prodrome of tingling, warmth or itching at the site usually heralds the recurrence. About 12 hours later, red-ness appears followed by papules and then vesicles.



Figure (2-27)Gingivostomatitis

Ocular Herpes

HSV causes a broad spectrum of ocular disease, ranging from mild superficial lesions involving the external eye, to severe sight-threatening diseases of the inner eye. Diseases caused include the following :

- Primary HSV keratitis – dendritic ulcers
- Recurrent HSV keratitis
- HSV conjunctivitis

Genital Herpes

- Genital lesions may be primary, recurrent or initial.
- Many sites can be involved which includes the penis, vagina, cervix, anus, vulva, bladder, the sacral nerve routes, the spinal and the meninges. The lesions of genital herpes are particularly prone to secondary bacterial infection eg. S.aureus, Streptococcus, Trichomonas and Candida Albicans.
- Dysuria is a common complaint, in severe cases, there may be urinary retention.
- Local sensory nerves may be involved leading to the development of a radiculitis. A mild meningitis may be present.
- 60% of patients with genital herpes will experience recurrences. Recurrent lesions in the perianal area tend to be more numerous and persists longer than their oral HSV-1 counterparts.

Herpes Simplex Encephalitis

- Herpes Simplex encephalitis is one of the most serious complications of herpes simplex disease. There are two forms:
- Neonatal – there is global involvement and the brain is almost liquefied. The mortality rate approaches 100%.
- Focal disease – the temporal lobe is most commonly affected. This form of the disease appears in children and adults. It is possible that many of these cases arise from reactivation of virus. The mortality rate is high (70%) without treatment.
- It is of utmost importance to make a diagnosis of HSE early. It is general practice that IV acyclovir is given in all cases of suspected HSE before laboratory results are available.

Other Manifestations

- Disseminated herpes simplex are much more likely to occur in immunocompromised individuals. The widespread vesicular resembles that of chickenpox. Many organs other than the skin may be involved e.g. liver, spleen, lungs, and CNS.
- Other cutaneous manifestations include
 - eczema herpeticum which is potentially a serious disease that occurs in patients with eczema.
 - Herpetic whitlow which arise from implantation of the virus into the skin and typically affect the fingers.
 - "zosteriform herpes simplex". This is a rare presentation of herpes simplex where HSV lesions appear in a dermatomal distribution similar to herpes zoster.

Laboratory Diagnosis

1. Direct Detection

- Electron microscopy of vesicle fluid - rapid result but cannot distinguish between HSV and VZV
- Immunofluorescence of skin scrapings - can distinguish between HSV and VZV
- PCR - now used routinely for the diagnosis of herpes simplex encephalitis

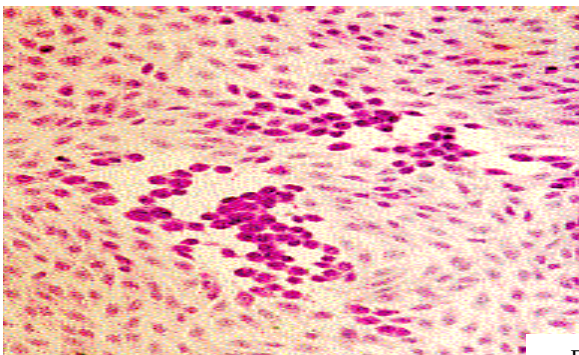
2. Virus Isolation

- HSV-1 and HSV-2 are among the easiest viruses to cultivate. It usually takes only 1 - 5 days for a result to be available.

3. Serology

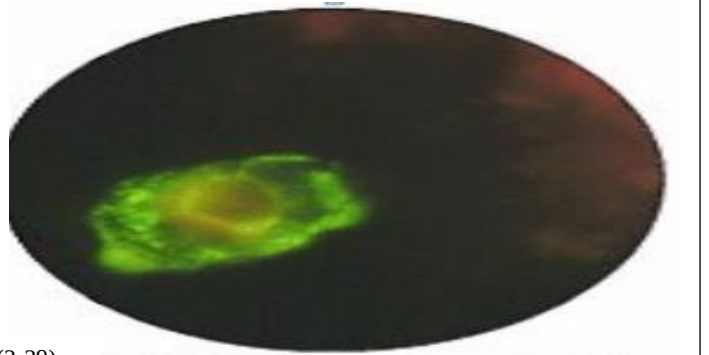
- Not that useful in the acute phase because it takes 1-2 weeks for before antibodies appear after infection. Used to document to recent infection.

A



A-Cytopathic Effect of HSV in cell culture: Note the ballooning of cells. (Linda Stannard, University of Cape Town, S.A)

B



B-Positive immunofluorescence test for HSV antigen in epithelial cell. (Virology Laboratory, New-Yale Haven Hospital)

Figure (2-29)

Management

At present, there are only a few indications of antiviral chemo-therapy, with the high cost of antiviral drugs being a main consideration. Generally, antiviral chemotherapy is indicated where the primary infection is especially severe, where there is dissemination, where sight is threatened, and herpes simplex encephalitis.

Acyclovir – this the drug of choice for most situations at present. It is available in a number of formulations:-

- I.V. (HSV infection in normal and immunocompromised patients)
- Oral (treatment and long term suppression of mucocutaneous herpes and prophylaxis of HSV in immunocompromised patients)
- Cream (HSV infection of the skin and mucous membranes)
- Ophthalmic ointment

Famciclovir and valacyclovir – oral only, more expensive than acyclovir.

Other older agents – e.g. idoxuridine, trifluorothymidine, Vidarabine (ara-A).

- These agents are highly toxic and is suitable for topical use for ophthalmic infection only

Varicella- Zoster Virus

Properties

- Belong to the alphaherpesvirus subfamily of herpesviruses
- Double stranded DNA enveloped virus
- Genome size 125 kbp, long and short fragments with a total of 4 isometric forms.
- One antigenic serotype only, although there is some cross reaction with HSV.

Epidemiology

- Primary varicella is an endemic disease. Varicella is one of the classic diseases of childhood, with the highest prevalence occurring in the 4 - 10 years old age group.
- Varicella is highly communicable, with an attack rate of 90% in close contacts.
- Most people become infected before adulthood but 10% of young adults remain susceptible.
- Herpes zoster, in contrast, occurs sporadically and evenly throughout the year.

Pathogenesis

- The virus is thought to gain entry via the respiratory tract and spreads shortly after to the lymphoid system.
- After an incubation period of 14 days, the virus arrives at its main target organ, the skin.

- Following the primary infection, the virus remains latent in the cerebral or posterior root ganglia. In 10 - 20% of individuals, a single recurrent infection occurs after several decades.
- The virus reactivates in the ganglion and tracks down the sensory nerve to the area of the skin innervated by the nerve, producing a varicellaform rash in the distribution of a dermatome.

Varicella

- Primary infection results in varicella (chickenpox)
- Incubation period of 14-21 days
- Presents fever, lymphadenopathy. a widespread vesicular rash.(Figure2-29)
- The features are so characteristic that a diagnosis can usually be made on clinical grounds alone.
- Complications are rare but occurs more frequently and with greater severity in adults and immunocompromised patients.
- Most common complication is secondary bacterial infection of the vesicles.
- Severe complications which may be life threatening include viral pneumonia, encephalitis, and haemorrhagic chickenpox.



Figure(2-29) Rash of Chickenpox

Herpes Zoster (Shingles)

- Herpes Zoster mainly affect a single dermatome of the skin (Figure2-30).
- It may occur at any age but the vast majority of patients are more than 50 years of age.
- The latent virus reactivates in a sensory ganglion and tracks down the sensory nerve to the appropriate segment.
- There is a characteristic eruption of vesicles in the dermatome which is often accompanied by intensive pain which may last for months (postherpetic neuralgia)
- Herpes zoster affecting the eye and face may pose great problems.

- As with varicella, herpes zoster is a far greater problem in immunocompromised patients in whom the reactivation occurs earlier in life and multiple attacks occur as well as complications.
- Complications are rare and include encephalitis and disseminated herpes zoster.
-



Figure(2-30) Shingles

Congenital VZV Infection

- 90% of pregnant women already immune, therefore primary infection is rare during pregnancy.
- Primary infection during pregnancy carries a greater risk of severe disease, in particular pneumonia.

First 20 weeks of Pregnancy

- Up to 3% chance of transmission to the fetus, recognised congenital varicella syndrome;
 1. Scarring of skin
 2. Hypoplasia of limbs
 3. CNS and eye defects
 4. Death in infancy normal

Laboratory Diagnosis

The clinical presentations of varicella or zoster are so characteristic that laboratory confirmation is rarely required. Laboratory diagnosis is required only for atypical presentations, particularly in the immunocompromised.

Virus Isolation - rarely carried out as it requires 2-3 weeks for a results.

Direct detection - electron microscopy may be used for vesicle fluids but cannot distinguish between HSV and VZV. Immunofluorescence on skin scrapings can distinguish between the two.

Serology - the presence of VZV IgG is indicative of past infection and immunity. The presence of IgM is indicative of recent primary infection.

Management

- Uncomplicated varicella is a self-limited disease and requires no specific treatment. However, acyclovir had been shown to accelerate the resolution of the disease and is prescribed by some doctors.
- Acyclovir should be given promptly immunocompromised individuals with varicella infection and normal individuals with serious complications such as pneumonia and encephalitis.
- herpes zoster in a healthy individual is not normally a cause for concern. The main problem is the management of the postherpetic neuralgia.
- The International Herpes Management Forum recommends that antiviral therapy should be offered routinely to all patients over 50 years of age presenting with herpes zoster.
- Three drugs can be used for the treatment of herpes zoster: acyclovir, valacyclovir, and famciclovir. There appears to be little difference in efficacy between them.

Prevention

- Preventive measures should be considered for individuals at risk of contracting severe varicella infection e.g. leukaemic children, neonates, and pregnant women
- Where urgent protection is needed, passive immunization should be given. Zoster immunoglobulin (ZIG) is the preparation of choice but it is very expensive. Where ZIG is not available, HNIG should be given instead.
- A live attenuated vaccine is available. There had been great reluctance to use it in the past, especially in immunocompromised individuals since the vaccine virus can become latent and reactivate later on.
- However, recent data suggests that the vaccine is safe, even in children with leukaemia provided that they are in remission.
- It is highly debatable whether universal vaccination should be offered since chickenpox and shingles are normally mild diseases.

Cytomegalovirus

Properties

1. Belong to the betaherpesvirus subfamily of herpesviruses
2. double stranded DNA enveloped virus
3. Nucleocapsid 105nm in diameter, 162 capsomers
4. The structure of the genome of CMV is similar to other herpesviruses, consisting of long and short segments which may be orientated in either direction, giving a total of 4 isomers.
5. A large no. of proteins are encoded for, the precise number is unknown.

Epidemiology

- CMV is one of the most successful human pathogens, it can be transmitted vertically or horizontally usually with little effect on the host.
- Transmission may occur in utero, perinatally or postnatally. Once infected, the person carries the virus for life which may be activated from time to time, during which infectious virions appear in the urine and the saliva.
- Reactivation can also lead to vertical transmission. It is also possible for people who have experienced primary infection to be reinfected with another or the same strain of CMV, this reinfection does not differ clinically from reactivation.

Pathogenesis

- Once infected, the virus remains in the person for life and may be reactivated from time to time, especially in immunocompromised individuals.
- The virus may be transmitted in utero, perinatally, or postnatally. Perinatal transmission occurs.
- Perinatal infection is acquired mainly through infected genital secretions, or breast milk. Overall, 2 - 10% of infants are infected by the age of 6 months worldwide. Perinatal infection is thought to be 10 times more common than congenital infection.
- Postnatal infection mainly occurs through saliva. Sexual transmission may occur as well as through blood and blood products and transplanted organ.

Laboratory Diagnosis (1)

Direct detection

- biopsy specimens may be examined histologically for CMV inclusion antibodies or for the presence of CMV antigens. However, the sensitivity may be low.
- The pp65 CMV antigenaemia test is now routinely used for the rapid diagnosis of CMV infection in immunocompromised patients.

- PCR for CMV-DNA is used in some centers but there may be problems with interpretation(Figure 2-31).

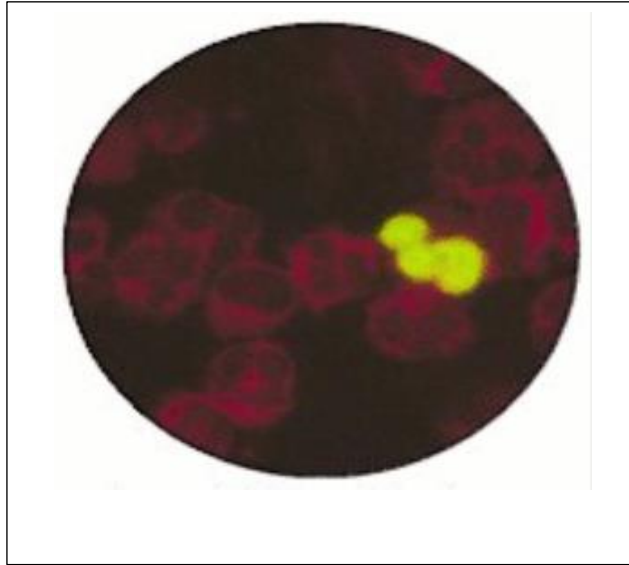


Figure (2-31)CMV pp65 detected in nuclei peripheral blood neutrophils

Laboratory Diagnosis (2)

Virus Isolation

- conventional cell culture is regarded as gold standard but requires up to 4 weeks for result.
- More useful are rapid culture methods such as the DEAFF test which can provide a result in 24-48 hours.

Serology

- the presence of CMV IgG antibody indicates past infection.
- The detection of IgM is indicative of primary infection although it may also be found in immunocompromised patients with reactivation.

Prevention

- No licensed vaccine is available. There is a candidate live attenuated vaccine known as the Towne strain but there are concerns about administering a live vaccine which could become latent and reactivates.
- Prevention of CMV disease in transplant recipients is a very complicated subject and varies from center to center. It may include the following measures.
 - Screening and matching the CMV status of the donor and recipient
 - Use of CMV negative blood for transfusions
 - Administration of CMV immunoglobulin to seronegative recipients prior to transplant
 - Give antiviral agents such as acyclovir and ganciclovir prophylactically.

Epstein-Barr Virus

Epstein-Barr Virus (EBV)

- Belong to the gammaherpesvirus subfamily of herpesviruses
- Nucleocapsid 100 nm in diameter, with 162 capsomers
- Membrane is derived by budding of immature particles through cell membrane and is required for infectivity.
- Genome is a linear double stranded DNA molecule with 172 kbp
- The viral genome does not normally integrate into the cellular DNA but forms circular episomes which reside in the nucleus.
- The genome is large enough to code for 100 - 200 proteins but only a few have been identified.

Epidemiology

- Two epidemiological patterns are seen with EBV.
- In developed countries, 2 peaks of infection are seen : the first in very young preschool children aged 1 - 6 and the second in adolescents and young adults aged 14 - 20 Eventually 80-90% of adults are infected.
- In developing countries, infection occurs at a much earlier age so that by the age of two, 90% of children are seropositive.
- The virus is transmitted by contact with saliva, in particularly through kissing.

Pathogenesis

- Once infected, a lifelong carrier state develops whereby a low grade infection is kept in check by the immune defenses.
- Low grade virus replication and shedding can be demonstrated in the epithelial cells of the pharynx of all seropositive individuals.
- EBV is able to immortalize B-lymphocytes in vitro and in vivo
- Furthermore a few EBV-immortalized B-cells can be demonstrated in the circulation which are continually cleared by immune surveillance mechanisms.
- EBV is associated with several very different diseases where it may act directly or one of several co-factors.

Disease Association

1. Infectious Mononucleosis
2. Burkitt's lymphoma
3. Nasopharyngeal carcinoma

4. Lymphoproliferative disease and lymphoma in the immunosuppressed.
5. X-linked lymphoproliferative syndrome
6. Chronic infectious mononucleosis
7. Oral leukoplakia in AIDS patients
8. Chronic interstitial pneumonitis in AIDS patients.

Infectious Mononucleosis

- Primary EBV infection is usually subclinical in childhood. However in adolescents and adults, there is a 50% chance that the syndrome of infectious mononucleosis (IM) will develop.
- IM is usually a self-limited disease which consists of fever, lymphadenopathy and splenomegaly. In some patients jaundice may be seen which is due to hepatitis. Atypical lymphocytes are present in the blood.
- Complications occur rarely but may be serious e.g. splenic rupture, meningoencephalitis, and pharyngeal obstruction.
- In some patients, chronic IM may occur where eventually the patient dies of lymphoproliferative disease or lymphoma.
- Diagnosis of IM is usually made by the heterophil antibody test and/or detection of EBV IgM.
- There is no specific treatment.

Burkitt's Lymphoma (1)

- Burkitt's lymphoma (BL) occurs endemically in parts of Africa (where it is the commonest childhood tumour) and Papua New Guinea. It usually occurs in children aged 3-14 years. It respond favorably to chemotherapy.
- It is restricted to areas with holoendemic malaria. Therefore it appears that malaria infection is a cofactor.
- Multiple copies of EBV genome and some EBV antigens can be found in BL cells and patients with BL have high titres of antibodies against various EBV antigens.

Burkitt's Lymphoma (2)

- BL cells show a reciprocal translocation between the long arm of chromosome 8 and chromosomes 14, 2 or 22.
- This translocation result in the c-myc oncogene being transferred to the Immunoglobulin gene regions. This results in the deregulation of the c-myc gene. It is thought that this

translocation is probably already present by the time of EBV infection and is not caused by EBV.

- Sporadic cases of BL occur, especially in AIDS patients which may or may not be associated with EBV.
- In theory BL can be controlled by the eradication of malaria (as has happened in Papua New Guinea) or vaccination against EBV.

Immunocompromised Patients

- After primary infection, EBV maintains a steady low grade latent infection in the body. Should the person become immunocompromised, the virus will reactivate. In a few cases, lymphoproliferative lesions and lymphoma may develop. These lesions tend to be extranodal and in unusual sites such as the GI tract or the CNS.
- **Transplant recipients** e.g. renal - EBV is associated with the development of lymphoproliferative disease and lymphoma.
- **AIDS patients** - EBV is associated with oral leukoplakia and with various Non-Hodgkin's lymphoma.
- **Duncan X-linked lymphoproliferative syndrome** - this condition occurs exclusively in males who had inherited a defective gene in the X-chromosome. This condition accounts for half of the fatal cases of IM.

Diagnosis

- Acute EBV infection is usually made by the heterophil antibody test and/or detection of anti-EBV VCA IgM.
- Cases of Burkitt's lymphoma should be diagnosed by histology. The tumour can be stained with antibodies to lambda light chains which should reveal a monoclonal tumour of B-cell origin. In over 90% of cases, the cells express IgM at the cell surface.
- Cases of NPC should be diagnosed by histology.
- The determination of the titre of anti-EBV VCA IgA in screening for early lesions of NPC and also for monitoring treatment.
- A patient with non-specific ENT symptoms who have elevated titres of EBV IgA should be given a thorough examination.

Vaccination

- A vaccine against EBV which prevents primary EBV infection should be able to control both BL and NPC.

- Such a vaccine must be given early in life. Such a vaccine would also be useful in seronegative organ transplant recipients and those developing severe IM, such as the male offspring of X-linked proliferative syndrome carriers.
- The vaccine should not preferably be a subunit vaccine since there is a danger that a live vaccine may still have tumorigenic properties.
- The antigen chosen for vaccine development is the MA antigen gp 340/220 as antibodies against this antigen are virus neutralizing.
- This vaccine is being tried in Africa.

Other Human Herpes Viruses

Properties of HHV-6 and 7

- Belong to the betaherpesvirus subfamily of herpesviruses
- Double stranded DNA genome of 170 kbp
- The main target cell is the T-lymphocyte, although B-lymphocytes may also be infected.
- HHV-6 and HHV-7 share limited nucleotide homology and antigenic cross-reactivity.
- It is thought that HHV-6 and HHV-7 are related to each other in a similar manner to HSV-1 and HSV-2.

Epidemiology and Pathogenesis

- HHV-6 and HHV-7 are ubiquitous and are found worldwide.
- They are transmitted mainly through contact with saliva and through breast feeding.
- HHV-6 and HHV-7 infection are acquired rapidly after the age of 4 months when the effect of maternal antibody wears off.
- By the time of adulthood, 90-99% of the population had been infected by both viruses.
- Like other herpesviruses, HHV-6 and HHV-7 remains latent in the body after primary infection and reactivates from time to time.

Clinical Manifestations (1)

- Primary HHV-6 infection is associated with Roseola Infantum, which is a classical disease of childhood.
- Most cases occur in infants between the ages of 4 months and two years.
- A spiking fever develops over a period of 2 days followed by a mild rash. The fever is high enough to cause febrile convulsions.
- There are reports that the disease may be complicated by encephalitis.

Clinical Manifestations (2)

- If primary infection is delayed until adulthood, there is a small chance that an infectious mononucleosis-like disease may develop in a similar manner to EBV and CMV.
- There is no firm evidence linking HHV-6 to lymphomas or lymphoproliferative diseases.
- There is no firm disease association with HHV-7 at present.
- Although both viruses may be reactivated in immunocompromised patients, it is yet uncertain whether they cause significant disease since CMV is almost invariably present.

Diagnosis and Management

- Rosela Infantum has a very characteristic presentation and a diagnosis can usually be made on clinical grounds alone.
- Therefore very few virology laboratories offer a diagnostic service for HHV-6 or HHV-7 infection.
- The technique for virus isolation is complicated and thus not practicable as a routine diagnostic procedure.
- Therefore serology is the mainstay of diagnosis where specific IgM and IgG are detected.
- There is no specific antiviral treatment for HHV-6 infection.

Human Herpes Virus 8

- Belong to the gammaherpesviruses subfamily of herpesviruses
- Originally isolated from cells of Kaposi's sarcoma (KS)
- Now appears to be firmly associated with Kaposi's sarcoma as well as some lesser known malignancies such as Castleman's disease and primary effusion lymphomas(Figure 2-32)
- HHV-8 DNA is found in almost 100% of cases of Kaposi's sarcoma
- Most patients with KS have antibodies against HHV-8
- The seroprevalence of HHV-8 is low among the general population but is high in groups of individuals susceptible to KS, such as homosexuals.
- Unlike other herpesviruses, HHV-8 does not have a ubiquitous distribution.

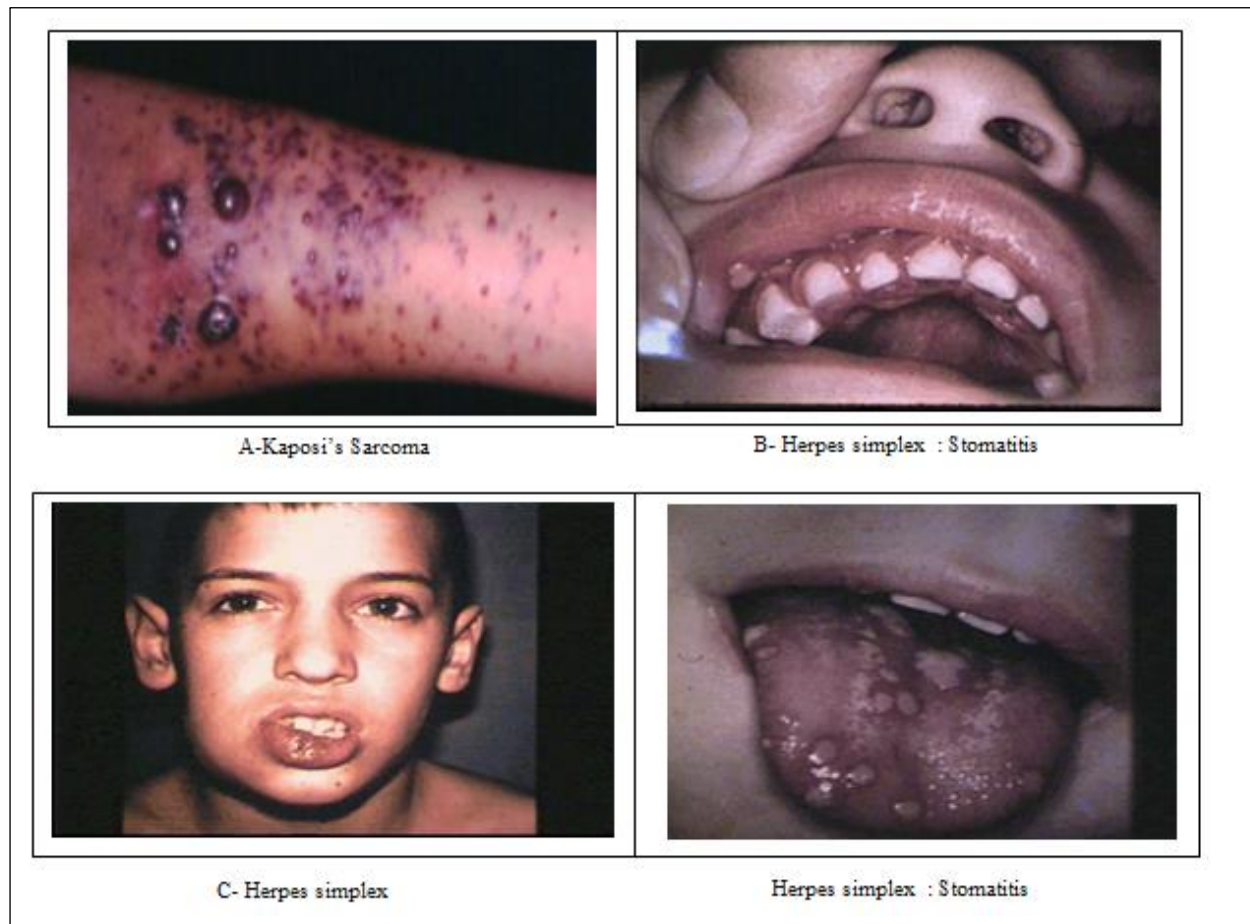


Figure (2-32) Diseases caused by Herpesvirus

paramyxoviruse virus

The paramyxoviruses include the most important agents of respiratory infections of infants and young children as well as the causative agents of two of the most common contagious diseases of childhood (mumps and measles).

Characters

Large enveloped viruses (150-300 nm)

Negative sense linear non-segmented RNA

Contain 2 types of glycoprotein spikes which are responsible for the attachment and fusion of the virus to the host cell:

Haemagglutinin-neuraminidase spikes (HN}

Fusion spikes (F)(Figure 2-33)

Classification

- 1.Parainfluenza viruses 1, 2, 3 and 4.
- 2.Mumps virus
- 3.Measles
- 4.Respiratory syncytial virus

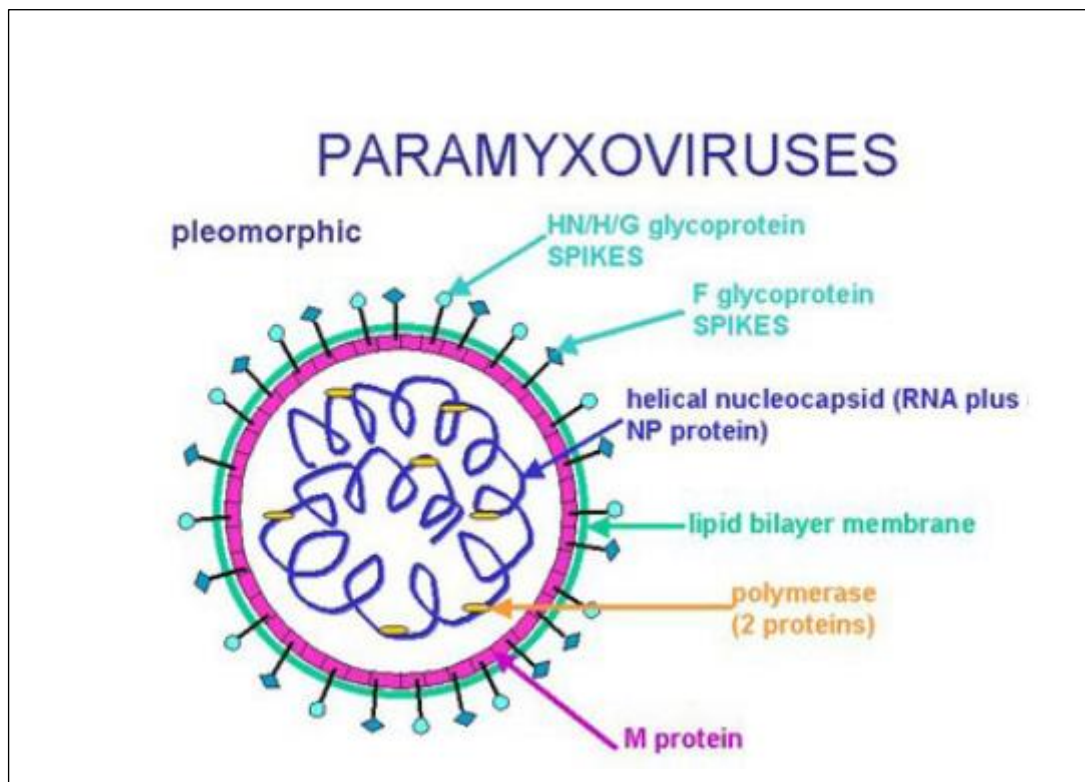


Figure (2-33) Structure of paramyxovirus

Four types of Parainfluenza viruses :

TYPE 1,2,& 3 are particularly considered major pathogens of severe respiratory tract disease in infants & young children

Type 4 does not cause severe disease even on primary infection.

Respiratory syncytial virus

- It lacks haemagglutinin & neuraminidase activity, but has F spikes. RSV is transmitted via droplet infection.
- Viral replication occurs in the epithelial cells of the nasopharynx.
- Viraemia has not been detected.
- RSV is the most important cause of lower respiratory tract infections in infants and young children.

Measles

Measles is a highly contagious disease caused by the paramyxovirus. 90% of the people exposed to the virus contract the disease. The symptoms are a fever, cough, runny nose, rash, and red eyes(Figure 2-34) . Again, there is no specific treatment for the disease, but it has become much less common with the increased use of the MMR vaccine.

Structure:

like other Paramyxoviruses but lacks the neuraminidase activity.

Complications

1. Post infection encephalitis: (rare) usually fatal.
2. Lower respiratory tract infection.
3. SSPE Subacute Sclerosing Panencephalitis:

(Occurs years after measles infection and very rare)



Figure (2-34) Child infected with Measles (Rubella)

Transmission

- ✓ Measles transmission is primarily person to person via large respiratory droplets. Airborne transmission via aerosolized droplet has been documented in closed areas (e.g., office examination room) for up to 2 hours after a person with measles occupied the area.
- ✓ Measles is highly communicable, with >90% among susceptible persons. Measles may be transmitted from 4 days prior to 4 days after rash onset. Maximum communicability occurs from onset of symptoms through the first 3-4 days of rash.

Virulence factors

Portal of entry:

- Respiratory mucus membrane.
- It first infects the respiratory mucosa, spreads through the lymphatics and bloodstream, and can then infect the conjunctiva, respiratory tract, urinary tract, GI tract, endothelial cells, and the central nervous system.

Attachment:

Hemagglutinin

Hemagglutinin is an integral membrane protein found on the surface of the measles virus.

Hemagglutinin binds to CD46, a glycoprotein found on the surface of most cells.

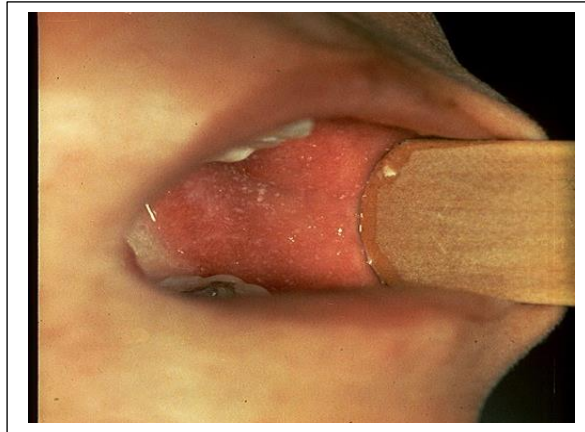
(CD46 protects host cells from autoimmune destruction by binding to C3b and C4b and cleaving them).

Destruction of tissue:

a serious febrile illness. The maculopapular rash, which starts at the hairline and spreads over the whole body, is caused by immune T-cells targeted to the infected endothelial cells of the small blood vessels. T-cell deficient individuals do not have the rash, but do have uncontrolled disease which usually results in death.

- ✓ The damage, as well as the control of the disease, is most probably caused by the immune system.

Koplik's spots



Figure(2-35) Koplik's spots

Koplik's spots :

Found in the mouth, these spots look like tiny grains of white sand, each surrounded by a red ring. They are found especially on the inside of the cheek (the buccal mucosa) opposite the 1st and 2nd upper molars.

Measles Laboratory Diagnosis

- Isolation of measles virus from a clinical specimen (e.g., nasopharynx, urine)
- Significant rise in measles IgG by any standard serologic assay (e.g., EIA, HA)
- Positive serologic test for measles IgM antibody

Mumps virus :

- Is acute infectious disease causing enlargement of one or both of the parotid glands.
- Other organs may be involved as the pancreas, testes, ovaries and even the CNS.
- Mumps has become less common since the MMR vaccine became more widespread.

Pathogenesis

1. Transmission by droplet infection
2. Then to the blood stream.
3. Then to the salivary glands and other organs.
4. Incubation period 18-21 days

Rubella

Rubella virus is single-stranded RNA of positive polarity which is enclosed by an icosahedral capsid with lipid bi-layer envelope.

- Rubella, or the **German measles** is the third most common disease caused by paramyxovirus. disease
- Rubella virus is the pathogenic agent of the disease Rubella, and is the cause of congenital rubella syndrome when infection occurs during the first weeks of pregnancy. Humans are the only known host of this virus.
- Spread by contact with an infected person, through coughing and sneezing

Two clinical forms:

- **Postnatal rubella** – malaise, fever, sore throat, lymphadenopathy, rash, generally mild, lasting about 3 days
- **Congenital rubella** – infection during 1st trimester most likely to induce miscarriage or multiple defects such as cardiac abnormalities, ocular lesions, deafness, mental and physical retardation
- **Diagnosis based on serological testing**
- **No specific treatment available**
- **Attenuated viral vaccine MMR**

Measles Vaccines

attenuated and killed vaccines

1965	Live further attenuated vaccine
1967	Killed vaccine withdrawn
1968	Live further attenuated vaccine (Edmonston-Enders strain)
1971	Licensure of combined measles- mumps-rubella vaccine
1989	Two dose schedule

- Composition Live virus
- Efficacy 95% (range, 90%-98%)
- Duration of
- Immunity Lifelong
- Schedule 2 doses

- Should be administered with mumps and rubella as MMR

MMR Vaccine

- First dose of MMR at 12-15 months
- Second dose of MMR at 4-6 years
- Second dose may be given any time >4 weeks after the first dose

Rabies

- Rhabdovirus family; genus Lyssavirus
- Enveloped, bullet-shaped virions(Figure 2-36)
- Slow, progressive zoonotic disease
- Primary reservoirs are wild mammals; it can be spread by both wild and domestic mammals by bites, scratches, and inhalation of droplets.

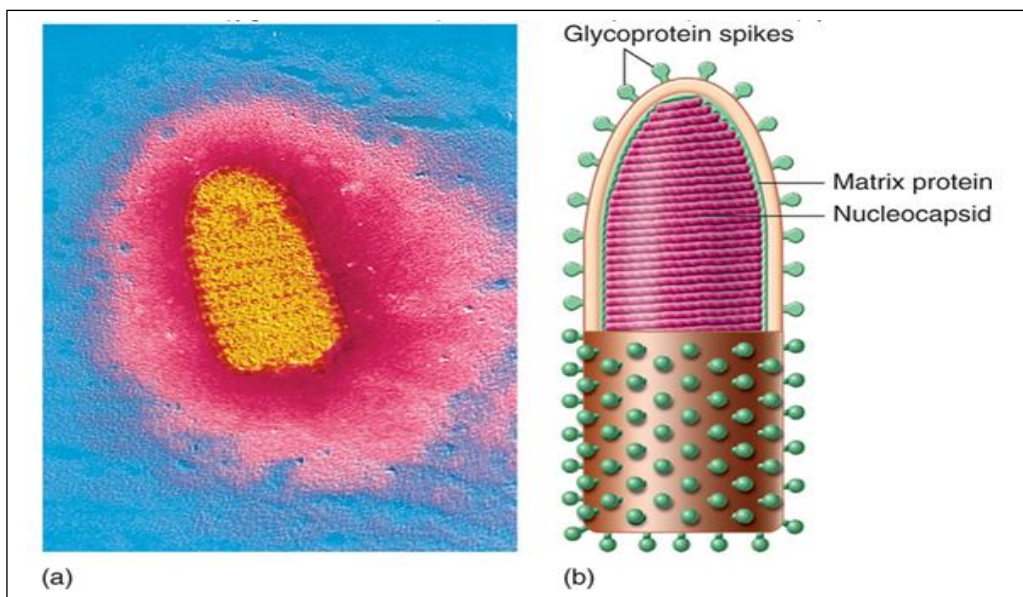


Figure (2-36) Enveloped bullet shaped Rubies virus

- Virus enters through bite, grows at trauma site for a week and multiplies, then enters nerve endings and advances toward the ganglia, spinal cord and brain.(Figure 2-37)
- Infection cycle completed when virus replicates in the salivary glands (Figure 2-38)
- Clinical phases of rabies:
- **Prodromal phase** – fever, nausea, vomiting, headache, fatigue; some experience pain, burning, tingling sensations at site of wound

- **Furious phase** – agitation, disorientation, seizures, twitching, hydrophobia
- **Dumb phase** – paralyzed, disoriented, stuporous
- Progress to coma phase, resulting in death

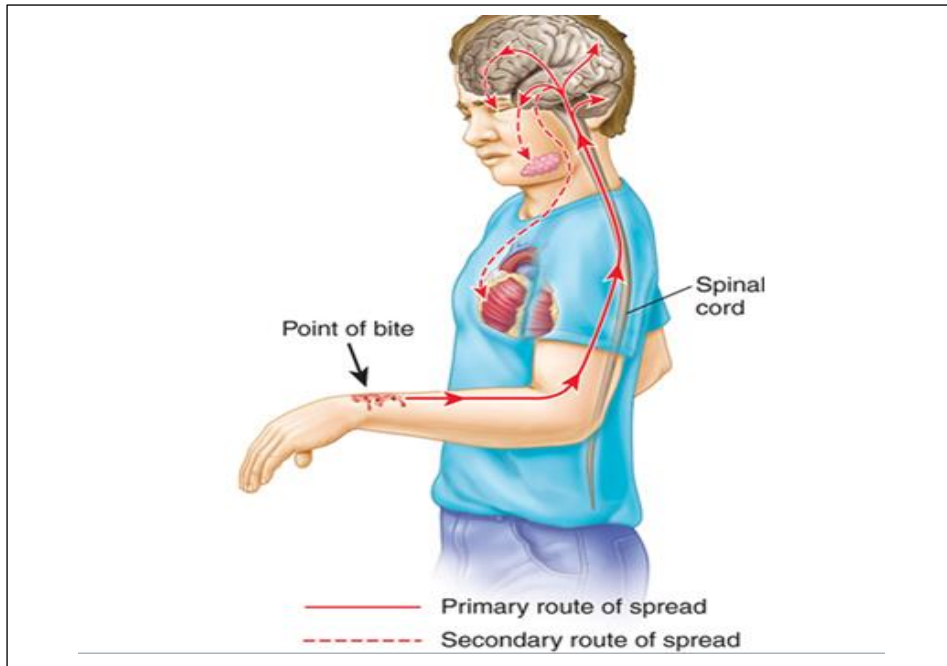
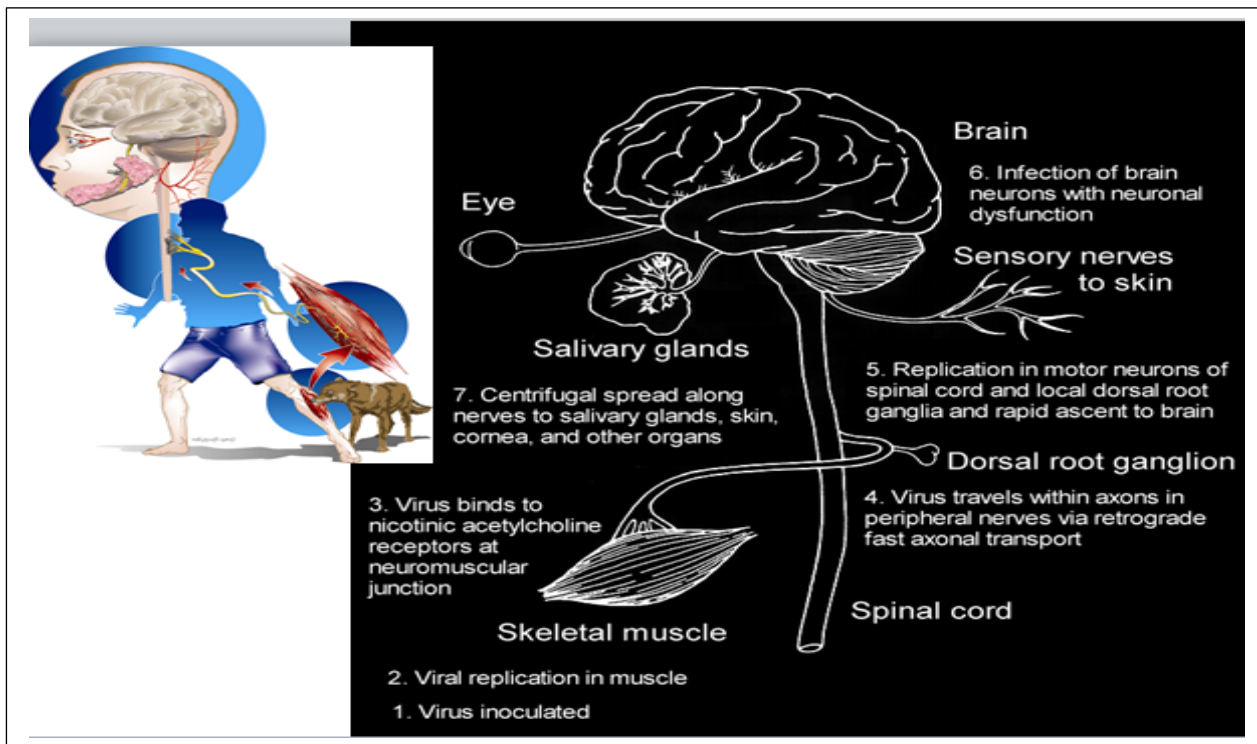


Figure (2-37) Rote spread of virus



Figure(2-38) Rabies virus inoculation and replication in infected person

Rabies Virus diagnosis

Laboratory diagnosis

1. PCR
2. Serology (IFA)
3. Animal control

Rabid or suspected rabid animals are killed and examined by histopathology for Negri bodies and viral antigen

- Vaccination of pets is required by law in most states

Immunity and protection

- Vaccines
- First one developed by Pasteur by using spinal cords from infected dogs
- Today's principal vaccine is the human diploid cell vaccine (HDCV) made in the WI-38 fibroblast cell line
- Virus is inactivated by β PL
- Post-exposure prophylaxis
- One dose of hyperimmune antiserum
- Five immunizations over 28 days

Recommended prophylaxis in exposed individuals not previously vaccinated against rabies

Wound site(s)	→	• Immediate thorough cleansing of all wounds with soap and water. • Tetanus prophylaxis
Human Rabies Immune Globulin (RIG)	→	- IU/kg body weight - should be infiltrated in wound(s) - The remainder should be given IM at a site distant from vaccine
Rabies Vaccine	→	IM (1 mL) in the deltoid area on days 0, 3, 7, 14, and 28

Laboratory diagnosis

- Diseased dog: viral antigen and Negri body in brain tissue (Figure2-39).
- Patient: IF assay, PCR.

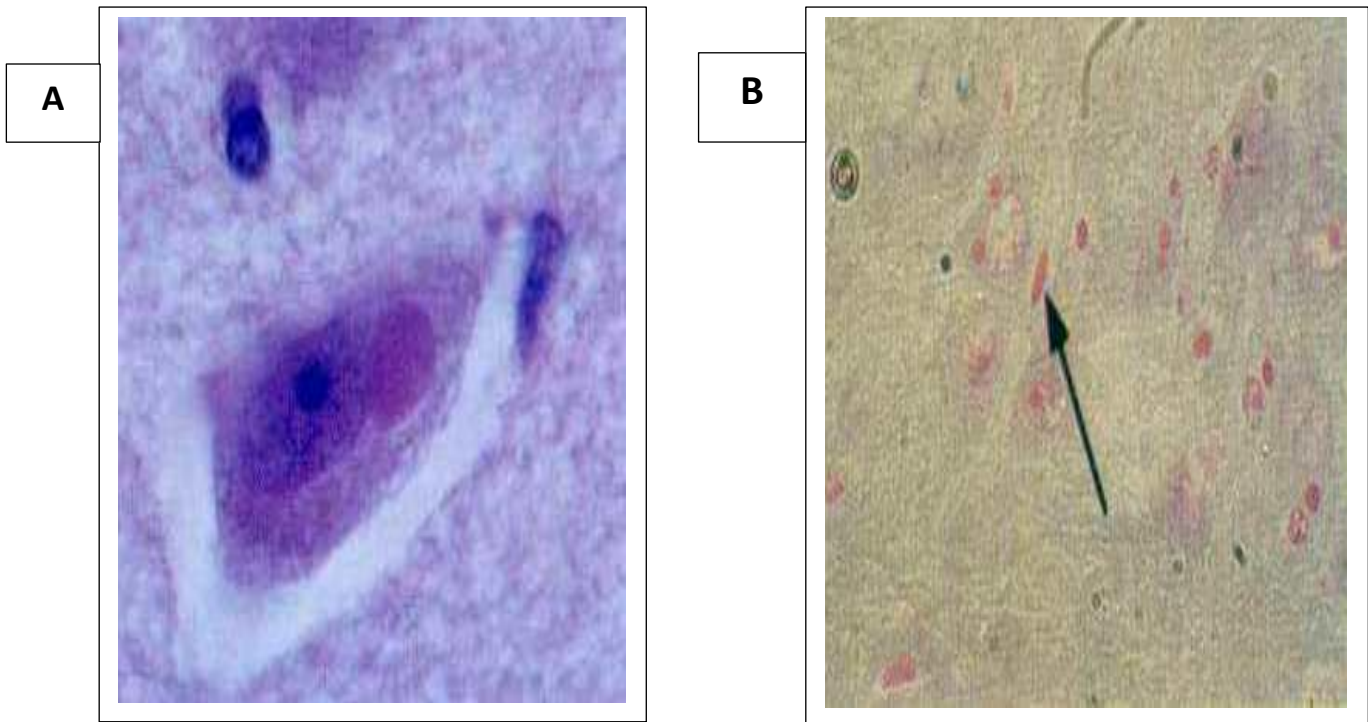


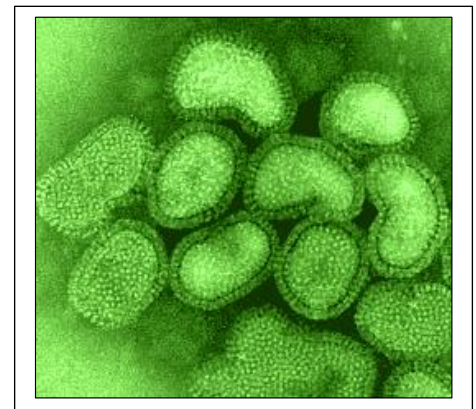
Figure (2-39) (A,B) Negri body brain tissue

Orthomyxoviruses (figure 2-40)(figure2-41)

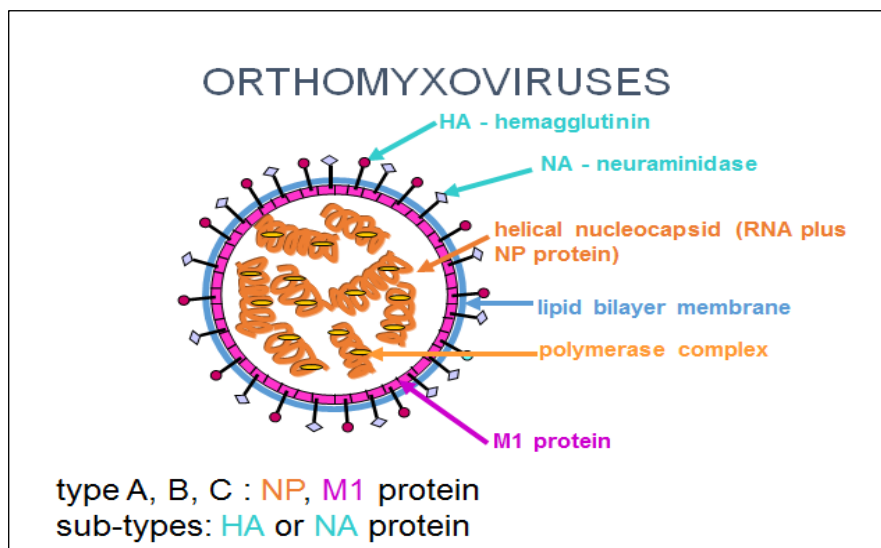
pleomorphic

influenza types A,B,C (Figure 2-42)

febrile, respiratory illness with systemic symptoms



Figure(2-41)Orthomyxoviruses



Figure(2-40) Orthomyxoviruses structure

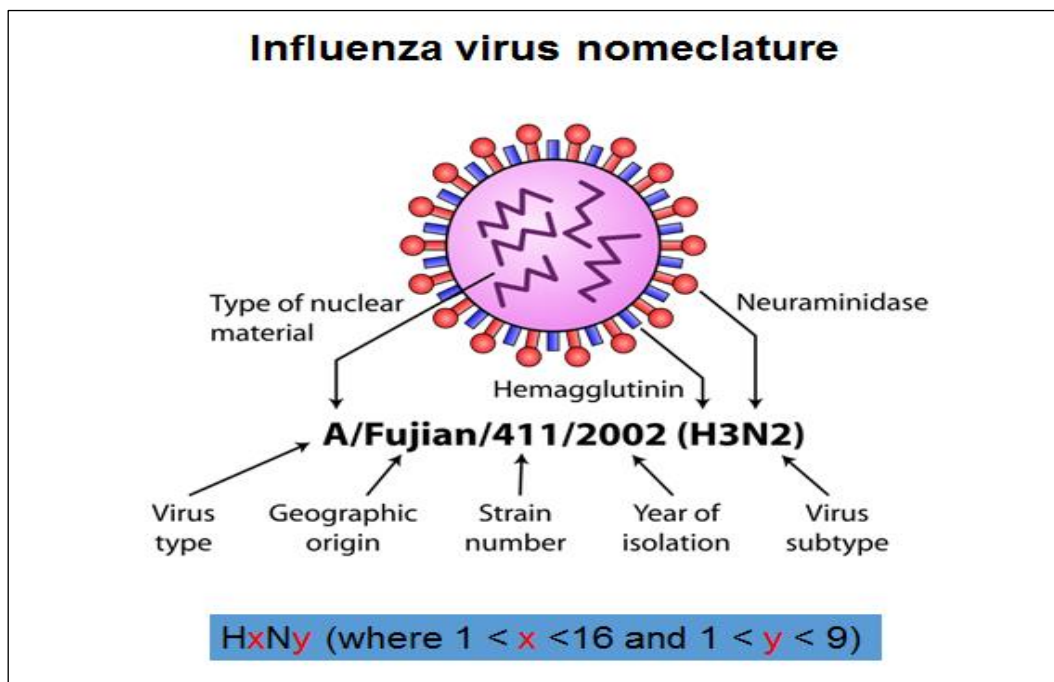
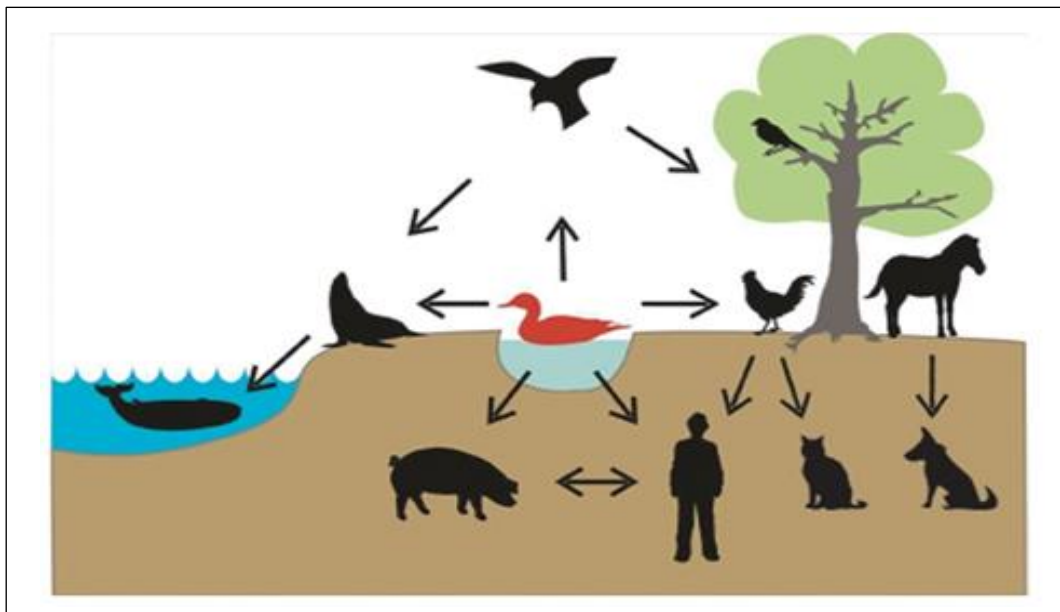


Figure (2-42) Influenza virus nomenclature



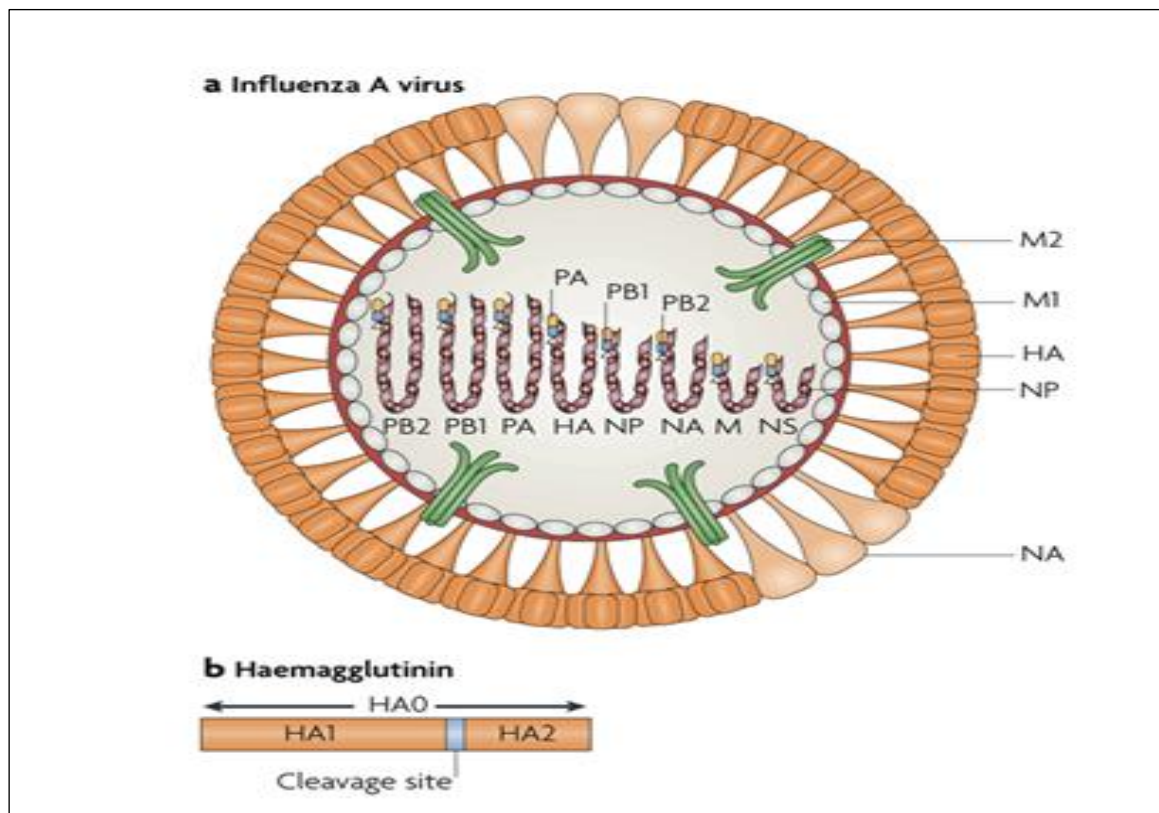
Figure(2-43) Influenza A reservoir

Wild aquatic birds are the main reservoir of influenza A viruses. Virus transmission has been reported from wild waterfowl to poultry, sea mammals, pigs, horses, and humans. Viruses are also transmitted between pigs and humans, and from poultry to humans. Equine influenza

viruses have recently been transmitted to dogs. (From Fields Virology (2007) 5th edition, Knipe, DM & Hawley, PM, eds, Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia Figure(2-43).

Hemagglutinin

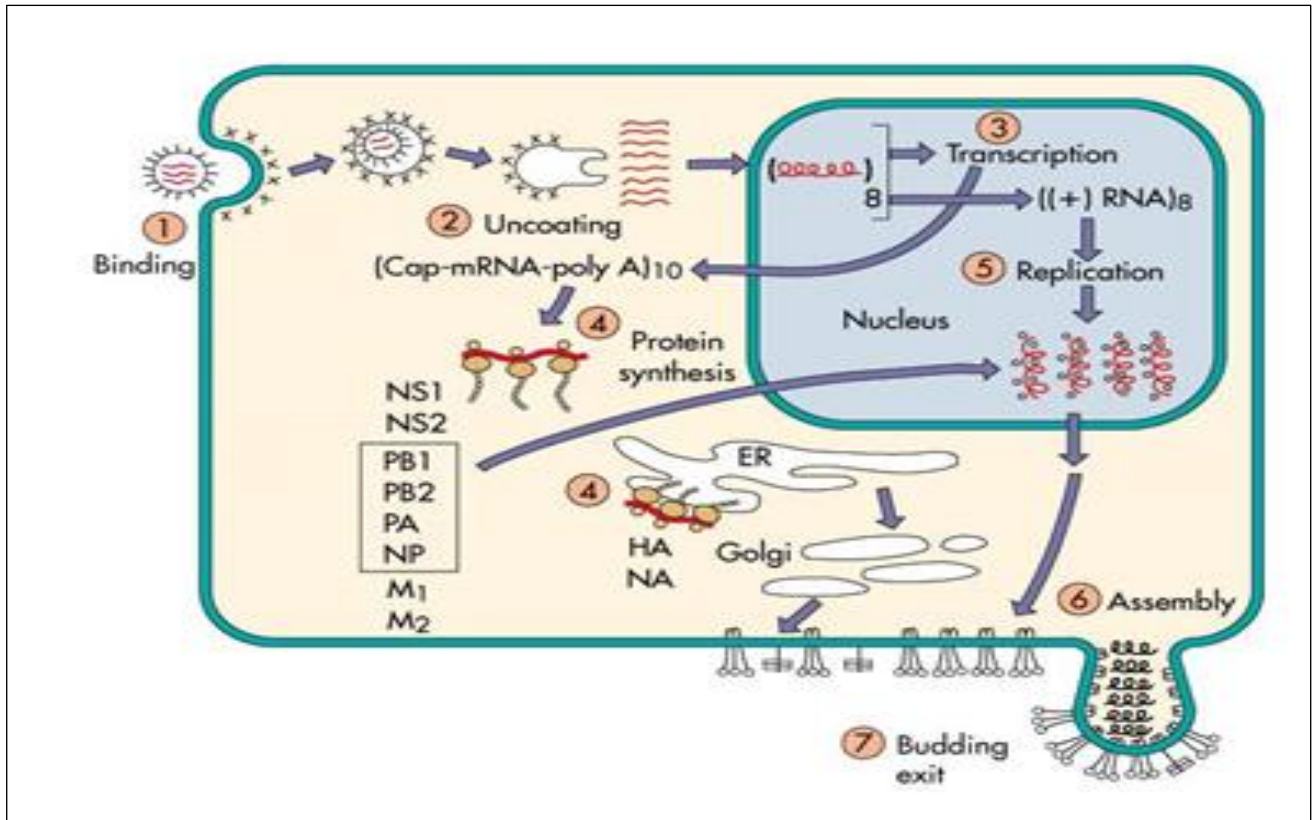
- Required for virus binding to cell surface sialyloglycolipids and sialyloglycoproteins.
 - Responsible for virus penetration.
 - Antibodies to HA neutralize virus.
 - Trimer in envelope.
 - Cleavage to HA1 and HA2 required for infectivity.
 - **Fiviteen HA subtypes**
1. Lowest homology is 25% (H1 and H3)
 2. Highest homology is 80% (H2 and H5)
 3. Less than 10% variation within subtype(Figure 2-44).



Figure(2-44) structural and non-structural coding by influenza virus genome

Neuraminidase

- Removes sialic acid from any glycoconjugate.
- Aids virus spread
- May remove “decoy” receptors on irrelevant cells during infection
- Prevents virus clustering at cell surface upon release
- High concentration of anti-NA antibody are necessary for virus neutralization(Figure 2-45).



Figure(2-45)Influenza replication

Different species harbor different strains of the flu virus:

Table(2-3)and figure(2-46)

- Human flu (Table2-3)
- Bird flu
- Swine flu
-

Table(2-3) Influenza A virus : HA subtypes

Subtype	Human	Swine	Horse	Bird
H1	✓	✓		✓
H2	✓			✓
H3	✓	✓	✓	✓
H4				✓
H5				✓
H6				✓
H7			✓	✓
H8				✓
H9				✓
H10				✓
H11				✓
H12				✓
H13				✓
H14				✓
H15				✓
H16				✓

Subtype	Human	Swine	Horse	Bird
N1				
N2				
N3				
N4				
N5				
N6				
N7				
N8				
N9				

Figure(2-46) NA subtypes in different animal species

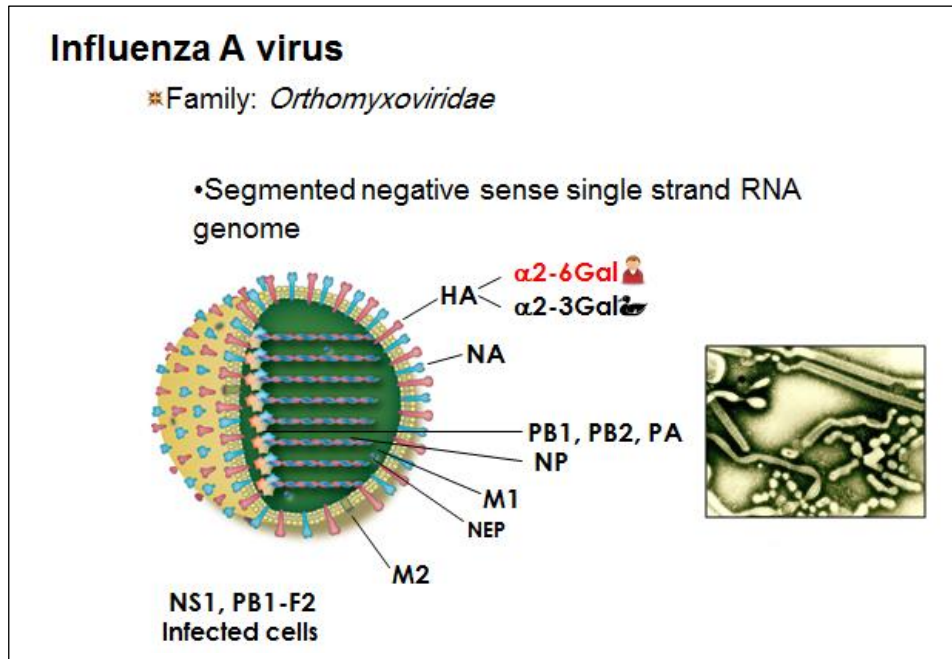


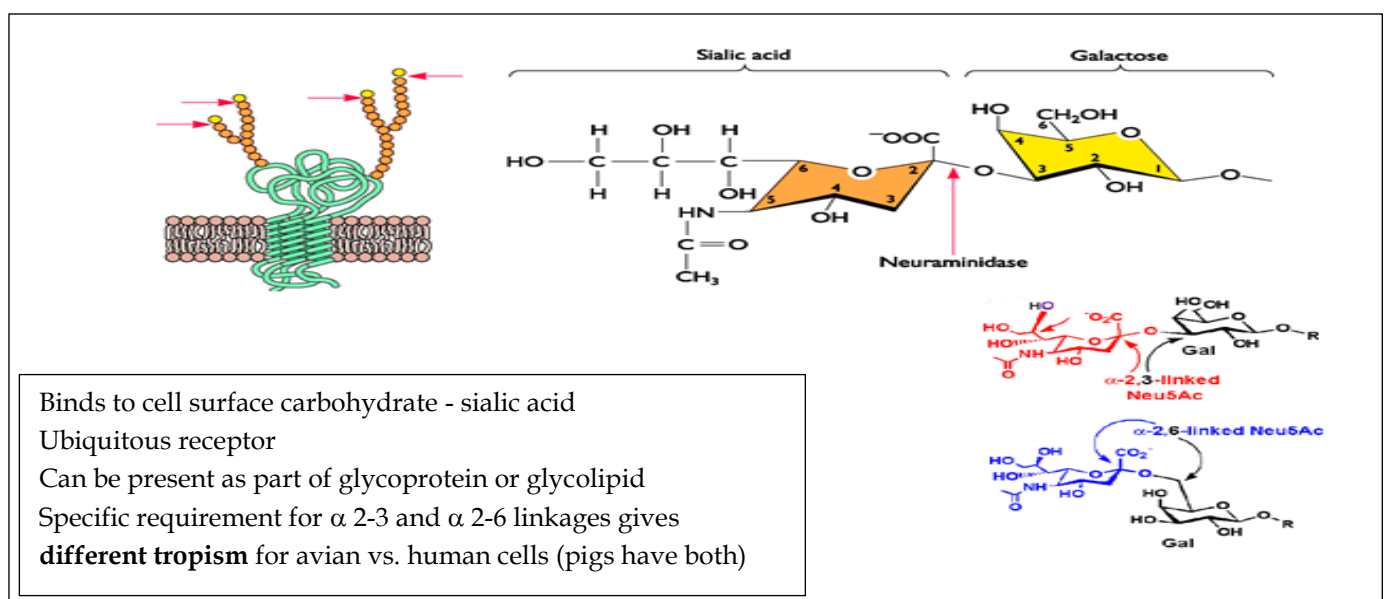
Figure (2-47) Influenza A virus , receptor of AH

Binds to cell surface carbohydrate - sialic acid

Ubiquitous receptor

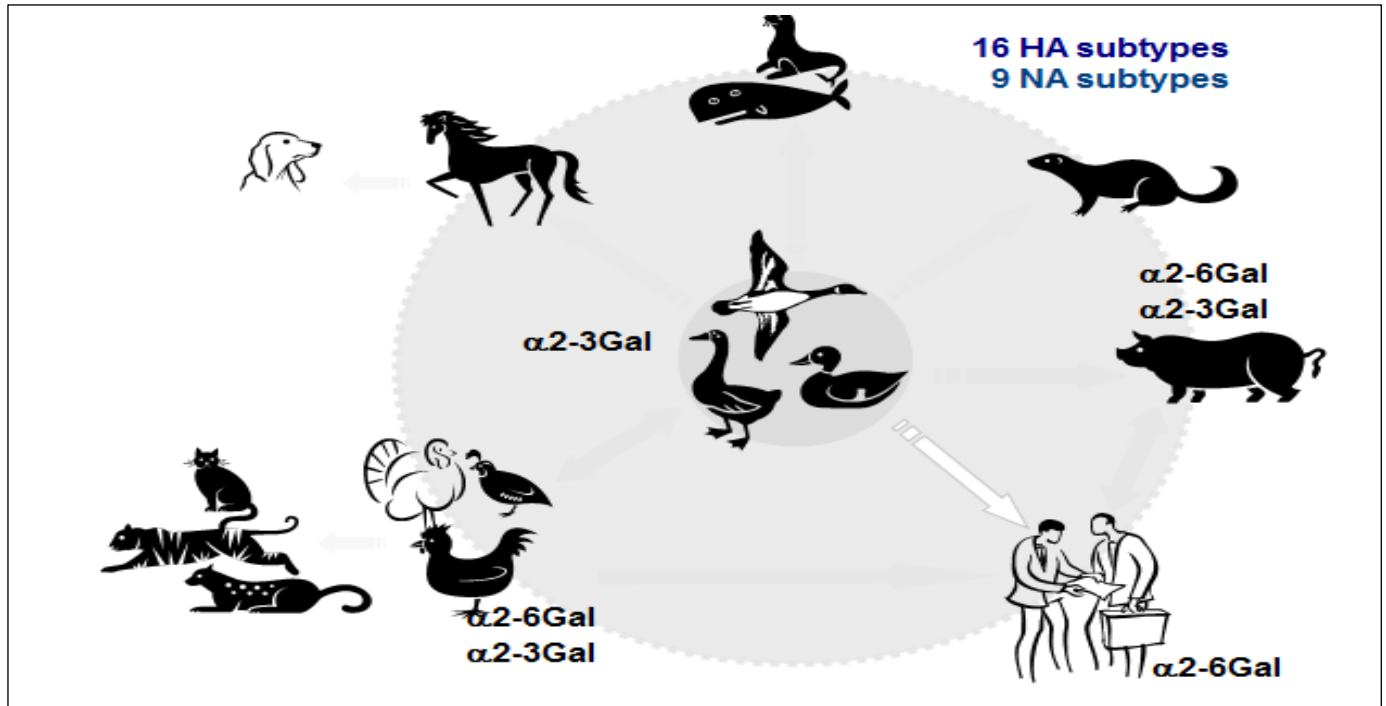
Can be present as part of glycoprotein or glycolipid

Specific requirement for $\alpha 2-3$ and $\alpha 2-6$ linkages gives **different tropism** for avian vs. human cells (pigs have both)Figure(2-48)



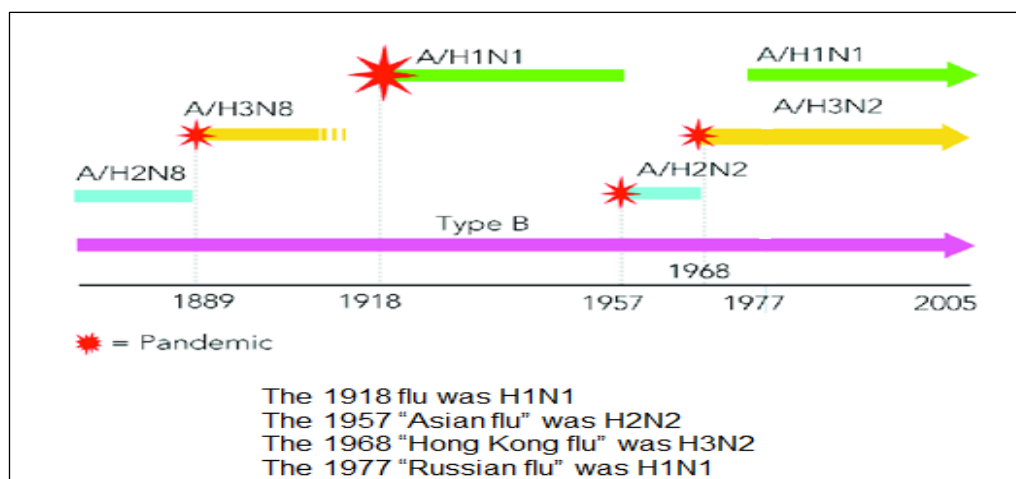
Figure(2-48) Influenza A virus: attachment

Arranged by Sarah Mohssen



Figure(2-49) Type A influenza cannot be eradicated

- On the surface of influenza virus reside two major proteins; Haemagglutinin (HA) and Neuraminidase (NA). Sixteen subtypes of HA (H1 to H16) and nine subtypes of NA (N1 to N9) are recognized in aquatic birds.
- Death mostly occurs as a consequence of primary viral pneumonia or of secondary respiratory bacterial infections, especially in patients with underlying pulmonary or cardiopulmonary diseases which causes death in different outbreak time(Figure2-50).



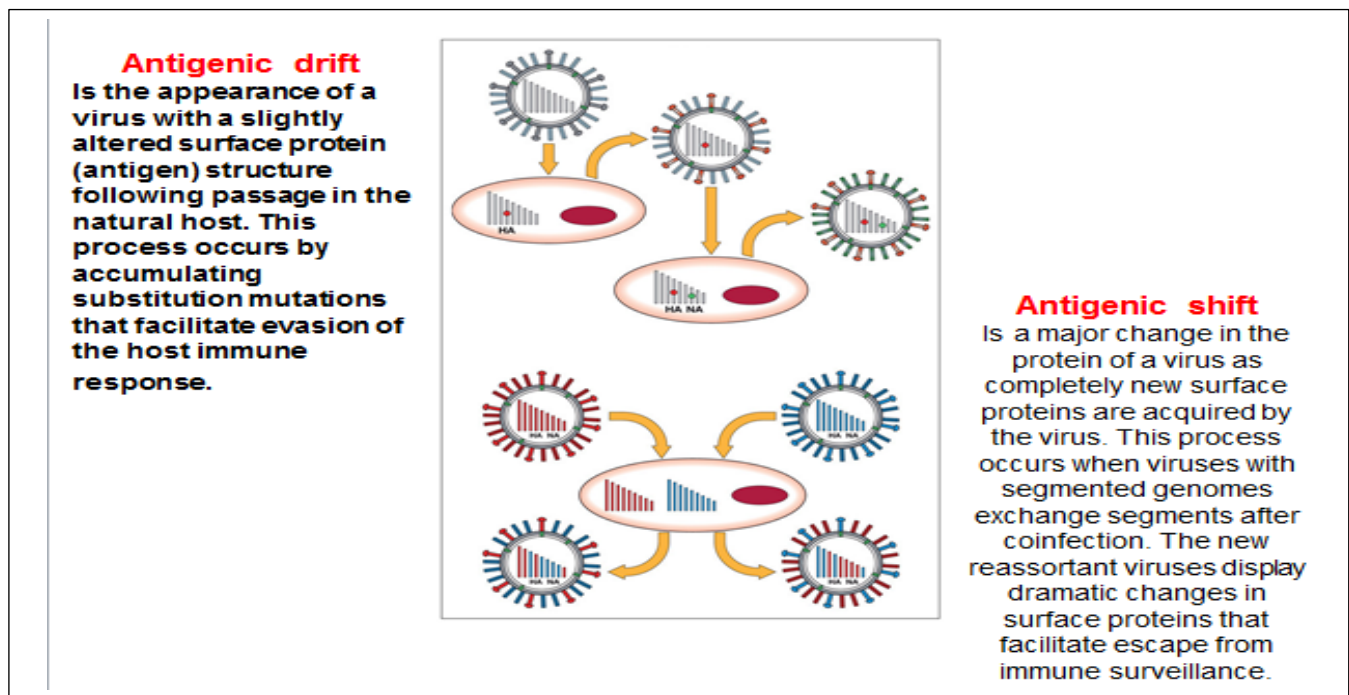
Figure(2-50) Eras of human Influenza A virus

Viruses undergo genetic change by several mechanisms

- Genetic drift where individual bases in the DNA or RNA mutate to other bases.
- Antigenic shift is where there is a major change in the genome of the virus. This occurs as a result of recombination (Figure 2-51).

Seasonal flu/ Pandemic flu

- **Epidemic** (seasonal) influenza which occurs annually and is attributable to minor changes in genes that encode proteins on the surface of circulating influenza viruses. These are known as inter pandemic epidemics.
- **Pandemic** influenza which occurs when more significant changes in the influenza A virus arises when human virus strains acquire genes from influenza viruses of other animal species. When this happens, everyone in the world is susceptible to the new virus, and a worldwide epidemic or pandemic can result.



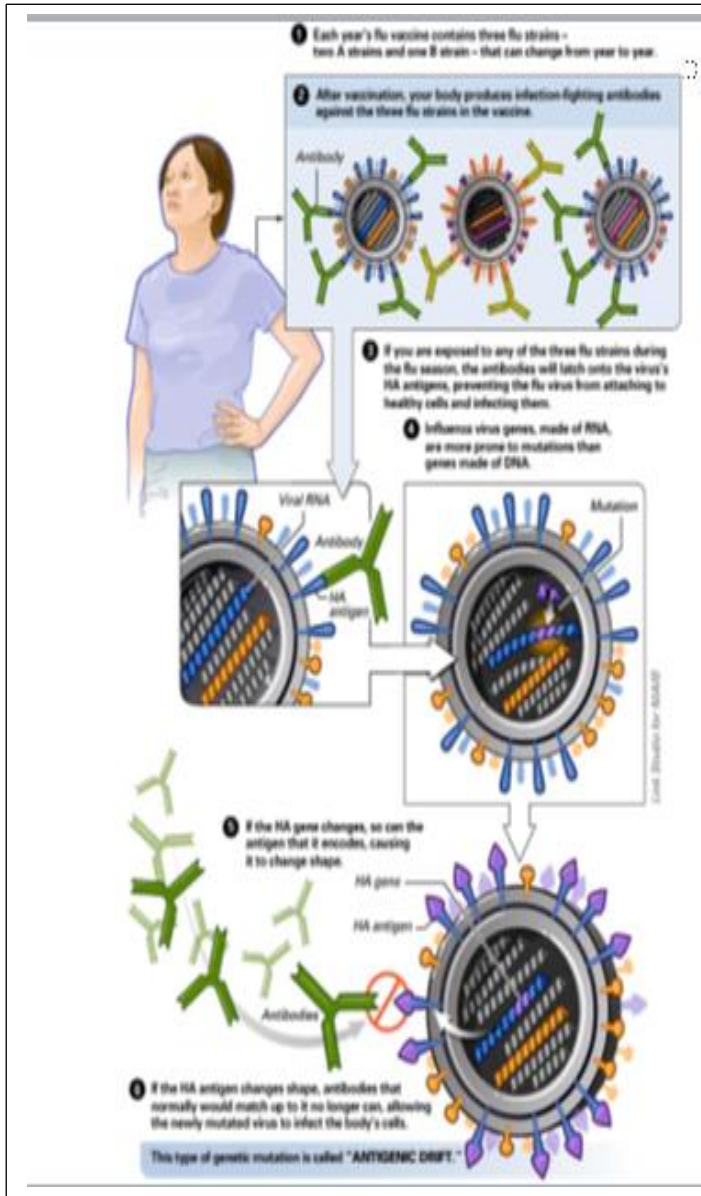
Figure(2-51)Antigenic variation of influenza virus envelope proteins

Reassortment of Gene Segments

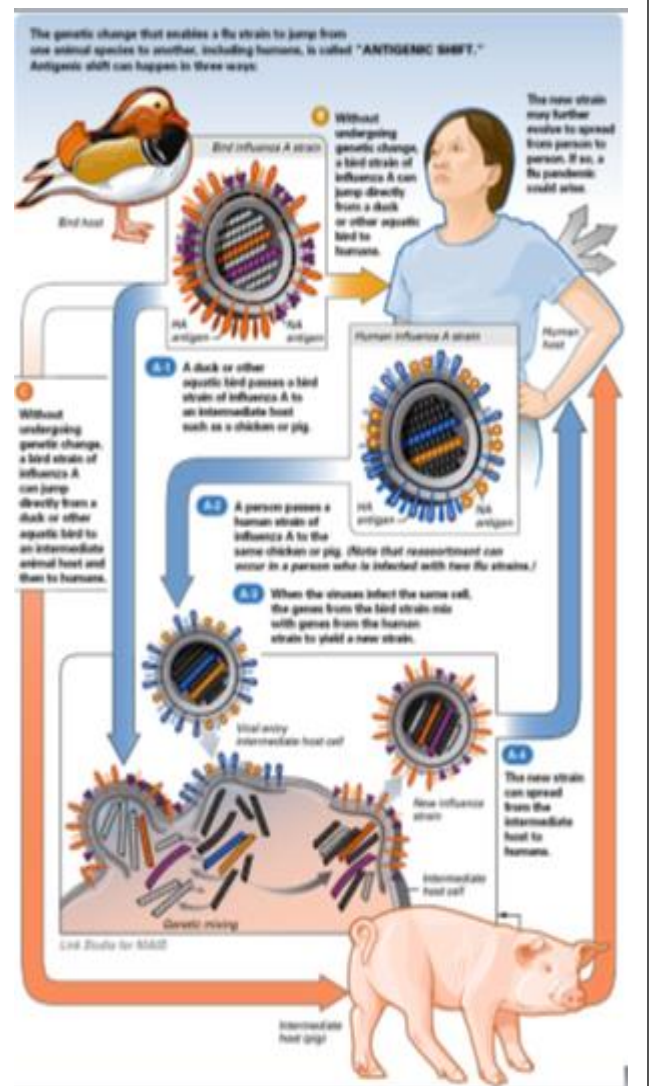
- Influenza has 8 separate gene segments that encode 10 different proteins
- When a host cell is infected with two different influenza viruses, the progeny virus can be a mixture of both “parent” viruses

- Reassortment provides for increased biological variation that increases the ability of the virus to adapt to new hosts (figure 2-51) which result a pandemic influenza (Figure 2-53)

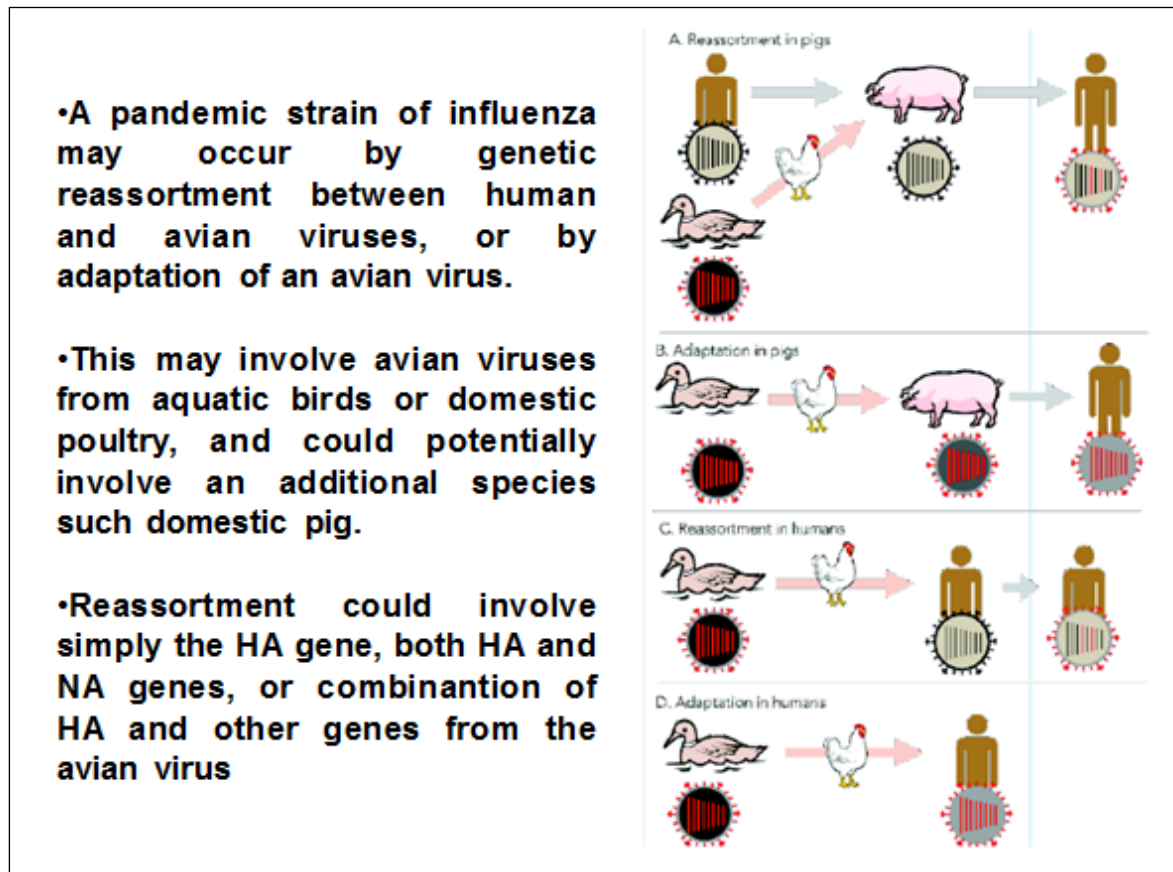
A



B



Figure(2-52)genetic mutation of influenza A-Antigenic drift B-Antigenic shift



Figure(2-53) Generation of pandemic influenza virus strains

Avian Influenza

- Avian influenza is an infectious disease of birds caused by type A strains of the influenza virus.
- These viruses occur naturally among wild aquatic birds worldwide and can infect domestic poultry and other bird and animal species. The disease, which was first identified in Italy more than 100 years ago.
- **Fifteen subtypes** of influenza virus are known to infect birds, thus providing an extensive reservoir of influenza viruses potentially circulating in bird populations.
- **H5N1**; the strain of avian flu known as has been behind outbreaks of deadly avian flu.
- Migratory water birds, especially wild ducks. They may do not show clinical disease. The virus colonizes the intestinal tract and is spread in the feces . They act as a reservoir for the infection of other species .
- Pigs can be infected by bird influenza (as well as by the form of influenza that affects humans) and can pass on the flu to humans.

•

Swine Flu

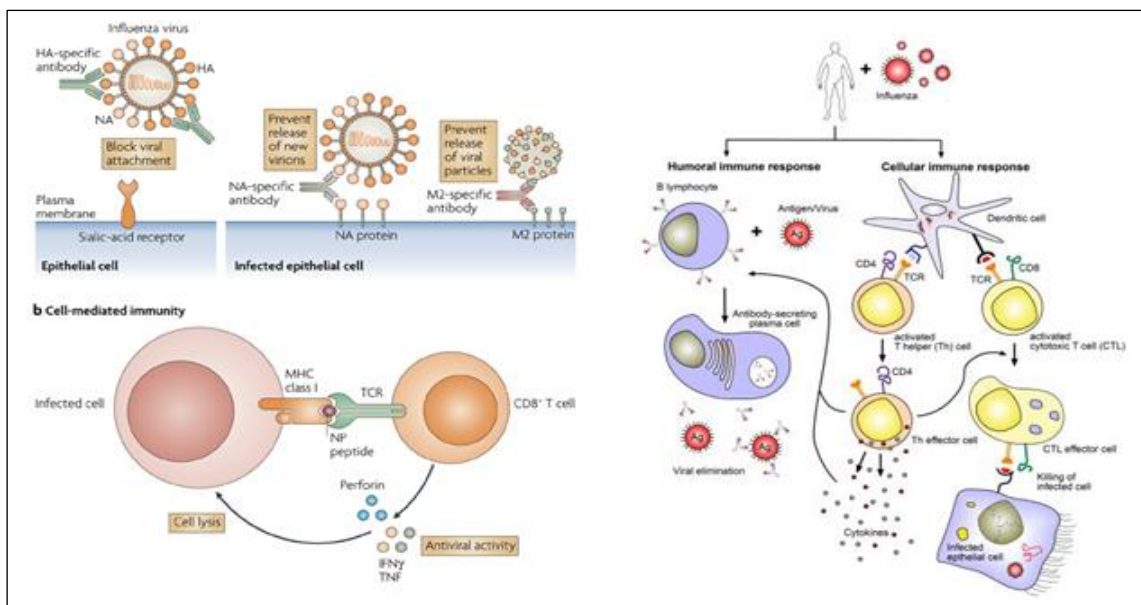
- Influenza in swine was first recognized as an epizootic disease in 1918.
- Swine influenza virus was first isolated from humans in 1974. Serologic evidence of infections with a swine influenza virus in humans has also been obtained. Viruses of swine may be a potential source of epidemic disease for humans.

Avian Influenza

- Avian influenza transmitted by birds usually through feces or saliva.
- Avian influenza is not usually passed on to humans, although it has been contracted by people who have handled infected birds or touched surfaces contaminated by the birds.

Swine Flu

- Swine influenza (swine flu) is a respiratory disease of pigs caused by type A influenza virus that regularly cause outbreaks of influenza in pigs.
- Like human influenza viruses, there are different subtypes and strains of swine influenza viruses. The main swine influenza viruses circulating in U.S. pigs in recent years are: H1N1 influenza virus, H3N2 virus, H1N2 virus.



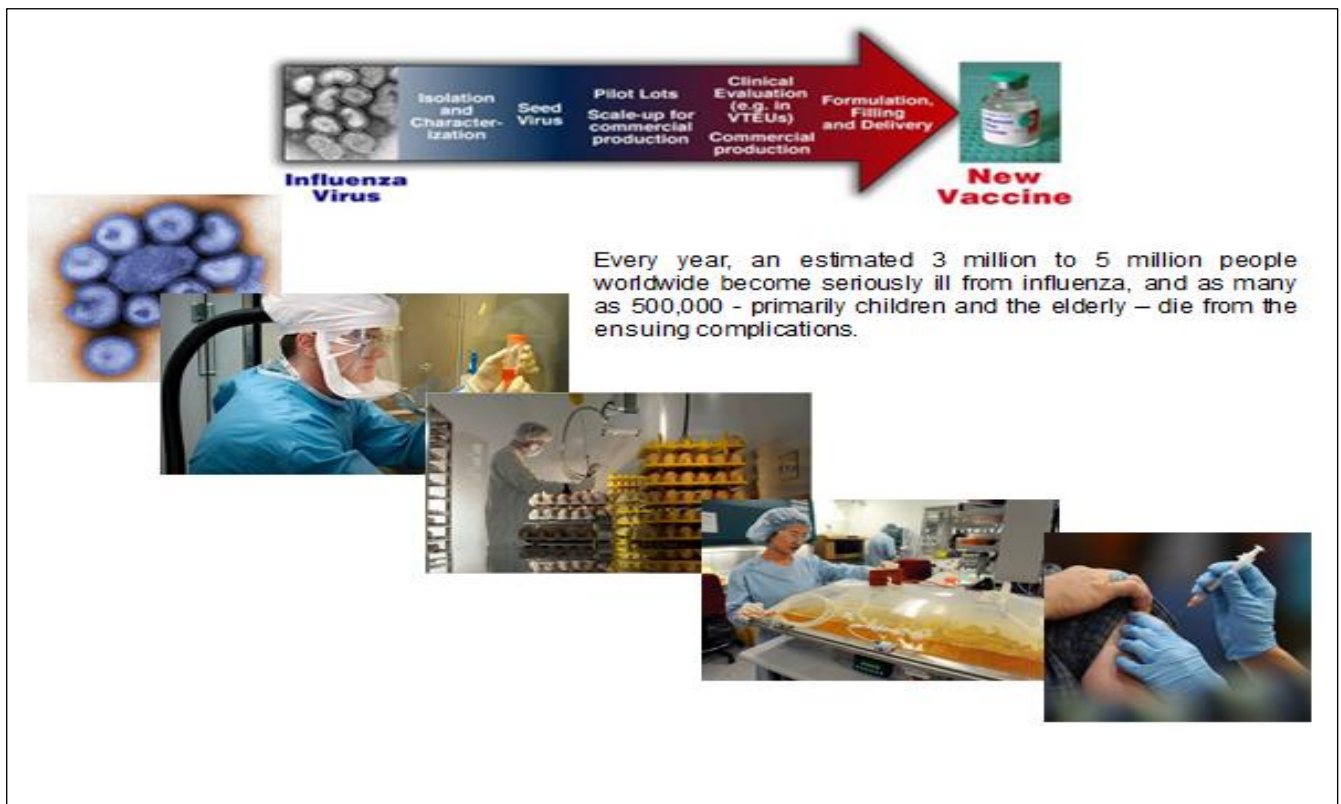
Figure(2-54) Influenza virus: the immune response

Influenza Prevention

- **Vaccination before the start of influenza season**
- Northern Hemisphere: October-November
- Southern Hemisphere: April-May
- **Antiviral treatment**
- Therapeutic
- Prophylactic

Vaccination

- ✓ **Trivalent: two current A strains and one current B strain.**
- **For 2010 season (Figure 2-55)**
- A/California/7/2009 (H1N1)-like virus
- A/Perth/16/2009 (H3N2)-like virus
- B/Brisbane/60/2008-like virus
- ✓ **Formalin fixed “wild type” virus approved for parenterally administered vaccination.**
- ✓ **Live attenuated vaccine (“Flumist”)**
- Temperature sensitive recombinant bearing relevant HA and NA genes.
- ✓ **Must anticipate shift and drift in order to identify appropriate vaccine strain.**



Figure(2-55) General steps for influenza vaccine production

Arranged by Sarah Mohssen

Hepatitis B Virus

Hepatitis (Liver-Attacking) Viruses

Hepatitis A – fecal/oral, contaminated food, vaccine available

Hepatitis B – blood, semen, vertical (mother-child), vaccine available

Hepatitis C – blood (IV drug use, transfusion, organ donation, unsterile injecting equipment, sexual intercourse)

Hepatitis D – survives only in cells co-infected with hepatitis B

Hepatitis E* – contaminated food or water, fecal/oral (Table 2-3)

*causes short-term disease and is not a chronic carrier state

Characteristics of Hepatitis Viruses

<u>Virus</u>	<u>Nucleic Acid</u>	<u>Routes of Transmission</u>	<u>Mortality</u>	<u>Risk of Chronic Illness</u>
HAV	Unenveloped single-stranded RNA	Fecal-oral	Low	None
HBV	Enveloped double-stranded DNA	Parenteral (sex, perinatal)	Moderate-high	High
HCV	Enveloped single-stranded RNA	Parenteral (sex, perinatal)	Moderate-high	High
HDV	Enveloped single-stranded RNA	Parenteral (sex)	High	High
HEV	Unenveloped single-stranded RNA	Fecal-oral	Low-moderate	None

Nelson KE, Thomas DL. Viral hepatitis. In *Infectious Disease Epidemiology*, 2nd ed., Nelson KE, Williams CM (eds). Jones & Bartlett, Sudbury MA, 2007; p. 898.

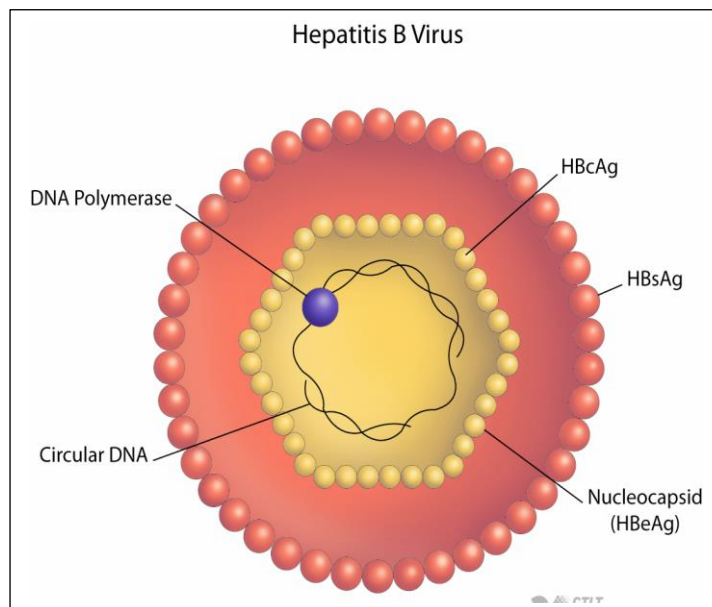
Table (2-3) Characteristics of hepatitis viruses

Hepatitis B

- Hepatitis B is caused by infection with the Hepatitis B virus (HBV), the prototype member of the hepadnavirus family
- It has a circular DNA genome of 3.2 kb (Figure 2-56)
- Currently, eight genotypes (A-H) are identified by a divergence of >8% in the entire genome (Figure 2-57)

Hepatitis B Characteristics

- A Hepadnaviridae – partially double-stranded DNA virus
- HBsAg – stimulates protective antibodies, a marker for current infection
- HBcAg – localized within liver cells, identifies acute infection, anti-HBcAg persists for life and is a marker of past infection
- HBeAg – a marker of active replication and infectivity



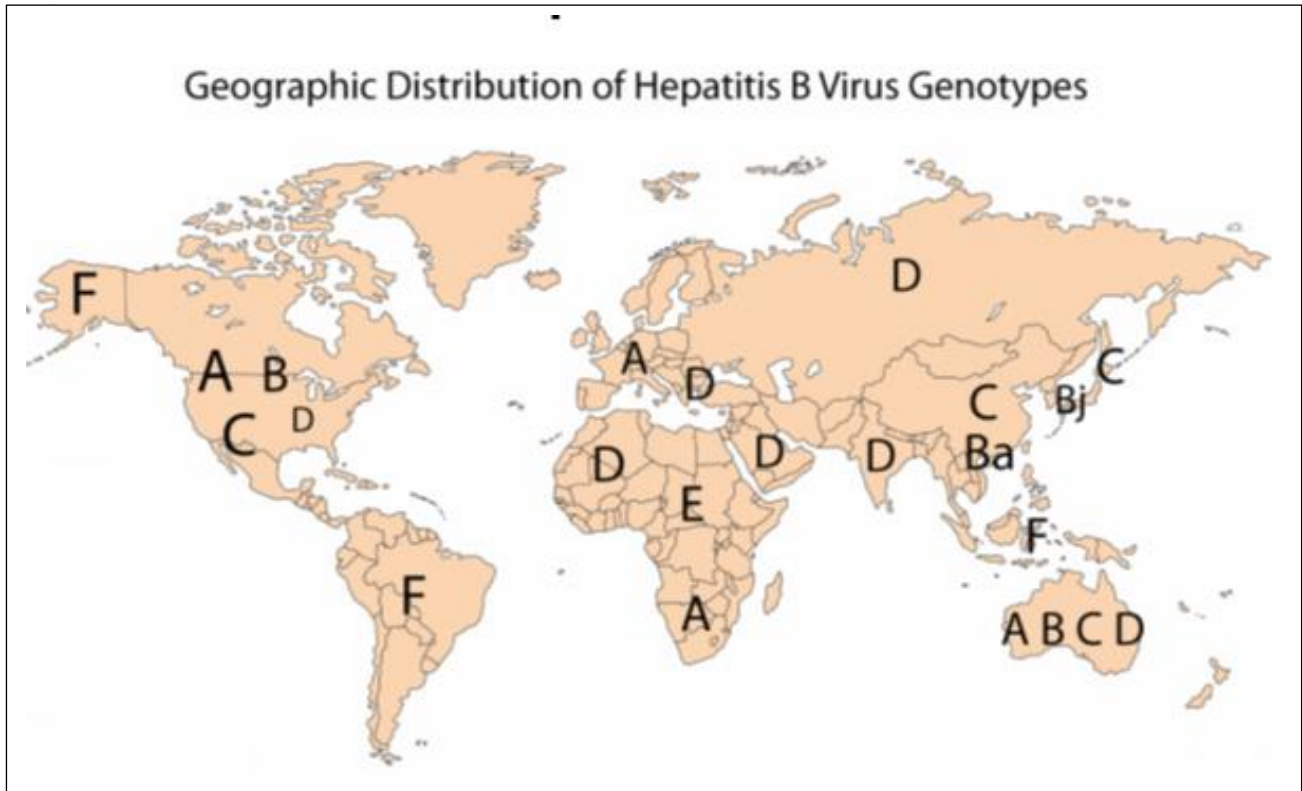
Figure(2-56)Hepatitis B

Hepatitis B Virus :

- Hepadnaviridae family (DNA)
- Numerous antigenic components
- Humans are only known hosts
- May retain infectivity for more than 7 days at room temperature

Hepatitis B virus infection :

- More than 350 million chronically infected worldwide
- Established cause of chronic hepatitis and cirrhosis
- Human carcinogen – cause of up to 80% of hepatocellular carcinomas
- More than 600,000 deaths worldwide in 2002



Figure(2-57) Geographic distribution of Hepatitis virus

Epidemiology

- **Worldwide, HBV is the primary cause of liver cancer**
1. For males, it is the third leading cause of cancer mortality
 2. For females, it is the sixth leading cause of cancer mortality

Epidemiology

- The incubation period from the time of exposure to onset of symptoms is 6 weeks to 6 months.
- HBV is found in highest concentrations in blood and in lower concentrations in other body fluids (e.g., semen, vaginal secretions, and wound exudates).

- HBV infection can be self-limited or chronic.
- In adults, only approximately half of newly acquired HBV infections are symptomatic, and approximately 1% of reported cases result in acute liver failure and death.

Diagnosis

- Hepatitis B is detected by looking for a number of different antigens and antibodies:

Hepatitis B surface antigen (HBsAg):

- A protein on the surface of HBV; it can be detected in high levels in serum during acute or chronic HBV infection.
- The presence of HBsAg indicates that the person is infectious.
- The body normally produces antibodies to HBsAg as part of the normal immune response to infection.
- HBsAg is the antigen used to make Hepatitis B vaccine.

Hepatitis B is detected by looking for a number of different antigens and antibodies:

Hepatitis B surface antibody (anti-HBs):

- The presence of anti-HBs is generally interpreted as indicating recovery and immunity from HBV infection.
- Anti-HBs also develops in a person who has been successfully vaccinated against Hepatitis B.

Total Hepatitis B core antibody (anti-HBc):

- Appears at the onset of symptoms in acute Hepatitis B and persists for life.
- The presence of anti-HBc indicates previous or ongoing infection with HBV in an undefined time frame.

Hepatitis B is detected by looking for a number of different antigens and antibodies:

IgM antibody to Hepatitis B core antigen (IgM anti-HBc):

- Positivity indicates recent infection with HBV (≤ 6 months).
- Its presence indicates acute infection.

Hepatitis B e antigen (HBeAg):

- A secreted product of the nucleocapsid gene of HBV that is found in serum during acute and chronic Hepatitis B.
- Its presence indicates that the virus is replicating and the infected person has high levels of HBV.

Hepatitis B is detected by looking for a number of different antigens and antibodies: (Table 2-4)

Hepatitis B e antibody (HBeAb or anti-HBe):

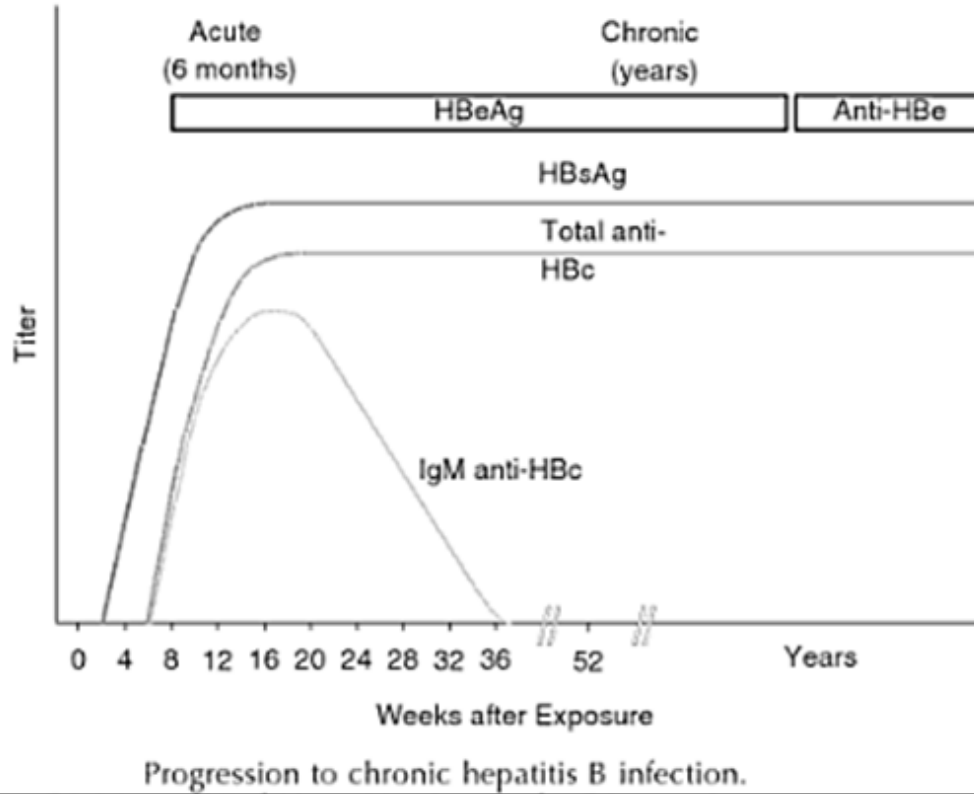
- Produced by the immune system temporarily during acute HBV infection or consistently during or after a burst in viral replication.
- Spontaneous conversion from e antigen to e antibody (a change known as seroconversion) is a predictor of long-term clearance of HBV in patients undergoing antiviral therapy and indicates lower levels of HBV. (Figure 2-58)

Table (2-4) Hepatitis B

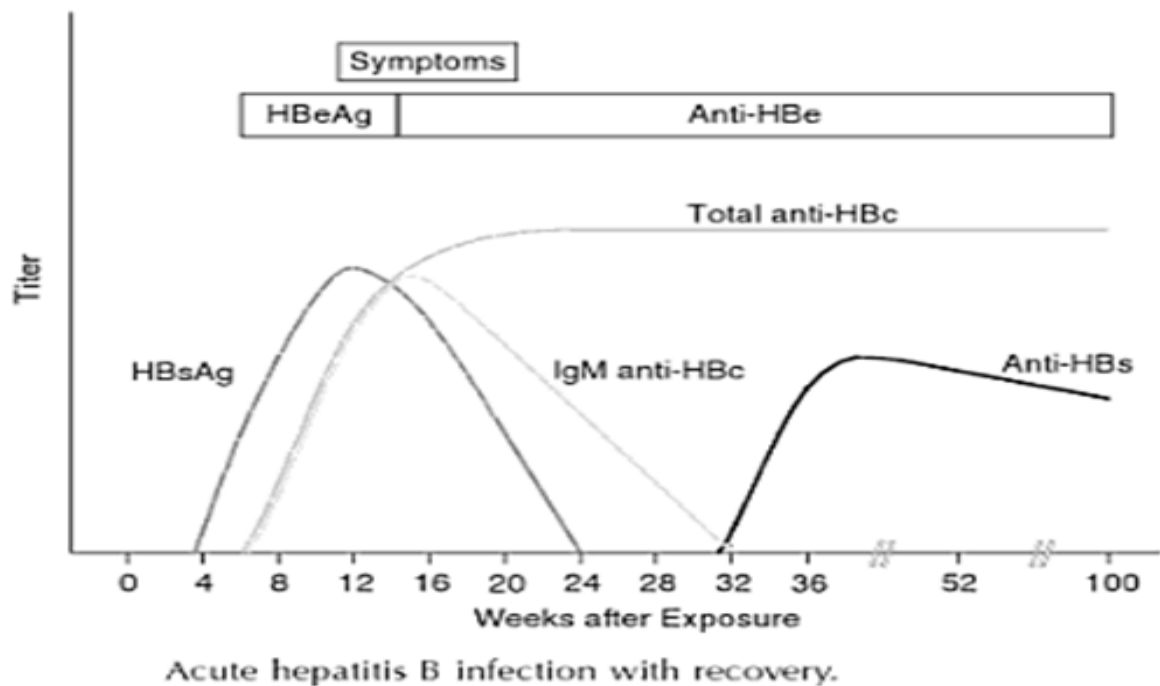
Typical interpretation of serologic test results for hepatitis B virus infection

Serologic Marker				Interpretation
HBsAg ¹	Total anti-HBc ²	IgM ³ anti-HBc	Anti-Hbs ⁴	
– ⁵	–	–	–	Never infected
+ ^{6,7}	–	–	–	Early acute infection; transient (up to 18 days) after vaccination
+	+	+	–	Acute infection
–	+	+	+ or –	Acute resolving infection
–	+	–	+	Recovered from past infection and immune
+	+	–	–	Chronic infection
–	+	–	–	False-positive (i.e., susceptible); past infection; "low-level" chronic infection; ⁸ or passive transfer of anti-HBc to infant born to HBsAg-positive mother
–	–	–	+	Immune if concentration is ≥10 mIU/mL after vaccine series completion; ⁹ passive transfer after hepatitis B immune globulin administration

A



B



Figure(2-58)Immune response of hepatitis B virus A-Chronic hepatitis B , B-Acute of hepatitis B virus

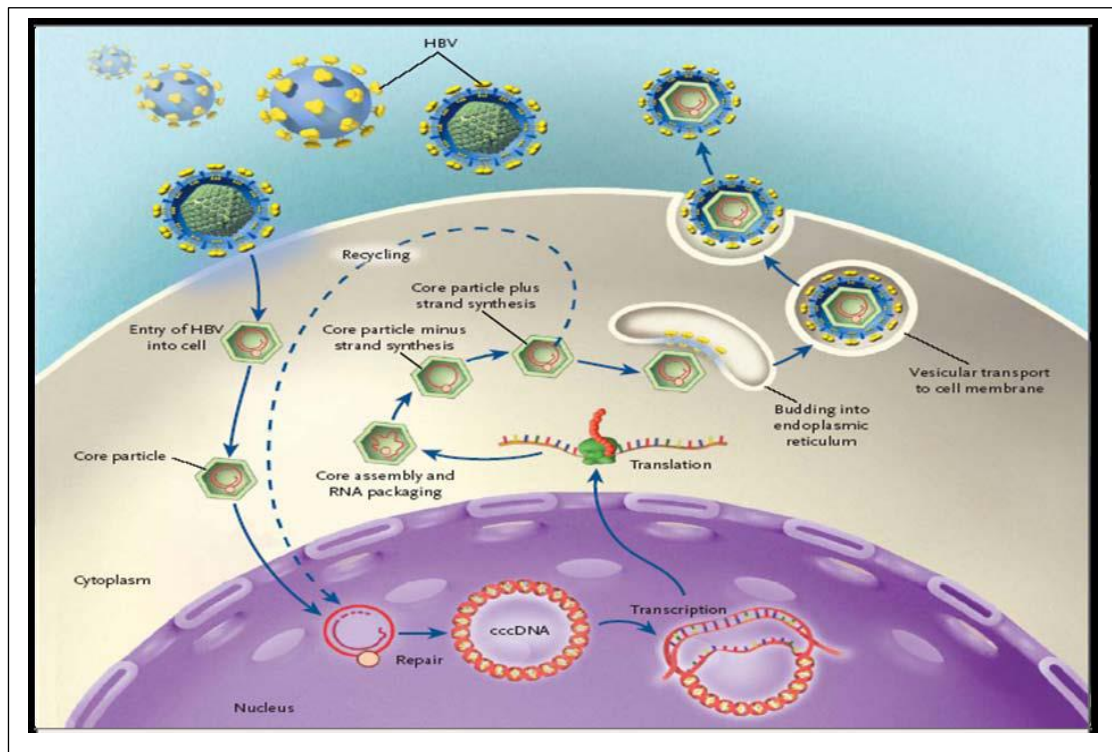


Figure (2-59) Hepatitis B virus multiplication

Hepatitis B complication :

- Fulminant hepatitis
- Hospitalization
- Cirrhosis
- Hepatocellular carcinoma
- Death

Chronic Hepatitis B virus infection :

- Chronic viremia(Figure 2-59)
- Responsible for most mortality
- Over risk 5% (Figure 2-60)
- Higher risk with early infection

Hepatitis B perinatal transmission :

- **If mother positive for HBsAg and HBeAg**
- 70%-90% of infected

- 90% of infected infants become chronically infected
- **If positive for HBsAg only**
- 5% - 20% of infants infected
- 90% of infected infants become chronically infected

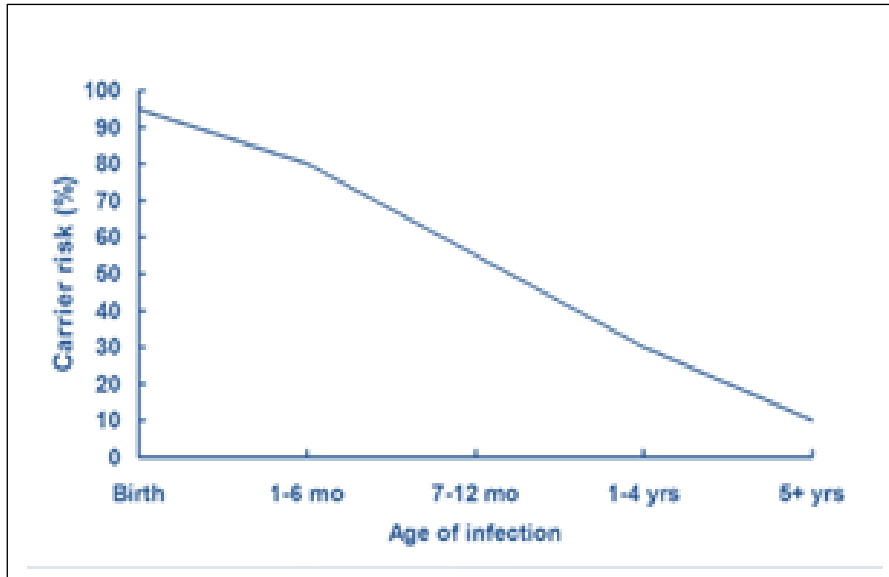


Figure (2-60) Risk of chronic HBV carriage by age of infection

Global patterns of chronic HBV infection :

- **High (> 8%) : 45% of global population**
 - Lifetime risk of infection >60%
 - Early childhood infections common
- **Intermediate (2%-7%):43% of global population**
 - Lifetime risk of infection 20%-60%
 - Infections occur in all age groups
- **Low(<20%):12% of global population**
 - Lifetime risk of infection <20%
 - Most infections occur in adult risk groups

Adult at risk for HBV infection :

- **Sexual exposure**
 - Sex partners of HBsAg-positive persons
 - Sexual active persons not in a long – term , mutually monogamous relationship.
 - Persons seeking evaluation or treatment for a sexually transmitted disease.

- Men who have sex with men.

adults at Risk for HBV Infection

- **percutaneous or mucosal exposure to blood**
- current for recent IDU
- household contacts of HBsAg-positive person
- residents and staff of facilities for development disabled persons.
- Healthcare and public safety workers with risk for exposure to blood or blood-contaminated body fluids.
- Persons with end-stage renal disease
- Persons with diabetes mellitus.

Hepatitis B Treatment

- For acute infection, no medication is available; treatment is supportive.
- For chronic infection, several antiviral drugs (adefovir dipivoxil, interferon alfa-2b, pegylated interferon alfa-2a, lamivudine, entecavir, and telbivudine) are available.
- Persons with chronic HBV infection require medical evaluation and regular monitoring to determine whether disease is progressing and to identify liver damage or hepatocellular carcinoma.

Hepatitis B Elimination

- ✓ CDC's national strategy to eliminate transmission of HBV infection includes:
- 1. Prevention of perinatal infection through routine screening of all pregnant women for HBsAg and immunoprophylaxis of infants born to HBsAg-positive mothers and infants born to mothers with unknown HBsAg status
- 2. Routine infant vaccination
- 3. Vaccination of previously unvaccinated children and adolescents through age 18 years
- 4. Vaccination of previously unvaccinated adults at increased risk for infection

Hepatitis B Vaccine

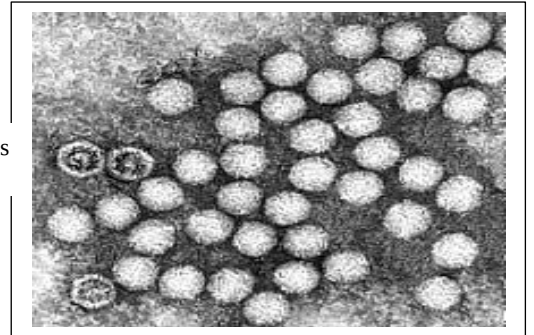
- Composition Recombinant HBsAg
- Efficacy 95% (Range , 80%-100%

- Duration of Immunity 20 years or more
- Schedule 3 Doses
- Booster doses not routinely recommended

Enteroviruses

- Enteroviruses are a genus of the picornavirus family which replicate mainly in the gut.(Figure2-61)
- Single stranded naked RNA virus with icosahedral symmetry
- Unlike rhinoviruses, they are stable in acid pH
- Capsid has 60 copies each of 4 proteins, VP1, VP2, VP3 and VP4 arranged with icosahedral symmetry around a positive sense genome.
- At least 71 serotypes are known: divided into 5 groups
 1. Polioviruses
 2. Coxsackie A viruses
 3. Coxsackie B viruses
 4. Echoviruses
 5. Enteroviruses (more recently, new enteroviruses subtype have been allocated sequential numbers (68-71) Figure(2-62)

Figure(2-61)Enterovirus Particles

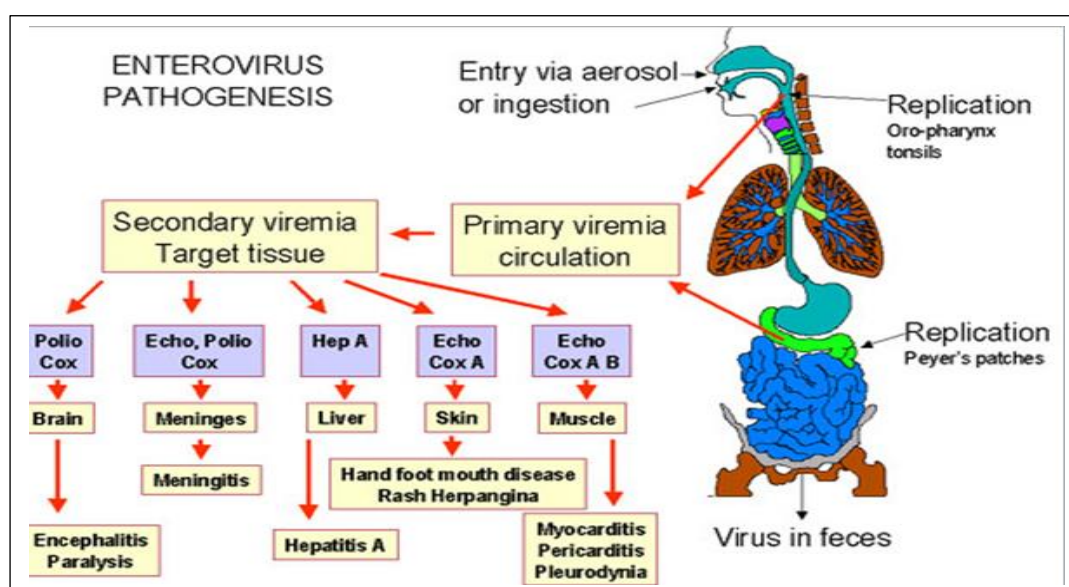


History

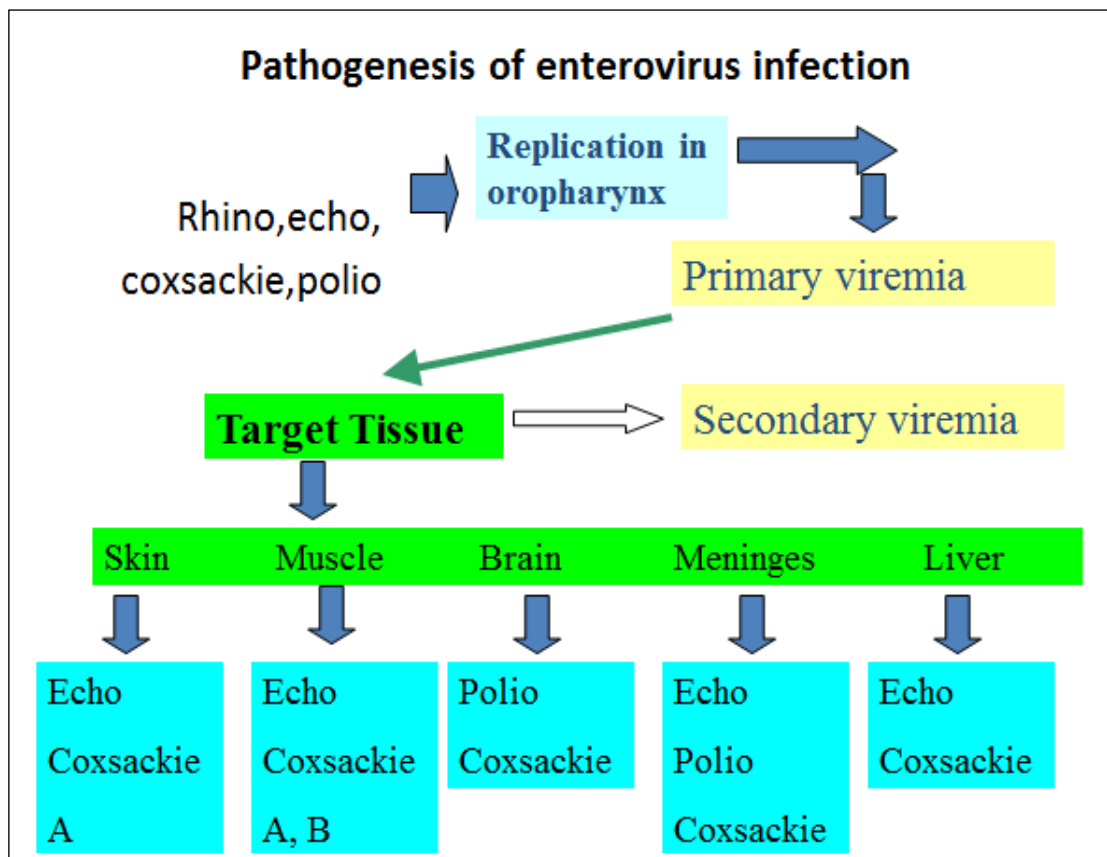
- **Poliovirus** - first identified in 1909 by inoculation of specimens into monkeys. The virus was first grown in cell culture in 1949 which became the basis for vaccines.
- **Coxsackieviruses** - In 1948, a new group of agents were identified by inoculation into newborn mice from two children with paralytic disease. These agents were named coxsackieviruses after the town in New York State. Coxsackieviruses A and B were identified on the basis of the histopathological changes they produced in Newborn mice and their capacity to grow in cell cultures.
- **Echoviruses** - were later identified which produced cytopathic changes in cell culture and was nonpathogenic for newborn mice and subhuman primates.
- More recently, new enterovirus types have been allocated sequential numbers (68 - 71).

Virus	Serotypes	Clinical Diseases
Polioviruses	3 types	Asymptomatic infection, viral meningitis, paraalytic disease, poliomyelitis
Coxsackie A viruses	23 types (A1-A22, A24)	Viral meningitis plus, rash, ARD, myocarditis, orchitis
Coxsackie B viruses	types (B1-B6)	Viral meningitis, but no orchitis
Echoviruses	32 types	Viral meningitis, with orchitis
Other Enteroviruses	4 types(68-71)	Viral meningitis

Table(2-5) Categories of Enteroviruses



Figure(2-62)



Figure(2-63)Pathogenesis of enterovirus infection

Poliovirus

- 3 serotypes of poliovirus (1, 2, and 3) but no common antigen.
- Have identical physical properties but only share 36-52% nucleotide homology.
- Humans are the only susceptible hosts.
- Polioviruses are distributed globally. Before the availability of immunization, almost 100% of the population in developing countries before the age of 5.
- The availability of immunization and the poliovirus eradication campaign has eradicated poliovirus in most regions of the world except in the Indian Subcontinent and Africa.
- Poliovirus is on course of being eradicated worldwide by the end of 2000 or 2001.

Pathogenesis

The incubation period is usually 7 - 14 days.

Following ingestion, the virus multiplies in the oropharyngeal and intestinal mucosa.

The lymphatic system, in particular the tonsils and the Peyer's patches of the ileum are invaded and the virus enters the blood resulting in a transient viraemia.

In a minority of cases, the virus may involve the CNS following dissemination. (Figure 2-63)

A



B



C



Figure (2-64) Population infection A-Indene children with poliomyelitis B-outbreak au early 20th country C- Evidence of poliomyelitis in the pharaohs

Born in 1882 at Hyde Park, New York--now a national historic site--he attended Harvard University and Columbia Law School. On St. Patrick's Day, 1905, he married Eleanor Roosevelt .

Following the example of his fifth cousin, President Theodore Roosevelt, whom he greatly admired, Franklin D. Roosevelt entered public service through politics, but as a Democrat. He won election to the New York Senate in 1910. President Wilson appointed him Assistant Secretary of the Navy, and he was the Democratic nominee for Vice President in 1920 .

In the summer of 1921, when he was 39, disaster hit--he was stricken with poliomyelitis. Demonstrating indomitable courage, he fought to regain the use of his legs, particularly through swimming. At the 1924 Democratic Convention he dramatically appeared on crutches to nominate Alfred E. Smith as "the Happy Warrior." In 1928 Roosevelt became Governor of New York .

He was elected President in November 1932, to the first of four terms.(Figure2-65)



Figure(2-65)Franklin D. Roosevelt

Immunity

- Antibody is the major protective immune response to the enteroviruses . Secretory antibody can prevent the initial establishment of infection in the oropharynx and gastrointestinal tract, and serum antibody prevents viremic spread to the target tissue and therefore disease.
- Cell-mediated immunity is not usually involved in protection but may play a role in pathogenesis.

Clinical Manifestations:

There are 3 possible outcomes of infection:

1. **Subclinical infection (90 - 95%)** - inapparent subclinical infection account for the vast majority of poliovirus infections.
2. **Abortive infection (4 - 8%)** - a minor influenza-like illness occurs, recovery occurs within a few days and the diagnosis can only be made by the laboratory. The minor illness may be accompanied by aseptic meningitis
3. **Major illness (1 - 2%)** - the major illness may present 2 - 3 days following the minor illness or without any preceding minor illness. Signs of aseptic meningitis are common. Involvement of the anterior horn cells lead to flaccid paralysis. Involvement of the medulla may lead to respiratory paralysis and death.

Laboratory Diagnosis

- **Virus Isolation**
 - a. Mainstay of diagnosis of poliovirus infection
 - b. poliovirus can be readily isolated from throat swabs, faeces, and rectal swabs. It is rarely isolated from the CSF
 - c. Can be readily grown and identified in cell culture
 - d. Requires molecular techniques to differentiate between the wild type and the vaccine type.
- **Serology**
 - Very rarely used for diagnosis since cell culture is efficient. Occasionally used for immune status screening for immunocompromised individuals.

Prevention (1)

No specific antiviral therapy is available. However the disease may be prevented through vaccination. There are two vaccines available.

Intramuscular Poliovirus Vaccine (IPV)

consists of formalin inactivated virus of all 3 poliovirus serotypes.

Produces serum antibodies only: does not induce local immunity and thus will not prevent local infection of the gut.

However, it will prevent paralytic poliomyelitis since viraemia is essential for the pathogenesis of the disease.

Oral Poliovirus Vaccine (OPV)

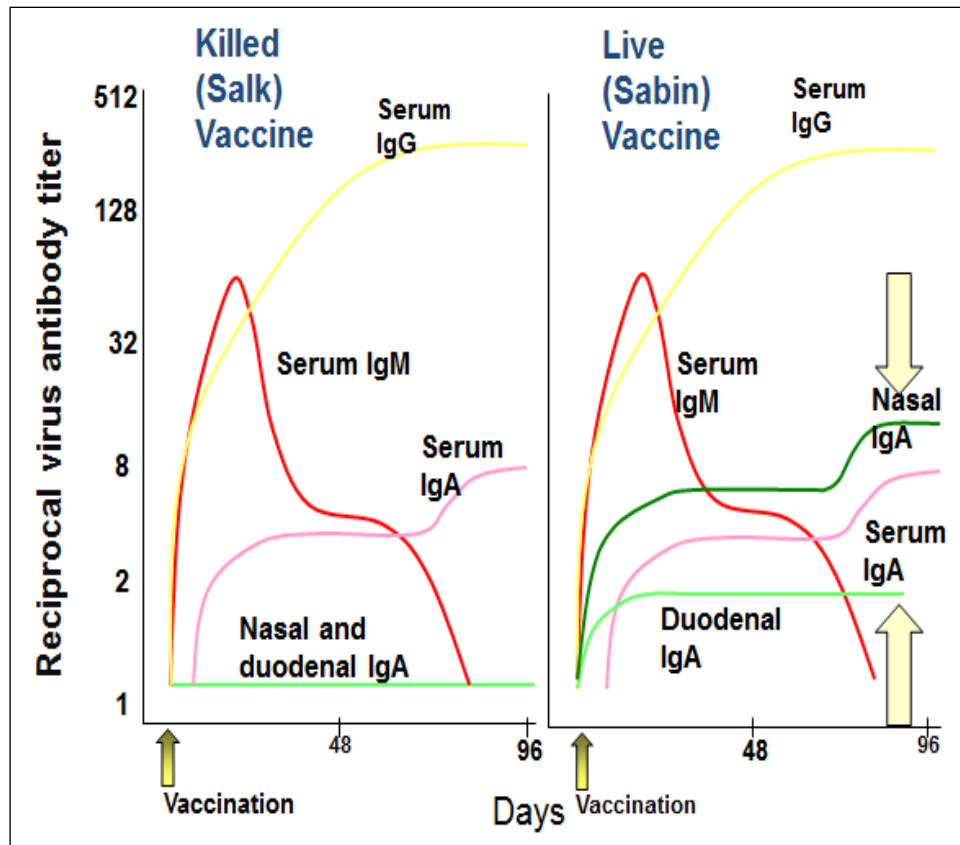
Consists of live attenuated virus of all 3 serotypes.

Produces local immunity through the induction of an IgA response as well as systemic immunity.

Rarely causes paralytic poliomyelitis, around 1 in 3 million doses.

Prevention (2)

- Most countries use OPV because of its ability to induce local immunity and also it is much cheaper to produce than IPV.
- The normal response rate to OPV is close to 100%. (Figure 2-64)
- OPV is used for the WHO poliovirus eradication campaign.
- Because of the slight risk of paralytic poliomyelitis, some Scandinavian countries have reverted to using IPV. Because of the lack of local immunity, small community outbreaks of poliovirus infections have been reported.
- Poliovirus was targeted for eradication by the WHO by the end of year 2000 (now 2005). To this end, an extensive monitoring network had been set up.
- Poliovirus has been eradicated from most regions of the world except the Indian subcontinent and sub-Saharan Africa. It is possible that the WHO target may be achieved.



Figure(2-64)The differences of immune response between OPV and IPV vaccination

Coxsackieviruses

- Coxsackieviruses are distinguished from other enteroviruses by their pathogenicity for suckling rather than adult mice. They are divided into 2 groups on the basis of the lesions observed in suckling mice.
- Group A viruses produce a diffuse myositis with acute inflammation and necrosis of fibers of voluntary muscles.
- Group B viruses produce focal areas of degeneration in the brain, necrosis in the skeletal muscles, and inflammatory changes in the dorsal fat pads, the pancreas and occasionally the myocardium.
- Each of the 23 group A and 6 group B coxsackieviruses have a type specific antigen.
- In addition, all from group B and one from group A (A9) share a group Ag. Cross-reactivities have also been demonstrated between several group A viruses but no common group antigen has been found.

Echoviruses

- The first echoviruses were accidentally discovered in human faeces, unassociated with human disease during epidemiological studies of polioviruses. The viruses were named echoviruses (enteric, cytopathic, human, orphan viruses).
- These viruses were produced CPE in cell cultures, but did not induce detectable pathological lesions in suckling mice.
- Altogether, There are 32 echoviruses (types 1-34; echovirus 10 and 28 were found to be other viruses and thus the numbers are unused)
- There is no group echovirus Ag but heterotypic cross-reactions occur between a few pairs.

New Enteroviruses

- Newly identified picornaviruses that are not polioviruses are no longer classified separated into the species coxsackie and echovirus because of the ambiguities presented by overlapping host range variations.
- 4 new enteroviruses have been identified (68 - 72). Enterovirus 70 is the causative agent epidemics of acute haemorrhagic conjunctivitis that swept through Africa, Asia, India and Europe from 1969 to 1974. The virus is occasionally neurovirulent.

- Enterovirus 71 appears to be highly pathogenic and has been associated with epidemics of a variety of acute diseases, including aseptic meningitis, encephalitis, paralytic poliomyelitis-like disease and hand-foot-mouth disease.
- Enterovirus 72 was originally assigned to hepatitis A virus, but it had now been assigned to a new family called heptoviruses.

Disease Associations (1)

- **Paralytic Disease** - most commonly associated with polioviruses but other enteroviruses may also be responsible, notably enterovirus 71
- **Meningitis** - caused by all groups of enteroviruses, most commonly seen in children under 5 years of age.
- **Encephalitis** - focal or generalized encephalitis may accompany meningitis. Most patients recover completely with no neurological deficit.
- **Undifferentiated febrile illness** - may be seen with all groups of enteroviruses.
- **Hand foot mouth disease** - usually caused by group A coxsackieviruses although group B coxsackieviruses and other enteroviruses have been caused outbreaks.
- **Herpangina** - caused by group A coxsackieviruses.
- **Epidemic Pleurodynia (Bornholm disease)** - normally caused by group B coxsackieviruses.

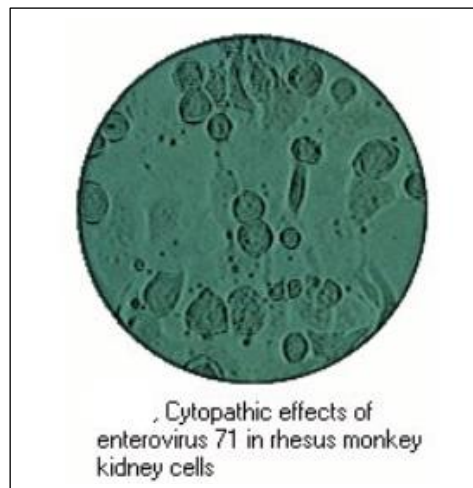
Disease Associations (2)

- **Myocarditis** - group B coxsackieviruses are the major cause of myocarditis, although it may be caused by other enteroviruses. It may present in neonates as part of neonatal infection and is often fatal. In adults, the disease is rarely fatal.
- **Respiratory Infections** - several enteroviruses are associated with the common cold.
- **Rubelliform rashes** - a rash disease resembling rubella may be seen with several coxsackie A, B, and echoviruses.
- **Neonatal Infection** - some coxsackie B viruses and echoviruses may cause infection in newborn infants. The virus is usually transmitted perinatally during the birth process and symptoms vary from a mild febrile illness to a severe fulminating multisystem disease and death.
- **Conjunctivitis** - associated with several types of enteroviruses, notably Coxsackie A24 and Enterovirus 70 (haemorrhagic conjunctivitis)

- **Pancreatitis/Diabetes** - associated with Coxsackie B virus infection. The extent of the role of the virus in diabetes is unknown.

Laboratory Diagnosis

- **Virus Isolation** (Figure 2-67)
 - Mainstay of diagnosis of enterovirus infection
 - Coxsackie B and Echoviruses can be readily grown in cell culture from throat swabs, faeces, and rectal swabs. They can also be isolated from the CSF
 - Coxsackie A viruses cannot be easily isolated in cell culture. They can be isolated readily in suckling mice but this is not offered by most diagnostic laboratories because of practical considerations. Molecular techniques may provide a better alternative.
- **Serology**
 - Very rarely used for diagnosis since cell culture is efficient.
 - Neutralization tests or EIAs are used but are very cumbersome and thus not offered by most diagnostic laboratories



Figure(2-67)Cytopathic Effect

Management and Prevention

There is no specific antiviral therapy available against enteroviruses other than polio.

Some authorities use IVIG in the treatment of neonatal infections or severe infections in immunocompromised individuals. However, the efficacy is uncertain.

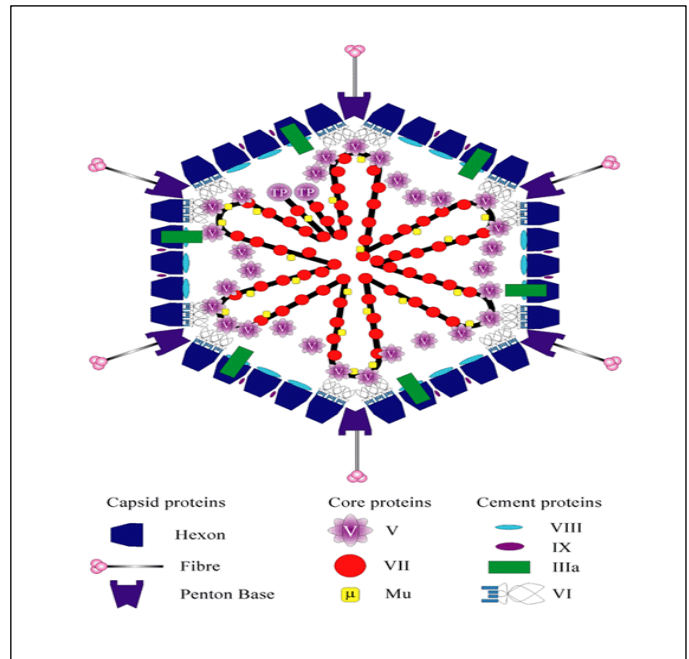
HNIG have been to prevent outbreaks of neonatal infection with good results.

There is no vaccine available mainly because of the multiplicity of serotypes. There is little interest in developing a vaccine except against enterovirus 71 and coxsackie B viruses.

Adenoviruses

Human Adenoviruses

- Adenoviruses were **first isolated in 1935** from human adenoid tissues.
- Since then, **at least 49 distinct antigenic types** have been isolated **from humans** and many other types from animals.
- **All human serotypes** are included in a single genus within the family Adenoviridae.



Figure(2-68) Adenovirus structure

Morphology

- ds-DNA viruses
- media sized in diameter
- icosahedral
- Nonenveloped

Antigenic structure

All human Adenoviruses share a common group-specific antigen.

- **Type specific antigens are important in serotyping.**
- Adenoviruses have a characteristic morphology (Stewart et al., 1993), with an icosahedral capsid consisting of three major proteins, hexon (II), penton base (III) and a knobbed fiber (IV), along with a number of other minor proteins, VI, VIII, IX, IIIa and IVa2 .The virus

genome is a linear, double-stranded DNA with a terminal protein (TP) attached covalently to the 5' termini (Rekosh et al., 1977)

Classification

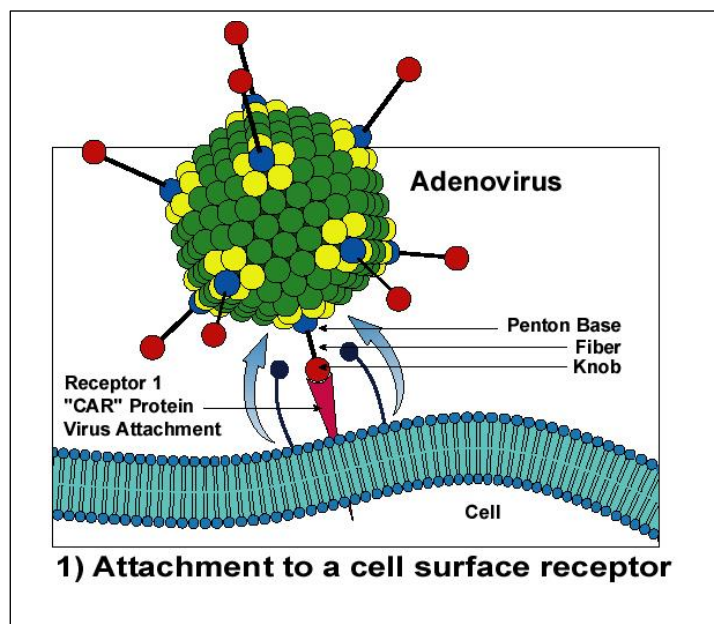
Adenoviruses are divided into six groups (A to F) based on:

1. physical
 2. chemical
 3. biological properties
- **Antigenic structure divides adenoviruses into:**
 - **49 serotypes:**
 - About 1/3 of the 49 known human serotypes are responsible for most cases of Adenovirus disease.(Figure 2-69)

Pathogenesis:

Adenoviruses spread by:

- direct contact
- respiratory droplets
- feco-oral route.



Figure(2-69) Adenovirus Attachment

Pathogenesis:

Adenoviruses infect and replicate in the epithelial cells of the:

1. pharynx
2. conjunctiva
3. urinary bladder
4. small intestine.

They usually do not spread beyond the regional lymph nodes **EXCEPT IN THE IMMUNE COMPROMIZED HOST.**

Pathogenesis:

- The virus has a tendency to become latent in lymphoid tissue.
- The virus can be reactivated by immunosuppression.

Clinical Syndromes:

- Adenoviruses cause primary infection in:
 - children
 - less commonly adults.
- Several distinct clinical syndromes are associated with Adenovirus infection.
 - a. Respiratory diseases:
 - b. Eye infections:
 - c. Gastrointestinal disease
 - d. Other diseases:
 - e. Adenoviral infections of the immune compromised host

A. Respiratory diseases:

- The most important etiological association of adenoviruses is with the respiratory diseases.
- They are responsible for 5% of acute respiratory diseases in:
 1. young children
 2. and much less in adults.

C. Gastrointestinal disease:

1. No disease association

Many Adenoviruses replicate in intestinal cells and are present in the stools without being associated with GIT disease.

2. Infantile gastroenteritis

Two serotypes (40, 41) have been etiologically associated with infantile gastroenteritis.

Laboratory Diagnosis

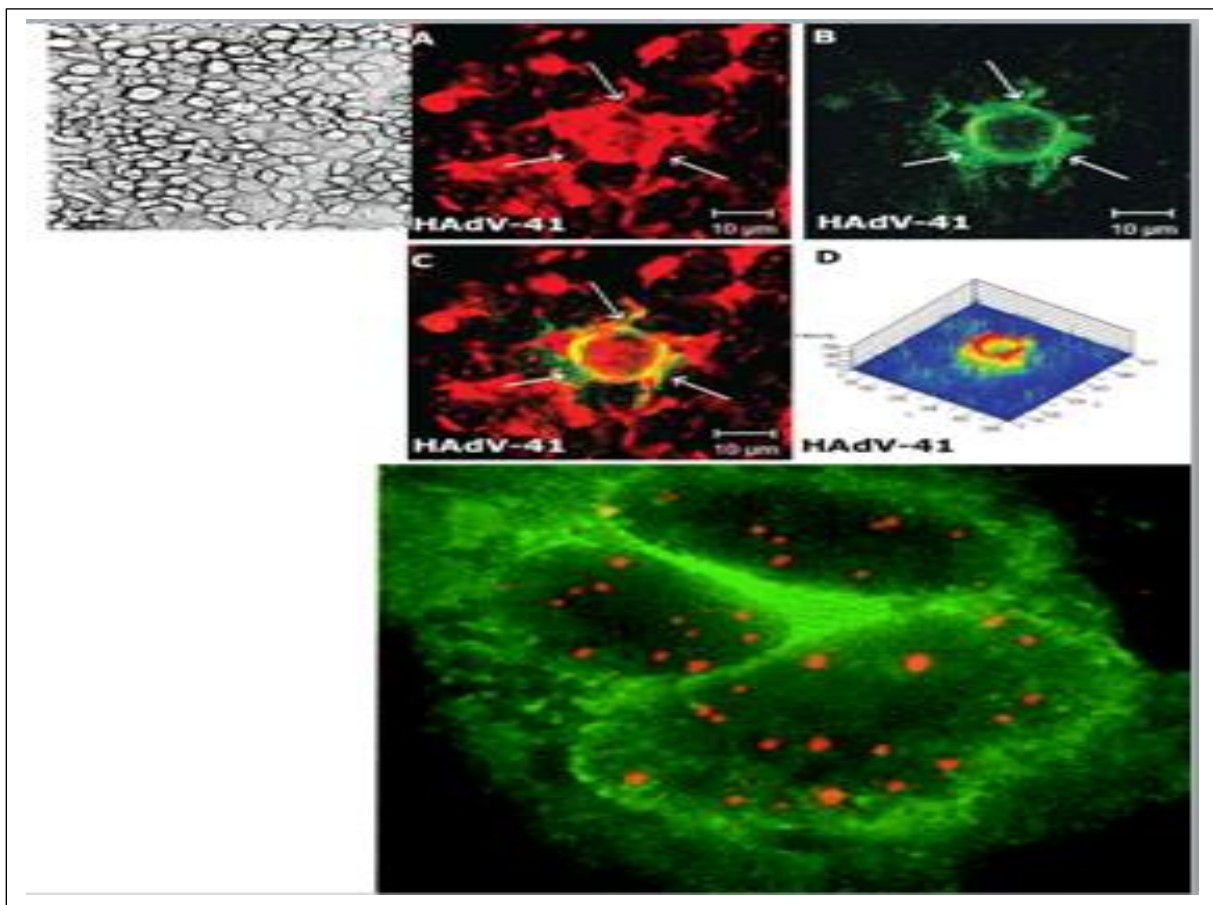
1. Direct detection:
2. Isolation
3. Serology

Direct detection:

- **Virus particle by EM** can be detected by direct examination of fecal extracts.
- **Detection of adenoviral antigens by ELISA.**
Enteric Adenoviruses
- **Detection of adenoviral NA by Polymerase chain reaction:** can be used for diagnosis of Adenovirus infections in tissue samples or body fluids.

Laboratory Diagnosis

- **Isolation** (Figure2-70)
- Isolation depending on the clinical disease, the virus may be recovered from **throat, or conjunctival swabs or and urine.**
- **Isolation is much more difficult from the stool or rectal swabs**



Figure(2-70) Diagnostic of adenovirus

Serology

1. Haemagglutination inhibition
&
2. Neutralization tests can be used to detect specific antibodies following Adenovirus infection.

Prevention and control

1. Careful **hand washing** is the easiest way to prevent infection.
2. **Disinfection of Environmental surfaces with hypochlorites.**
3. The risk of water borne outbreaks of conjunctivitis can be minimized by chlorination of swimming pools.
4. Epidemic keratoconjunctivitis can be controlled **by strict asepsis during eye examination.**

Human Immunodeficiency Virus

HIV

Human Immunodeficiency Virus

- **H** = Infects only Human beings
- **I** = Immunodeficiency virus weakens the immune system and increases the risk of infection
- **V** = Virus that attacks the body

AIDS

Acquired Immune Deficiency Syndrome

- **A** = Acquired, not inherited
- **I** = Weakens the Immune system
- **D** = Creates a Deficiency of CD4+ cells in the immune system
- **S** = Syndrome, or a group of illnesses taking place at the same time

HIV and AIDS

- When the immune system becomes weakened by HIV, the illness progresses to AIDS
- Some blood tests, symptoms or certain infections indicate progression of HIV to AIDS

HIV-1 and HIV-2

- HIV-1 and HIV-2 are
 - Transmitted through the same routes
 - Associated with similar opportunistic infections
- HIV-1 is more common worldwide
- HIV-2 is found in West Africa, Mozambique, and Angola
 - HIV-2 is less easily transmitted
 - HIV-2 is less pathogenic

Transmission of HIV

HIV is transmitted by:

1. Direct contact with infected blood
2. Sexual contact: oral, anal, or vaginal
3. Direct contact with semen or vaginal and

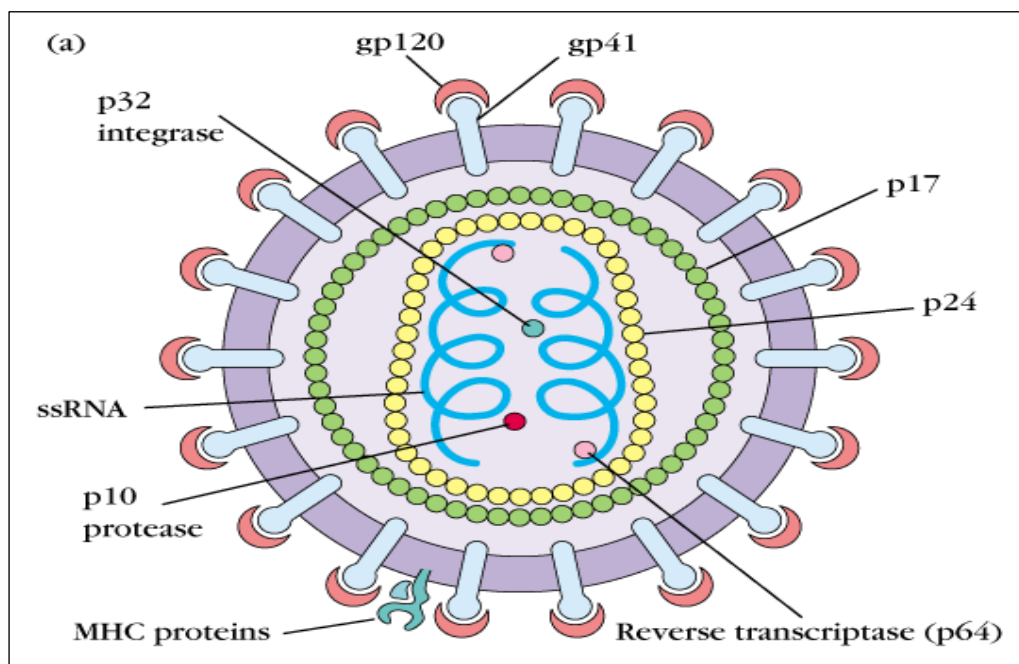
4. cervical secretions
5. HIV-infected mothers to infants during
6. pregnancy, delivery, or breastfeeding

Pathophysiology of HIV

- RNA virus discovered in 1983
- Virus binds to specific CD4 receptor sites and then enters the cell
- Reverse transcriptase assists to make a single viral DNA and it copies itself to make a double-stranded viral DNA

Pathophysiology of HIV

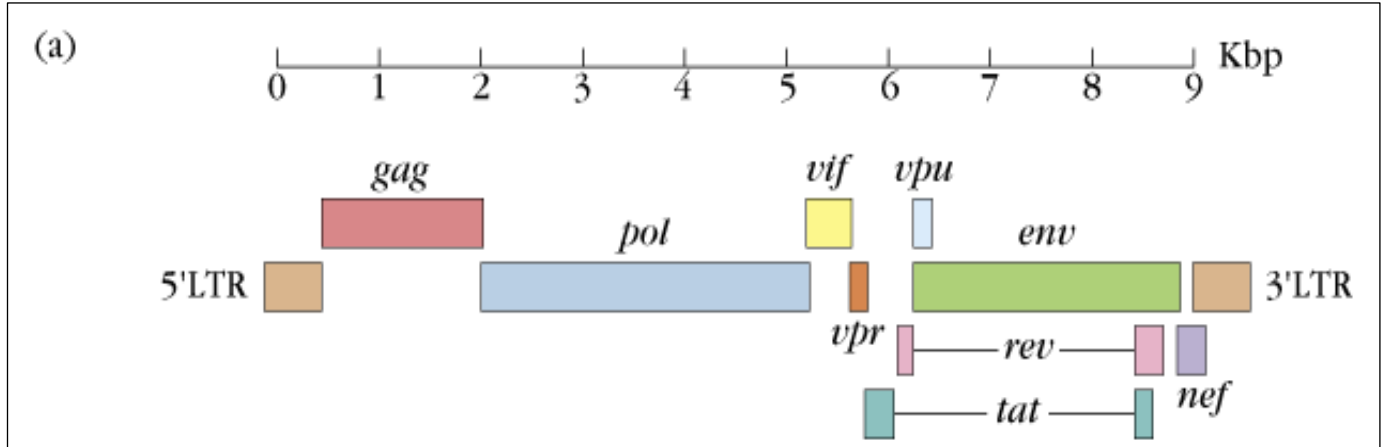
- Virus enters the cell nucleus
- Using integrase the virus splices itself into genome to become part of the cell's genetic structure (Figure 2-71)



Figure(2-71)HIV-1 Virus structure

- The virus that causes AIDS
- It is a retrovirus with two copies of single stranded RNA genome
- It uses reverse transcriptase to transform its ss-RNA genome into a ds-DNA for integration into its host genome

- It has marker proteins (gp120) in the protein coat that allow it to recognize specific cells in the human body
- The protein coat also contains MHC-I and MHC-II molecules



Figure(2-72) HIV genome

- gag gene codes for nucleocapsid proteins
- env gene codes for envelope glycoproteins, i.e. gp41 (transmembrane protein) and gp120 (surface protein)
- pol gene codes for enzymes such as reverse transcriptase, protease and integrase (Figure 2-72)
- Other genes code for various activators and accessory proteins

Pathophysiology of HIV

- **HIV destroys CD4+ cells 3 ways**
 1. Viral replication leaves holes in cell membranes
 2. Infected cells fuse with other cells
 - Combine to form a syncytium that destroys all affected cells .
 3. Antibodies against HIV bind to the infected cells and activate the complement system, which destroy the infected cells

Consequences

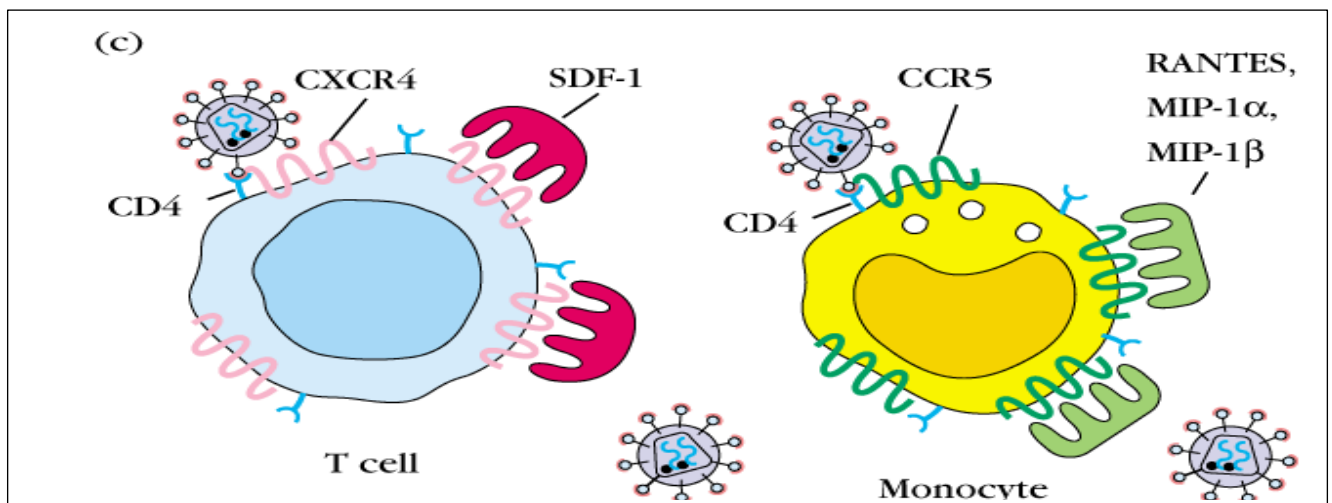
- All daughter cells from infected cell are infected
- Genetic codes can direct the cell to make HIV

Initial infection

- Viremia (large amount of virus in blood)
- Few clinical symptoms
- Steady state of viral load can be maintained for many years

Pathophysiology of HIV

- HIV destroys about 1 billion CD4⁺ T cells every day
- Immune problems start when CD4⁺T cell counts drop below 500 cells/ μ l
- While CD4 is recognized by the virus, it is not sufficient for viral attack; it needs a costimulatory signal.
- T cells: coreceptor is CXCR4, which also acts as a receptor for the chemokine SDF-1; there is competitive inhibition between chemokine and HIV for binding; the HIV strain is called T-tropic
- Monocytes: coreceptor is CCR5, which is a receptor for chemokines, which also act as competitive inhibitors to HIV; the HIV strain is called M-tropic
- T-tropic HIV strains cause syncytia: formation of giant cells as a result of fusion of cells via the gp120 protein on viral coats. (Figure2-73)

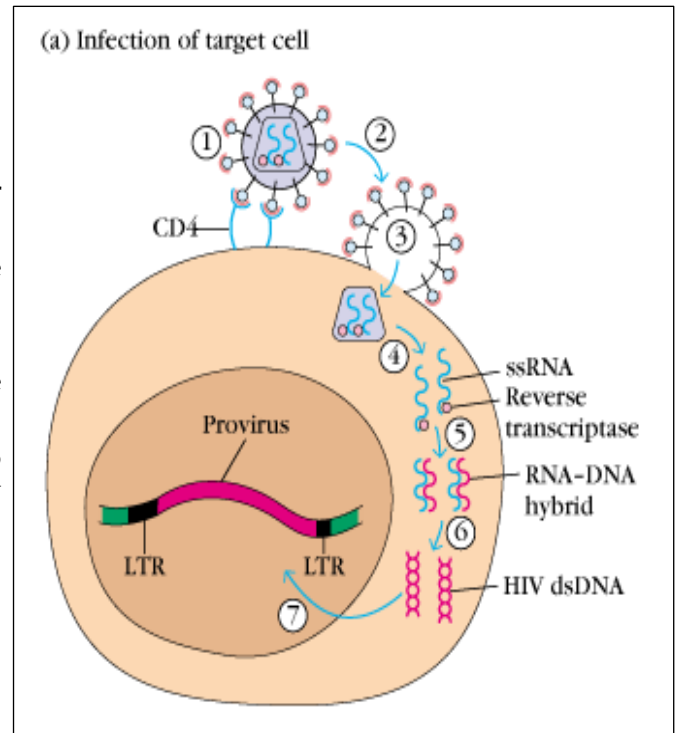


Figure(2-73) Complete Activation of HIV

Infection of Human Cell with HIV

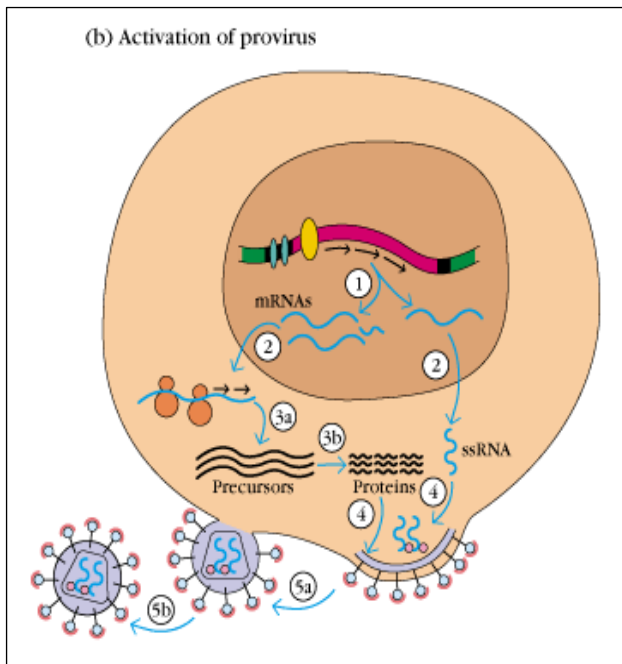
- HIV gp120 surface protein binds CD4 on target cell
- Transmembrane component, gp41, binds coreceptor CXCR4 to enhance fusion
- Viral genome and other proteins are able to enter the cell via nucleocapsid
- RT transcribes the ssRNA genome
- The next DNA strand is made, making a double stranded DNA molecule called a provirus
- The dsDNA is transferred to the nucleus to be added to the host genome via the viral integrase protein at HIV LTR sites(Figure2-74)

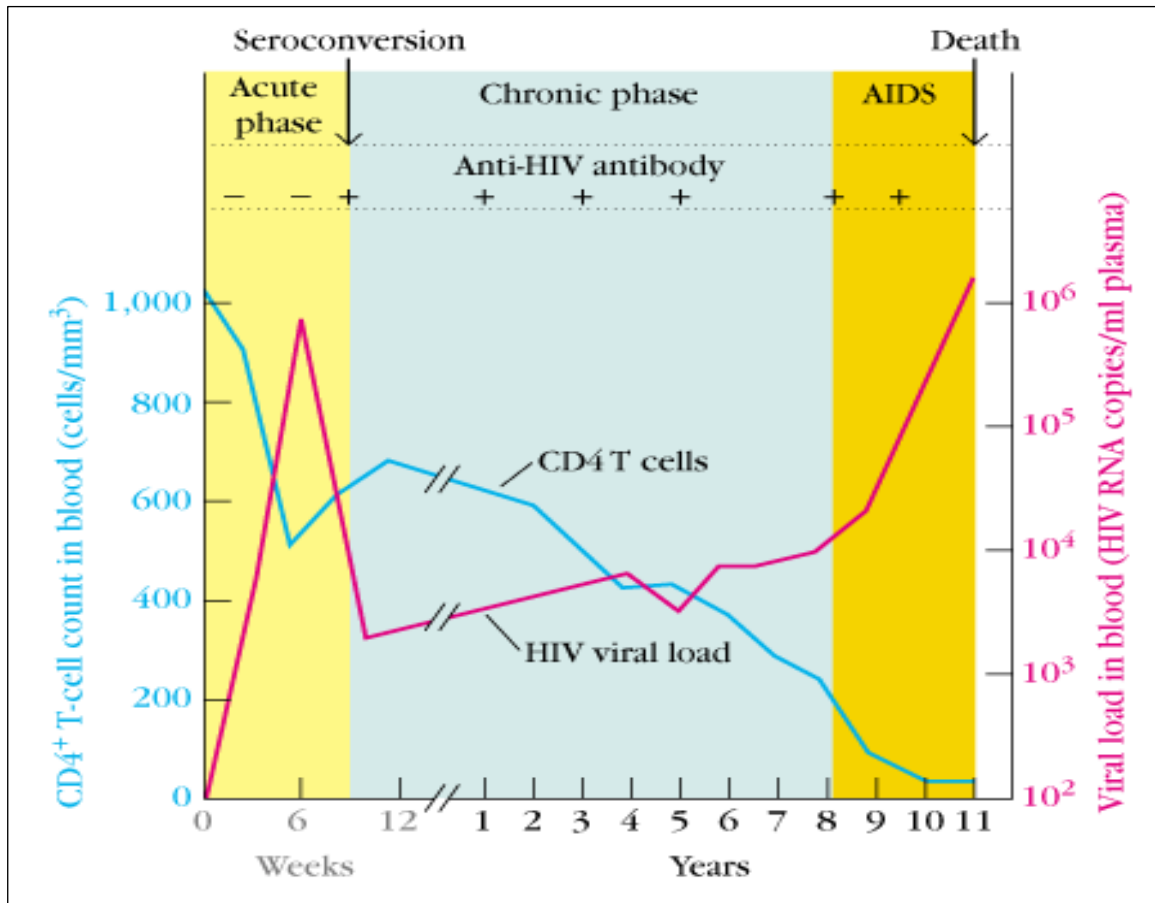
Figure(2-73)Infection of Human Cell with HIV



- In a latent cell, the integrated provirus must be activated by transcriptional factors to make genomic ssRNA and mRNAs
- Genomic RNA is exported
- Host ribosomes transcribe viral mRNAs, and the proteins are either with the genomic RNA or part of the membrane
- The membrane buds to form a viral envelope
- The mature virus is released outside the cell
- These latent cells are dangerous because they can remain latent for long periods of time(Figure 2-75)

Figure(2-75)Activation of Provirus





Figure(2-76)Progression of HIV to AIDS

Progression from HIV infection to stage of AIDS (figure 2-76)

Normal Healthy Individual



Gets infected with HIV



WINDOW PERIOD (3-12 weeks or even 6 months)

(Antibodies to HIV not yet developed, test does not capture the real status but person can infect others)



HIV Positive

(Development of antibodies, can be detected in test)

No exclusive symptoms (mild fever or flu like features in some cases)



May take up to 10 to 12 years to reach the stage of **AIDS**, the period can be prolonged through available treatment

HIV Testing

- Tests should be taken 12 weeks after high-risk behavior, repeated 6 months after an uncertain result
- Types of Tests
 - A. ELISA: enzyme-linked immunosorbent assay
 - B. Western Blot: rechecks ELISA results
 - C. Viral load tests measure HIV in bloodstream (PCR)

Major Signs / Symptoms of AIDS:

A. Major Signs:

1. Weight loss (> 10% of body weight)
2. Fever for longer than a month
3. Diarrhea for longer than a month

B. Minor Signs:

1. Persistent cough
2. General itchy skin diseases
3. Thrush in mouth and throat
4. Recurring shingles (herpes zoster)
5. Long lasting, spreading and severe cold sores
6. Long lasting swelling of the lymph glands
7. Loss of memory
8. Loss of intellectual capacity
9. Peripheral nerve damage

Treatment

- **Antiretroviral Medications**
 - Nonnucleoside reverse transcriptase inhibitors
 - Nucleoside reverse transcriptase inhibitors
 - Protease inhibitors

- Fusion Inhibitor
- **HAART - Highly Active Antiretroviral Therapy**
- Combination of three or more medications
- **Atripla - newest antiretroviral**
- Combination of three medications in one pill
- Lowers the amount of HIV (called viral load) by interfering with the way HIV makes copies of itself.

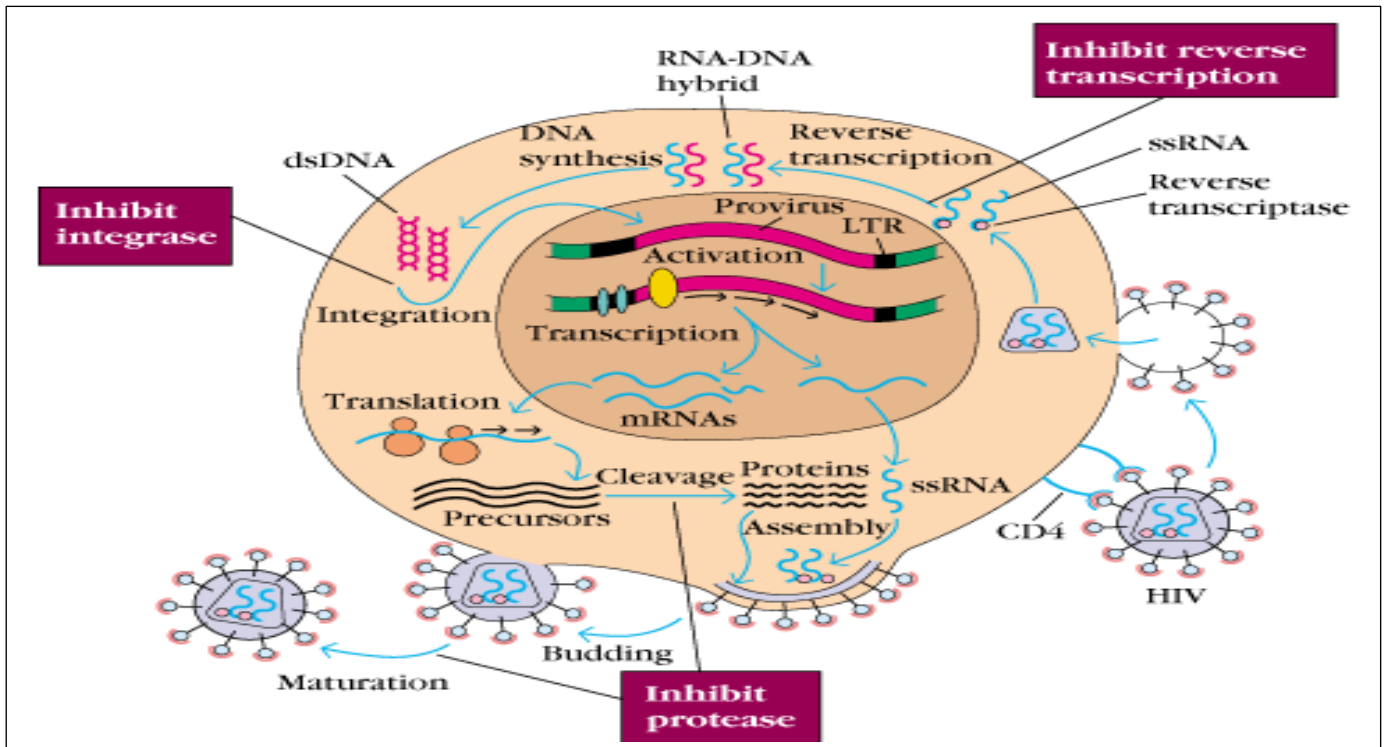


Figure (2-77) 3Points In HIV Cell Cycle Where Replication Can be Stopped

3Points In HIV Cell Cycle Where Replication Can be Stopped

- Nucleoside Reverse Transcriptase Inhibitors (NRTIs)
- Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
- Protease Inhibitors
- All 3 of these treatments are usually prescribed at once. Known as HAART, the combination of all 3 fights the ability of the virus to rapidly mutate.(Figure 2-77)

Reverse Transcriptase Inhibitors

- Reverse Transcriptase Inhibitors interfere with the reverse transcriptase (RT) enzyme that HIV needs to make copies of itself. There are 2 types of inhibitors each working differently .

Type 1: NRTI's – nucleoside drugs provide faulty DNA building blocks, stopping the DNA chain the virus uses to make copies of itself .

Type 2: NNRTI's- non-nucleoside RT inhibitors bind RT so the virus cannot carry out its copying function

Examples Include: AZT, 3TC, Combivir, Nevirapine

Protease Inhibitors

- **Protease Inhibitors (PI)**, discovered in 1995, block the protease enzyme. When protease is blocked, HIV makes copies of itself that can't infect new cells .
- **PI Side Effects:** PI's can cause high blood sugar and consequently diabetes. Another main concern is lipodystrophy, where your body absorbs fats and nutrients in an irregular manner. Latent HIV can hide out in these fat cells .

Can HIV be Vaccinated Against?

Challenges

- HIV thrives in the presence of circulating antibodies directed against it .(Figure 2-78)



- HIV integrates its genome into the host cell's DNA, making it difficult to remove
- HIV mutates and can show up to 109 viruses per day, while the common cold with 100 subtypes has proven to be difficult to make a vaccine for

The AIDS logo demonstrates :

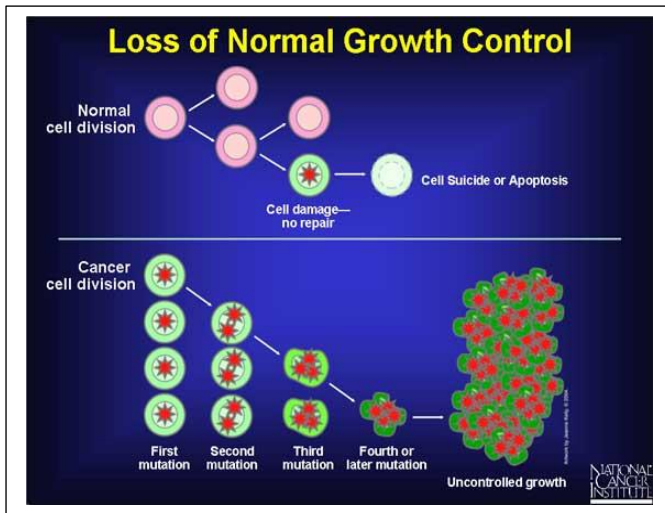


Figure(2-79)

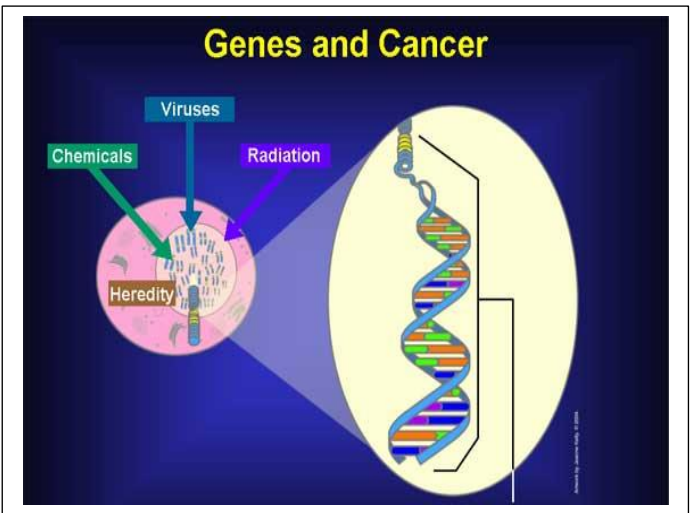
Arranged by Sarah Mohssen

- **Care and concern** about HIV and AIDS for those who are living with HIV, for those who are ill, for those who have died and for those who care for and support those directly affected.
- **Hope** - that the search for a vaccine and cure to halt the suffering will be successful.(Figure 2-79)
- **Support** for those living with HIV, for the continuing education of those not infected, for maximum efforts to find effective treatments, cures or vaccines, and for those who have lost friends, family members or loved ones to AIDS.

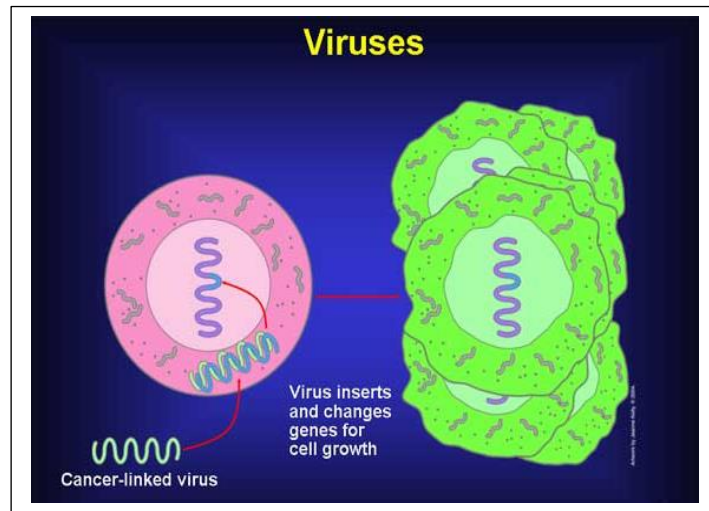
Human Cancer Viruses



A-Carcinogenesis



B-Factors in Carcinogenesis



C-Viruses

Figure (2-80) Human causes by viruses

Viruses

Viruses contribute to development of some cancers. Typically, the virus can cause genetic changes in cells that make them more likely to become transformed .

These cancers and viruses are linked

1. Cervical cancer and the genital wart virus, HPV
2. Primary liver cancer and the Hepatitis B virus

3. T cell leukaemia in adults and the Human T cell leukaemia virus(Figure 2-80)

Table (2-5) Viruses Associated With Human Cancers

Family	Virus	Cancer
<i>Papillomaviridae</i>	Human papillomaviruses	Genital tumors Squamous cell carcinomas Oropharyngeal carcinomas
<i>Herpesviridae</i>	Epstein-Barr virus	Nasopharyngeal carcinoma African Burkitt's lymphoma B cell lymphoma
<i>Hepadnaviridae</i>	Hepatitis B virus	Hepatocellular carcinoma
<i>Retroviridae</i>	Human T lymphotropic viruses Human immunodeficiency viruses	Adult T cell leukemias AIDS-associated tumors (due to impaired T cell responses)
<i>Flaviviridae</i>	Hepatitis C virus	Hepatocellular carcinoma

Retroviruses

- **Structure and composition**
 - Diploid single-stranded RNA viruses (5-8 kb)
 - Helical ribonucleoprotein
 - Icosahedral symmetry (100 nm)
 - Enveloped
- **Genetics**
 - Only diploid viruses
 - Nonsegmented
 - About 10 genes, 16 proteins
- **reverse transcriptase**
 - protease
 - envelope
 - gag
 - tax

- rex
- Genes encoded in both directions

Retroviruses

- Epidemiology
 - Typical infectious viruses (exogenous)
 - Sexual transmission
 - IV drug abusers
 - Other, unknown transmission mechanisms
 - Classification
 - Leukemia viruses
 - Alpharetrovirus
 - Gammaretrovirus
 - Nontransforming retroviruses
 - Deltaretrovirus
 - Lentivirus

Mechanisms of Retroviral Carcinogenesis

- Infection leads to uncoating in the cytoplasm
- Reverse transcriptase makes a double-stranded DNA copy
- The ds-DNA translocates into the nucleus where it randomly integrates in host cell chromosome
- This version of the viral genome is termed the provirus
- Two replication strategies
 - Induce cell division - leads to copies of the viral genome in each daughter cell
 - Productive infection - spread of virus to other cells.

DNA Tumor Viruses

Virus	Viral Oncoproteins	Cellular Targets
Polyomavirus SV40	Large T antigen Small t antigen	p53 and pRb tumor suppressor genes PP2A
Human papillomavirus	E6 E7	p53, DLG, MAGI-1, MUPP1 pRb
Bovine papillomavirus	E5	PDGF β receptor
Adenovirus	E1A E1B-55k	pRb p53
Adenovirus 9	E4ORF1	DLG, MAGI-1, MUPP1
Epstein-Barr virus	LMP1 vIL10 BCL2 homolog	TRAFs IL-10 receptor (soluble viral cytokine) Rescues cell from apoptosis

Table (2-6) DNA tumor viruses

DNA Tumor Viruses

- **Papillomaviruses**
- **Features**
 - Nonenveloped icosahedral (55 nm)
 - Circular ds-DNA (8 kb)
 - Nuclear replication
 - Stimulate cellular DNA synthesis
 - Highly restricted host range and tissue range
- **Many human types**
- Only a few are known to cause cancers
- **Cervical cancer** is the most important
- **Vaccine is now available** (Gardasil; types 6, 11, 16, 18)

- Cause **warts** (abnormal cellular proliferation)
- Replicate in **basal stem cells and keratinocytes** of the skin and mucosa
 - HeLa cells are cervical cancer cells from Helen Lang (fatal)

Papillomaviruses Encode Two Structural Proteins

L1: the major structural protein. Each viral particle has 360 copies in 72 pentamers.

L2: the minor structural protein. Up to 72 copies per particle.(Figure2-81)

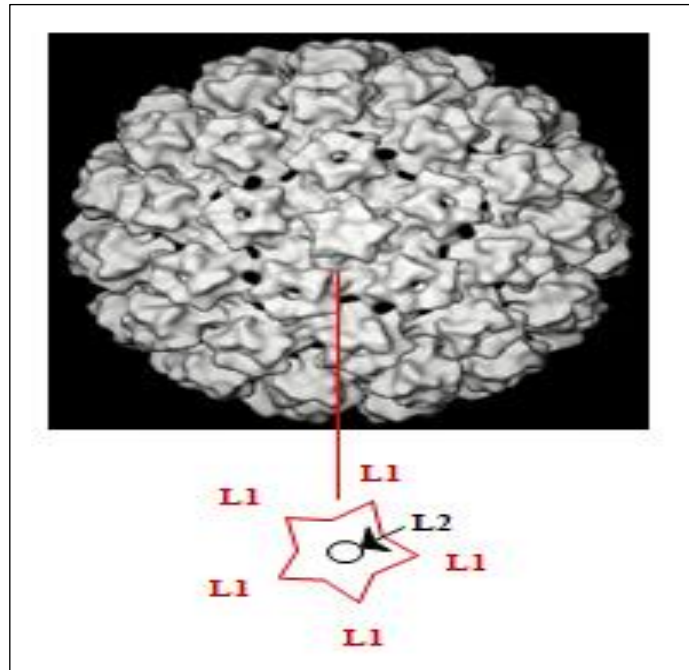
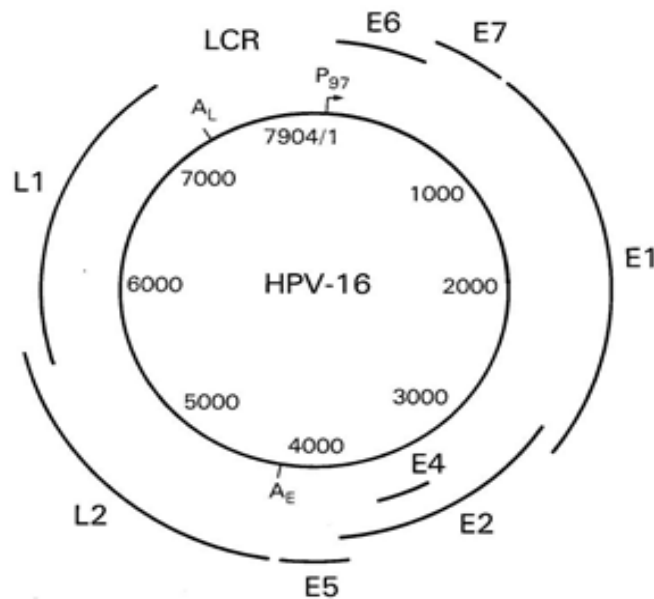


Figure (2-81) Papillomavirus Particle

Late genes

- Region of greatest genetic conservation
- L1 is major capsid protein
 - Capsid is 72 pentamers of L1
 - Expressed L1 assembles into viral conformation, viral-like particles (VLPs)
- L2 is minor capsid protein(Table 2-7)
- Required for encapsidation of viral genome(Figure 2-82)



- **Small (8 kb) closed circular dsDNA, complexed with cellular histones in nucleosome structure.**
 - *Small genome, genetic simplicity, implies strong host dependence, few targets for antivirals, stimulates host to assist in replication.*
- **Ten genes, 8 early (E) and 2 late (L)**
 - Early genes control transcription, DNA replication, transformation
 - Late genes are capsid proteins
- **Long control region contains replication origin and other cis acting signals**
- **All papilloma viruses have similar organization**

Genomic map of HPV-16. The genome is a double-stranded circular DNA molecule of 7904 base pairs. Transcription occurs in a clockwise manner; the only transcriptional promoter presently mapped for HPV-16 is designated P97. The open reading frames deduced from the DNA sequence are designed E1 to E7, L1, and L2 and are indicated outside of the circular genome. AE and AL represent the early and late polyadenylation sites. The viral long control region (LCR) contains transcriptional and replication regulatory elements.

Figure (2-82) HPV16 genome

Table (2-8) Papilloma virus gene function

ORF	Function
L1	L1 protein, major capsid protein
L2	L2 protein, minor capsid protein
E1	Initiation of viral DNA replication
E2	Transcriptional regulatory protein with an auxillary role in viral DNA replication
E3	No known function
E4	Late protein; disrupts cytokeratins
E5	Membrane-transforming protein; interacts with growth factor receptors
E6	Transforming protein of HPVs; targets degradation of p53
E7	Transforming protein of HPVs; binds to the retinoblastoma protein
E8	No known function
ORF, open reading frame; HPV, human papillomavirus.	

HPV DNA integration and cervical carcinoma

- Strong association between integrated HPV DNA and cervical carcinoma. (HPV-16, HPV-18, HPV-31, HPV-45)
- DNA integration anywhere in chromosome
- Integration upregulates E6 and E7 gene expression
- Disrupts E2 function: loss transcriptional repression of E6 and E7
- E6 and E7 expressed in cells from cervical carcinoma
- E7 is the HPV oncoprotein
- Binds Rb (a cellular anti-oncogene), releasing E2F (a cellular transcription factor)
- E6 cooperates in transformation
- Stimulates degradation of p53 (a cellular anti-oncogene, transcription factor)
- Unregulated expression of HPV E6 and E7 results in unregulated cell cycling (Figure 2-84)

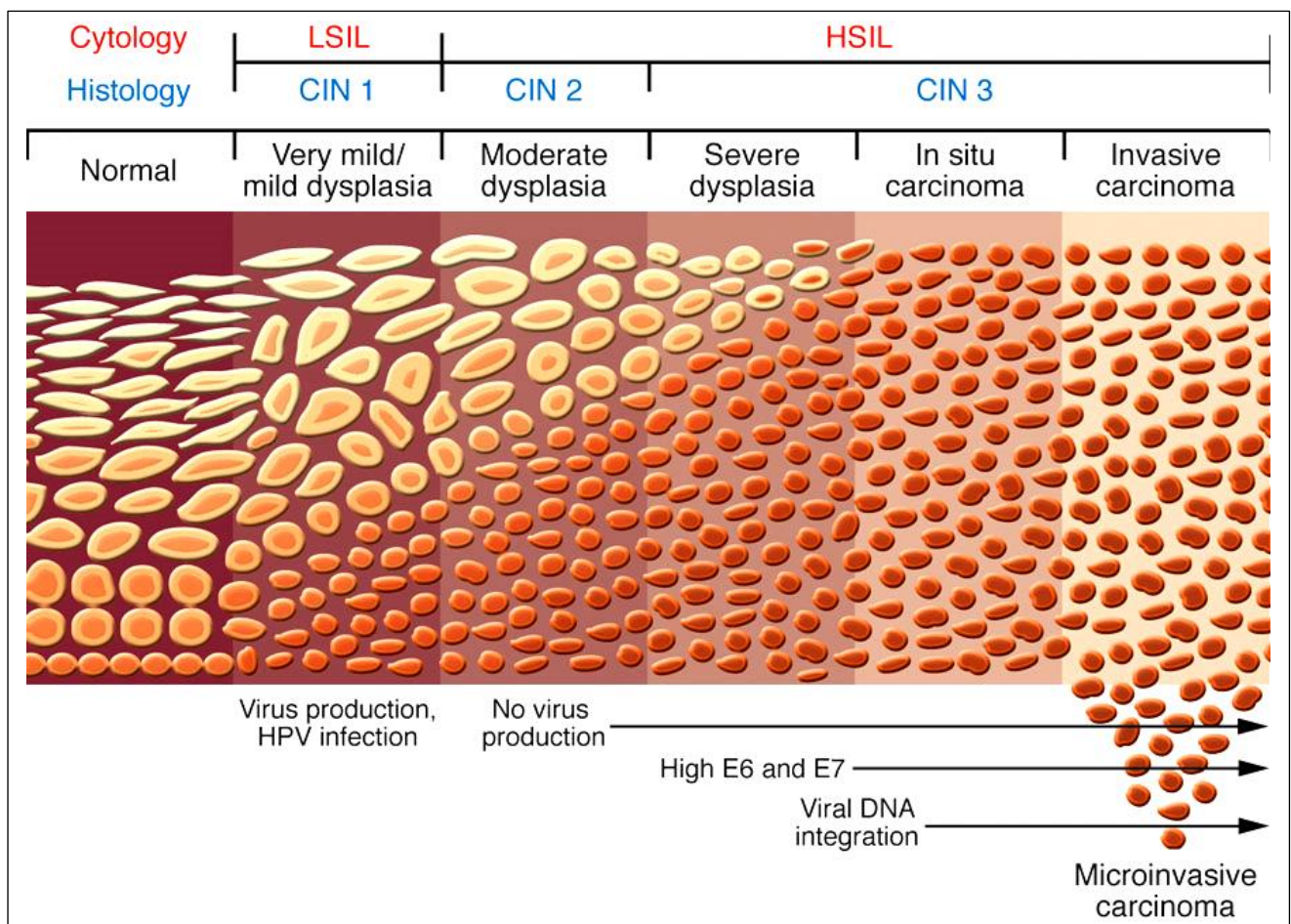


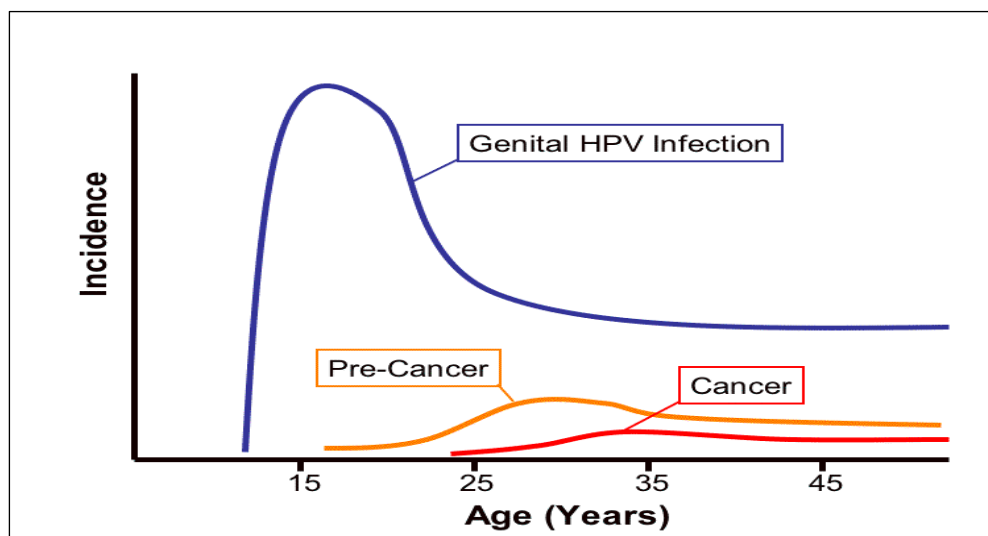
Figure (2-84) Precursor lesions for cervical cancer

How do you know you have HPV?

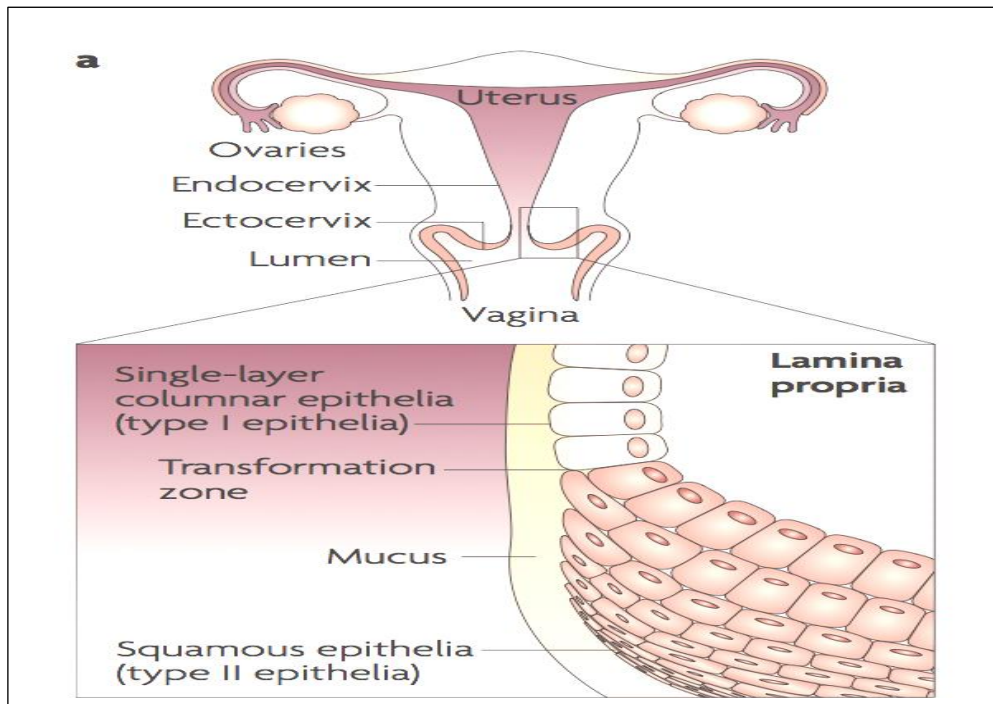
- There are no tests to detect the HPV virus.
- Most people who contract HPV will never know they have it.
- Having HPV does not mean you have a disease – most people don't have any signs or symptoms.
- Some low risk types cause genital and anal warts.
- In rare instances, the virus persists, especially the high risk types of the HPV virus that can develop pre-cancerous lesions and cancer.(Figure2-85)(Figure2-86)(Figure2-87).

Can you prevent HPV?

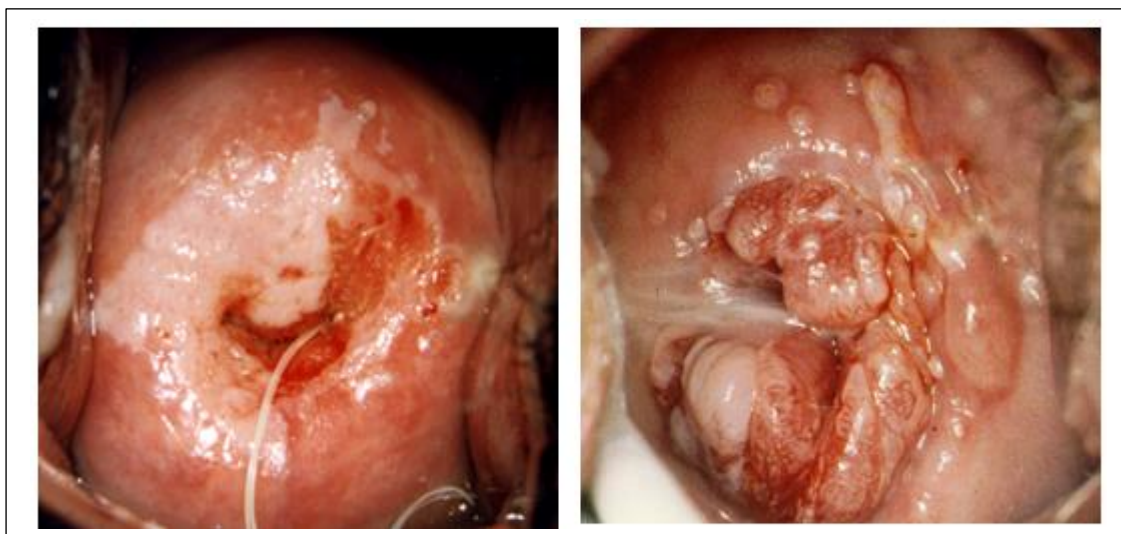
1. Absolutely no skin-to-skin sexual contact.
2. One sexual / intimate partner forever.
3. The more sexual partners, the higher the chance of contracting HPV.
4. Using condoms is excellent protection against STI, but does not cover all the skin.
5. Pap testing will detect abnormal cells.(Figure 2-88)
6. Vaccination is now available to prevent certain low risk types that cause genital warts certain high risk types that cause cancer.



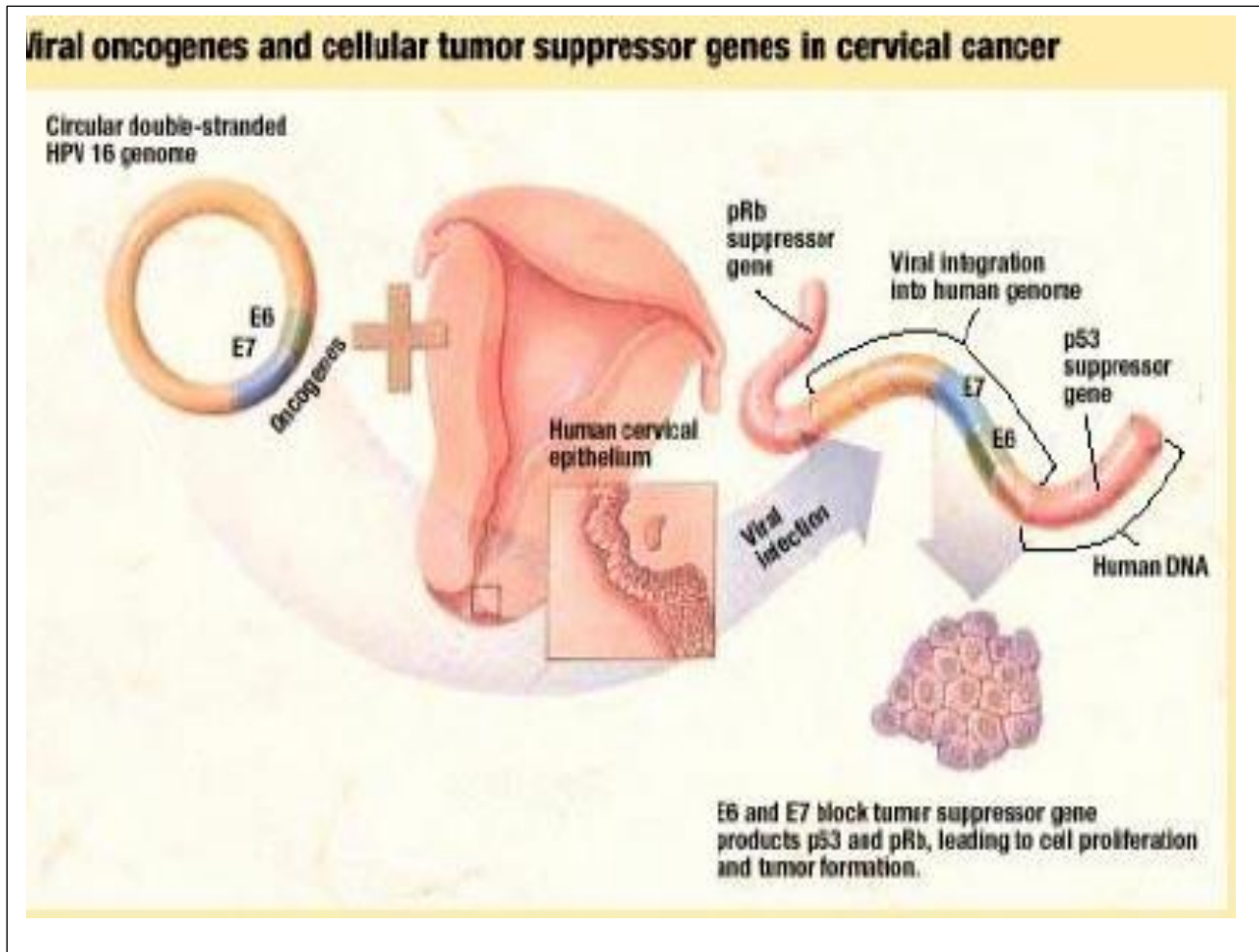
Figure(2-90) HPV Infection and Cervical Cancer



Figure(2-86)HIV infection and cervical cancer



Figure(2-87)Cervical Cancer Develop at the Transition Zone Between Squamous and Columnar Epithelium



Figure(2-88) Cervical oncogenes and cellular tumor suppressor genes in cervical cancer

Cervical Cancer Vaccines

Two Distinct HPV VLP Vaccines Were Developed Commercially

GlaxoSmithKline: HPV16

Cervarix

HPV18

ASO4 Adjuvant (Aluminum + MPL)

Made in insect cells

Merck:	HPV16
Gardasil	HPV18
	HPV6
	HPV11
	Aluminum Adjuvant
	Made in yeast

IM Injections at 0, 1 or 2, and 6 months

Prophylactic HPV Vaccines Are L1 Virus Like Particles (VLPs)

L1 Insertion in Baculovirus

Expression Vector

Production in

Insect Cells

Spontaneous assembly

of L1 into VLPs

Induce high titers

of virion neutralizing antibodies

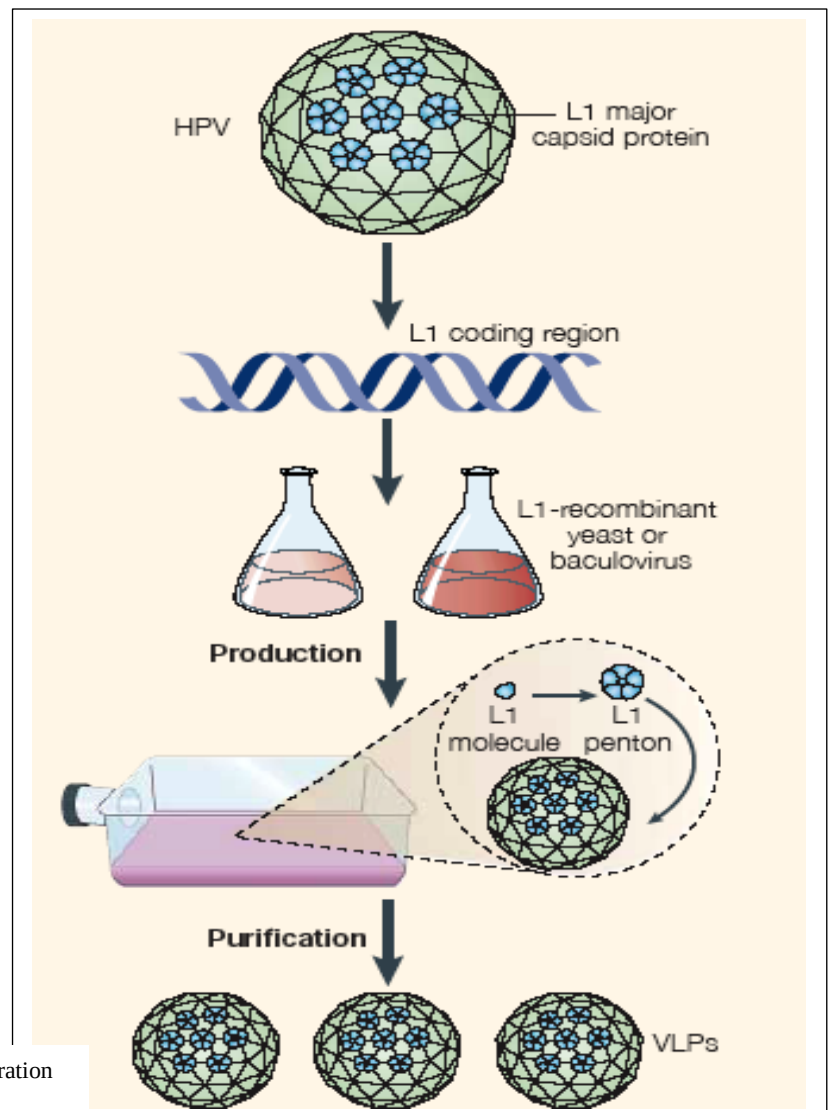


Figure (2-89) Cervical cancer vaccine preparation

Live Attenuated Viruses Are Not Suitable For an HPV Prophylactic Vaccine

Papillomavirus cannot be efficiently

grown in cultured cells

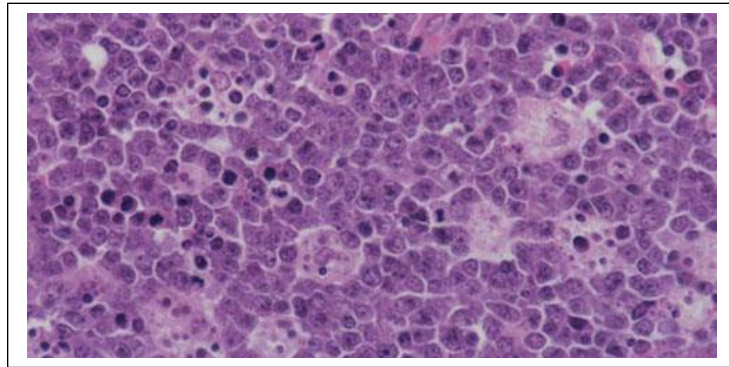
The viral genomes contain oncogenes

Virion protein-based subunit vaccines

are preferable, if they could efficiently induce neutralizing antibodies .

EBV-associated malignancies

- The strongest evidence linking EBV and cancer formation is found in Burkitt's lymphoma and Nasopharyngeal carcinoma(Figure2-90)



Kaposi's sarcoma

Figure(2-90)EBV-associated indigence

- form of skin cancer that can involve internal organs. It most often is found in patients with acquired immunodeficiency syndrome (AIDS), and can be fatal (Figure 2-91)



Hepatocellular carcinoma

- cancer that arises from hepatocytes, the major cell type of the liver .
- Hepatocellular carcinoma is one of the major cancer killers .
- It affects patients with chronic liver disease who have established cirrhosis, and currently is the most frequent cause of death in these patients .
- The main risk factors for its development are hepatitis B and C virus infection, alcoholism and aflatoxin intake.

What is a vaccine

- A vaccine is any preparation intended to produce immunity to a disease by stimulating the production of antibodies. Vaccines include, for example, suspensions of killed or attenuated microorganisms, or products or derivatives of microorganisms.
- **The most common method of administering vaccines is by injection (Figure 2-92), but some are given by mouth or nasal spray.**



Figure (2-92) vaccine administration by injection

Diseases Caused by Bacteria

- Diphtheria (Figure 2-93)
- Haemophilus influenzae type b (Figure 2-94)
- Meningococcal disease (Figure 2-95)
- Pertussis (Figure 2-96)
- Pneumococcal disease
- Polio (Figure 2-97)
- Tetanus (Figure 2-98)

Diphtheria

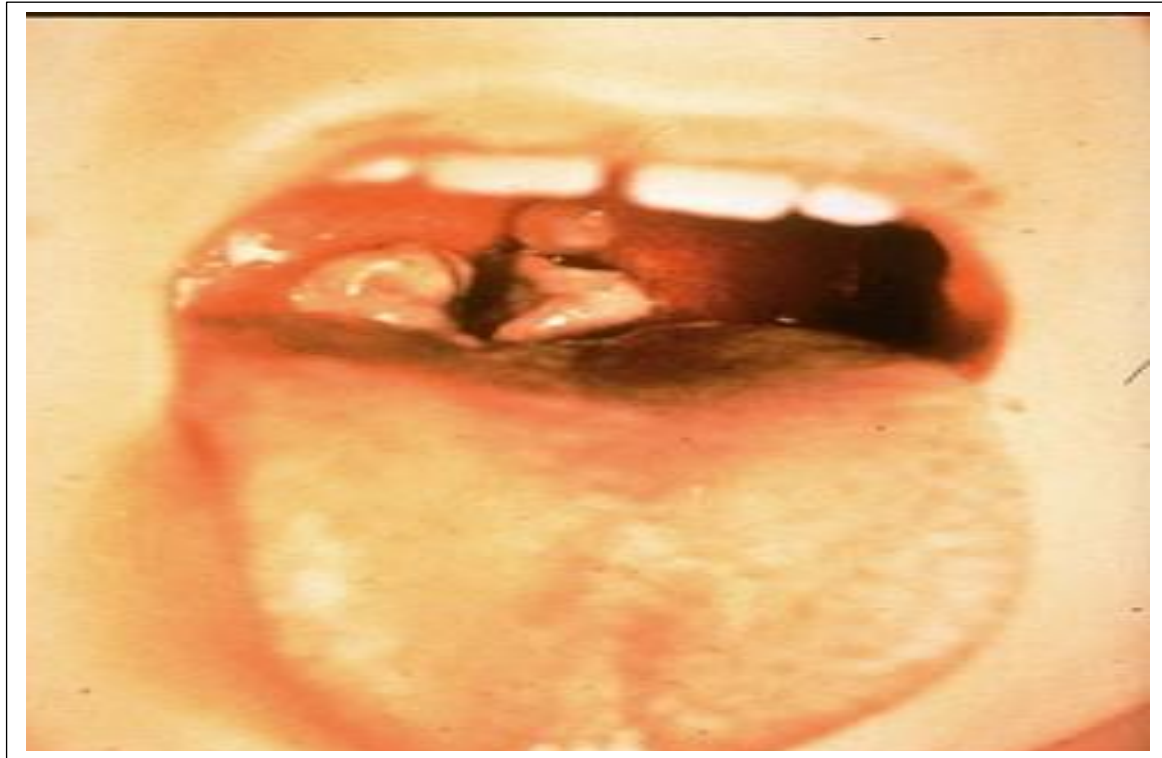


Figure (2-93) This child has diphtheria and has developed a pseudo-membrane, a thick gray coating over the back of his throat.

Haemophilus influenzae type b



Figure(2-94) This child has a swollen face due to Hib infection.

Meningococcal Disease



Figure(2-95) This 4-month-old has gangrene due to infection with meningococcus.

Pertussis



Figure(2-96) Child with broken blood vessels in eyes and bruising on face due to severe coughing caused by pertussis.

Pneumococcal Disease

- Caused by the bacterium *Streptococcus pneumoniae*
- Can infect different parts of the body leading to:
 1. Pneumonia
 2. Bacteremia (blood infection)
 3. Meningitis
 4. Ear infection

Polio



Figure (2-97) This young man suffers from upper extremity paralysis due to infection with poliovirus.

- Inactivated vaccines also ight viruses. These vaccines are made by
- inactivating, or killing, the virus during the process of making
- the vaccine. The inactivated polio vaccine is an example of this
- type of vaccine. Inactivated vaccines produce immune responses
- in different ways than live, attenuated vaccines. Often, multiple
- doses are necessary to build up and/or maintain immunity

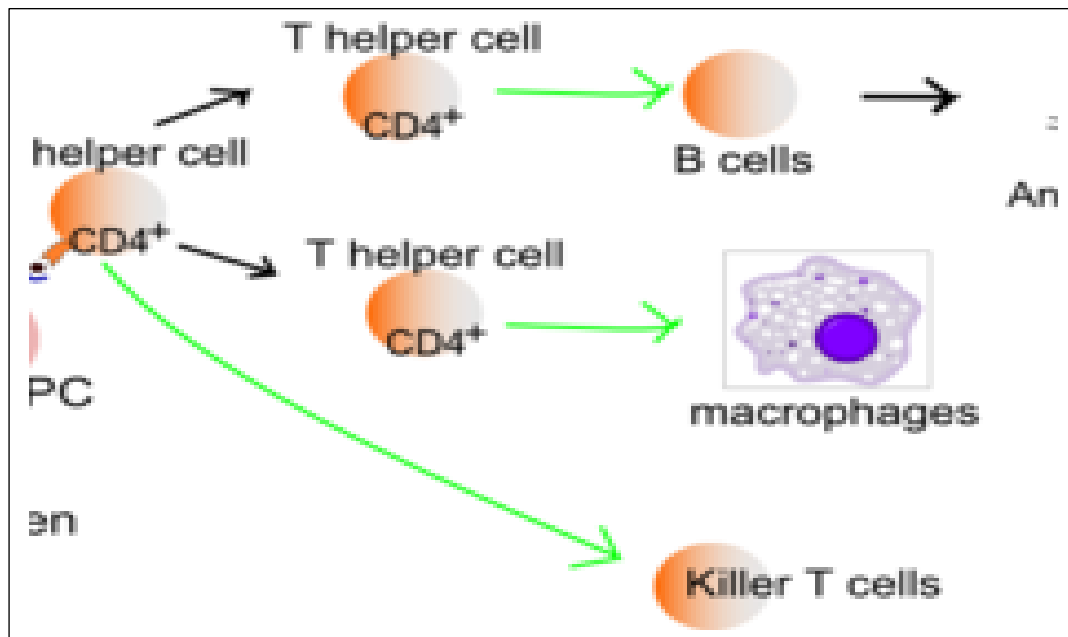
Tetanus



Figure (2-98) This child was experiencing painful muscle spasms due to infection with tetanus.

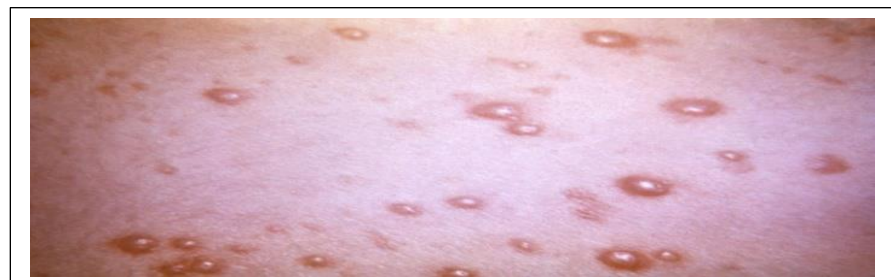
Diseases Caused by Viruses

1. Hepatitis A (Figure 2-95)
2. Hepatitis B
3. Shingles (Figure 2-99)
4. Human papillomavirus (HPV)
5. Influenza (Figure 2-100)
6. Measles
7. Mumps
8. Rotavirus
9. Chickenpox



Figure(2-98) Importance of T helper cells in an immune response: T helper cells recognize antigens from antigen-presenting cells (APCs) and then release cytokines and activate other immune cells. Parasitic worms influence what kinds of T helper cells are activated.

Shingles



Figure(2-99) This woman has a skin rash from shingles.

Human Papillomavirus (HPV)

- HPV is the most common sexually transmitted disease
- Most people who are infected do not have any symptoms
- Pap tests are performed on females to evaluate cells from the cervix under a microscope
 - Cells are examined for abnormal changes that if left untreated may develop into cervical cancer.

Influenza



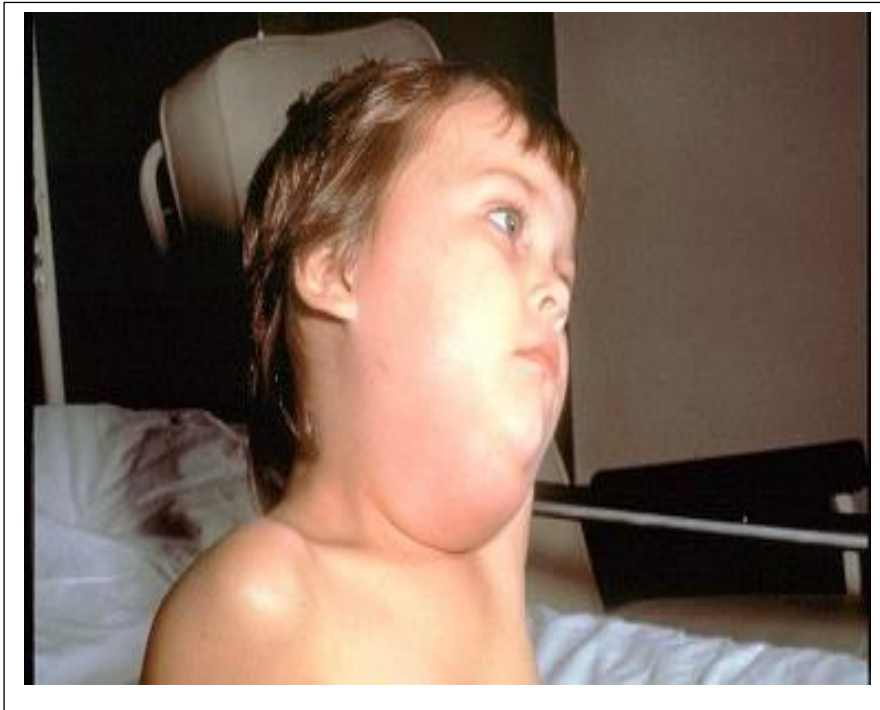
Figure(2-100) This photo shows how influenza germs spread through the air when someone coughs.

Measles (Figure 2-101)



Figure (2-101) Head and shoulders of a boy with measles

Mumps (Figure 2-102)



Figure(2-102) This child is very swollen under the jaw and in the cheeks due to mumps.

Rubella(Figure 2-104)



Figure (2-104) This child was born with cataracts caused by a rubella infection his mother .transmitted to him before birth

Rotavirus

- Causes severe vomiting and diarrhea that can lead to dehydration.
- Dehydration can lead to death particularly in countries where medical care is not readily accessible.
- Before there was a vaccine virtually all children had this infection by the age of five.

Chickenpox (Figure 2-105)

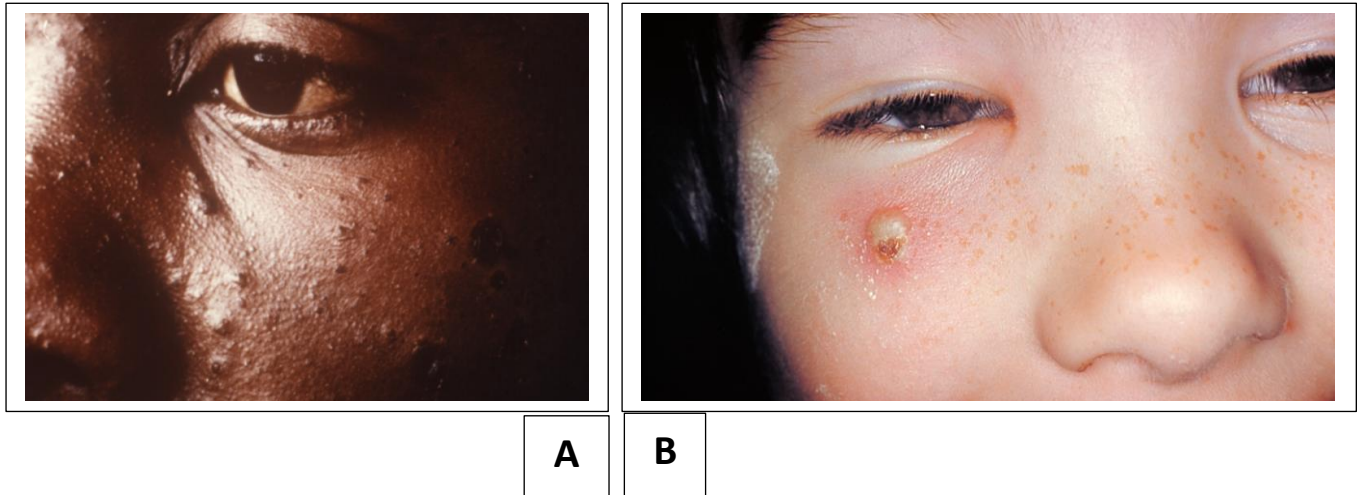


Figure (2-105) Illustrated A- Patient with chickenpox infection B- One of the chickenpox lesions became infected leading to complications.

Purpose of Vaccination

1. Protect the individual from disease.
2. Reduce the severity of disease.
3. Protect the community.
4. Eradication of the disease.

History of vaccine development (Table 2-7)

In 1796 Edward Jenner, used matter from cowpox pustules to inoculate patients successfully against smallpox, which is caused by a related virus.

By 1900, there were two human virus vaccines, against smallpox and rabies, and three bacterial vaccines against typhoid, cholera, and plague.

During the 20th century, other vaccines that protect against once commonly fatal infections such as pertussis, diphtheria, tetanus, polio, measles, rubella, and several other communicable diseases were developed. The initial EPI goals were to ensure that every child received protection against six childhood diseases (i.e. tuberculosis, polio, diphtheria, pertussis, tetanus and measles) by the time they were one year of age and to give tetanus toxoid vaccinations

to women to protect them and their newborns against tetanus.

Since then, new vaccines have become available. Some of them, such as hepatitis B, rotavirus, Haemophilus

influenzae type b (Hib) and pneumococcal vaccines, are recommended by the WHO for global use. Others, such as yellow fever vaccine, are recommended in countries where disease burden data indicate they should be used.

Regulatory and safety issues of vaccines before and after licenses

How the immune system works

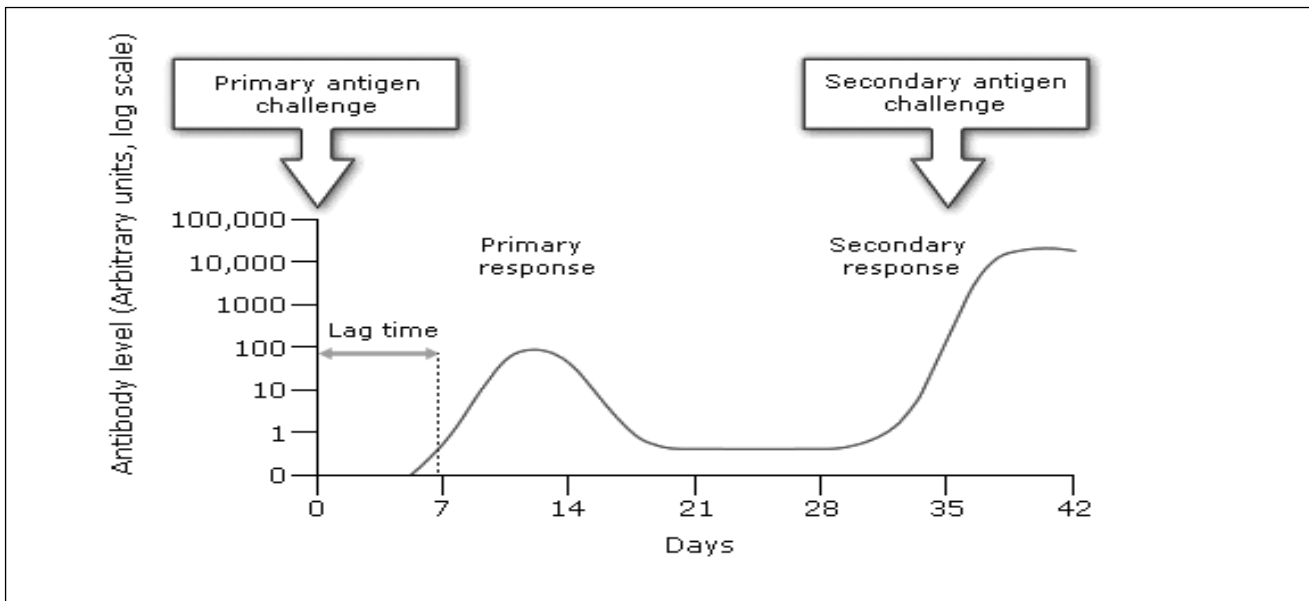
Table (2-7) History of vaccine development

		1955 Polio (IPV)		
		1962 Polio (OPV)		
		1963 Measles		
		1967 Mumps		
		1969 Meningitis A		
	1923 Diphtheria	1970 Rubella	1981 Hepatitis B	
1798 Smallpox	1923 Tuberculosis	1972 <i>Haemophilus influenzae</i>	1986 Meningitis B	
1885 Cholera	1924 Tetanus	1976 Viral influenza	1989 Hepatitis A	
1885 Rabies	1926 Pertussis	1976 Pneumococcal polysaccharide	1995 Varicella zoster	2000 Pneumococcal conjugate
1891 Anthrax	1927 Tetanus	1977 Meningitis C (polysaccharide)	1998 Rotavirus	2006 Human papilloma virus
1896 Typhoid	1935 Yellow fever		1999 Meningitis C (conjugate)	
1897 Plague	1943 Typhus			
1800-1899	1900-1949	1950-1979	1980-1999	2000

How the immune system works ?

The pathogens causing the vaccine-preventable are mainly microorganisms such as bacteria or viruses.

- Bacteria are single-celled life-forms that can reproduce quickly on their own.
- Viruses, on the other hand, cannot reproduce on their own. They are ultramicroscopic infectious agents that replicate themselves only within cells of living hosts.



Figure(2-106) Primary and secondary immune responses to the same pathogen

Types of vaccine

Live attenuated (LAV)

- Tuberculosis (BCG)
- Oral polio vaccine (OPV)
- Measles
- Rotavirus
- Yellow fever

Inactivated (killed antigen)

- Whole-cell pertussis (wP)
- Inactivated polio virus (IPV)

Subunit (purified antigen)

- Acellular pertussis (aP),
- Haemophilus influenzae type b (Hib),
- Pneumococcal (PCV-7, PCV-10, PCV-13)
- Hepatitis B (HepB)

Toxoid (inactivated toxins)

- Tetanus toxoid (TT),
- Diphtheria toxoid

Arranged by Sarah Mohssen

Mono and polyvalent vaccines

Vaccines may be monovalent or polyvalent. A monovalent vaccine contains a single strain of a single antigen (e.g. Measles vaccine), whereas a polyvalent vaccine contains two or more strains/serotypes of the same antigen (e.g. OPV).

Combination vaccines

Some of the antigens above can be combined in a single injection that can prevent different diseases or that protect against multiple strains of infectious agents causing the same disease (e.g. combination vaccine DPT combining diphtheria, pertussis and tetanus antigens). Combination vaccines can be useful to overcome logistic constraints of multiple injections, and accommodate for a children's fear of needles and pain.

Live attenuated vaccines

Available since the 1950s, live attenuated vaccines (LAV) are derived from disease-causing pathogens (virus or bacteria) that have been weakened under laboratory conditions. They will grow in a vaccinated individual, but because they are weak, they will Live microorganisms provide continual antigenic stimulation giving sufficient time for memory cell production. cause no or very mild disease.

Attenuated pathogens have the very rare potential to revert to a pathogenic form and cause disease in vaccinees or their contacts. Examples for this are the very rare, serious adverse events of: vaccine-associated paralytic poliomyelitis (VAPP) and

disease-causing vaccine-derived poliovirus (VDPV) associated with oral polio vaccine (OPV).

Sustained infection, for example tuberculosis (BCG) vaccination can result in local lymphadenitis or a disseminated infection.

- If the vaccine is grown in a contaminated tissue culture it can be contaminated by other viruses (e.g. retro viruses with measles vaccine).

Many LAVs require strict attention to the cold chain for the vaccine to be are subject to program failure when this is not adhered to.

Protein-based subunit vaccines

- Protein based subunit vaccines present an antigen to the immune system without viral particles, using a specific, isolated protein of the pathogen .

- Commonly used protein-based subunit vaccines are the following:
- **Acellular pertussis (aP)** vaccines contain inactivated pertussis toxin (protein) and may contain one or more other bacterial components. The pertussis toxin is detoxified either by treatment with a chemical or by using molecular genetic techniques.
- **Hepatitis B** vaccines are composed of the hepatitis B virus surface antigen (HBsAg), a protein produced by hepatitis B virus. Earlier vaccine products were produced using purified plasma of infected individuals. This production method has been replaced by recombinant technology that can produce HBsAg without requiring human plasma increasing the safety of the vaccine by excluding the risk from potential contamination of human plasma.

Polysaccharide vaccines

Some bacteria when infecting humans are often protected by a polysaccharide (sugar) capsule that helps the organism evade the human defense systems especially in infants and young children.

Polysaccharide vaccines create a response against the molecules in the pathogen's capsule. These molecules are small, and often not very immunogenic. As a consequence they tend to:

Not be effective in infants and young children (under 18–24 months),

Induce only short-term immunity (slow immune response, slow rise of antibody levels, no immune memory).

Examples of polysaccharide vaccines include Meningococcal disease caused by *Neisseria meningitidis* groups A, C, W135 and Y, as well as Pneumococcal disease.

Conjugate subunit vaccines

Conjugate subunit vaccines also create a response against the molecules in the pathogen's capsule. In comparison to plain polysaccharide vaccines, they benefit from a technology that binds the polysaccharide to a carrier protein that can induce a long-term protective response even in infants.

Various protein carriers are used for conjugation, including diphtheria and tetanus toxoid. Conjugate subunit vaccines, can therefore prevent common bacterial infections for which plain polysaccharide vaccines are either ineffective in those most at risk (infants) or provide only short-term protection (everyone else).

The advent of conjugate subunit vaccines heralded a new age for immunization against diseases caused by encapsulated organisms such as meningococcus, Haemophilus influenzae type b (Hib) and pneumococcus.

WHO recommends that children receive Haemophilus influenzae type b (Hib) and pneumococcal conjugate vaccines. In addition,.

Toxoid vaccines

Toxoid vaccines are based on the toxin produced by certain bacteria (e.g. tetanus or diphtheria).

The toxin invades the bloodstream and is largely responsible for the symptoms of the disease. The protein-based toxin is rendered harmless (toxoid) and used as the antigen in the vaccine to elicit immunity.

To increase the immune response, the toxoid is adsorbed to aluminium or calcium salts, which serve as adjuvants.

Components of a vaccine

Vaccines include a variety of ingredients including antigens, stabilizers, adjuvants, antibiotics, and preservatives.

- **Antigens**

Antigens are the components derived from the structure of disease-causing organisms, which are recognized as 'foreign' by the immune system and trigger a protective immune response to the vaccine.

- **Stabilizers**

Stabilizers are used to help the vaccine maintain its effectiveness during storage. Bacterial vaccines can become unstable due to hydrolysis and aggregation of protein and carbohydrate molecules. Stabilizing agents include MgCl₂ (for OPV), MgSO₄ (for measles), lactose-sorbitol and sorbitol-gelatine.

Adjuvants

Adjuvants are added to vaccines to simulate the production of antibodies against the vaccine to make it more effective.

Adjuvants have been used for decades to improve the immune response to vaccine antigens, most often in inactivated (killed) vaccines. In conventional vaccines, adding adjuvants into vaccine formulations is aimed at enhancing, accelerating and prolonging the specific immune response to vaccine antigens.

Aluminium salts example

Aluminium salts are among the oldest adjuvants that are commonly used. They slow the escape of the antigen

from the site of injection thereby lengthening the duration of contact between the antigen and the immune system

(i.e. macrophages and other antigen-receptive cells).

Antibiotics

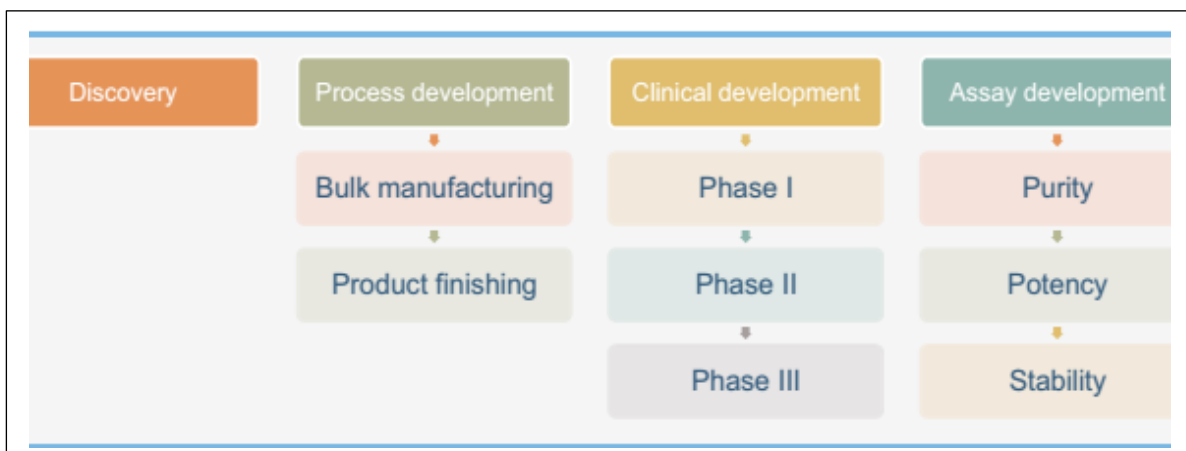
Antibiotics (in trace amounts) are used during the manufacturing phase to prevent bacterial contamination of the tissue culture cells in which the viruses are grown. Usually only trace amounts appear in vaccines, for example, MMR vaccine and IPV each contain less than 25 micrograms of neomycin per dose. Persons who are known to be allergic to neomycin should be closely observed after vaccination so that any allergic reaction can be treated at once.

Preservatives

Preservatives are added to multidose vaccines to prevent bacterial and fungal growth. They include a variety of substances, for example Thiomersal, Formaldehyde, or Phenol derivatives.

Vaccine Development

Table (2-8) The main steps for vaccine development



The development process for vaccines is unique. Vaccine development is highly capital intensive and risky. Given the importance of safety with biologics, the vaccine industry is highly regulated

Research to discover new vaccine antigens and novel approaches to immunization usually takes several years, and costs tens of millions of dollars. (Table 2-8)

Once a discovery is made, several developments must be undertaken to reach the licensing stage. The development of each of these processes is very lengthy, requiring on average 10–15 years. The total development costs can reach close to \$US1 billion

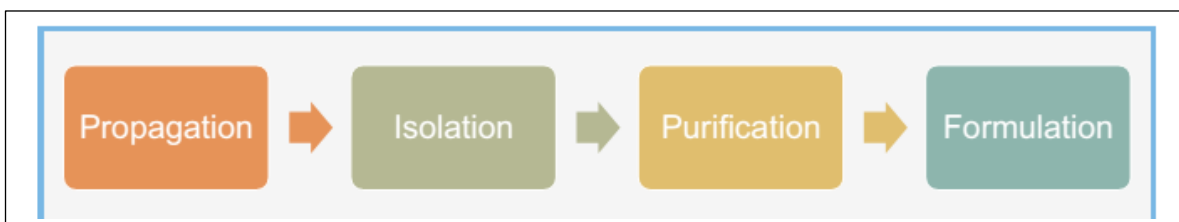
Vaccine Manufacturing

The manufacture of vaccines is achieved from the propagation of living microorganisms. Some of these may be dangerous human pathogens. Therefore, the manufacture of vaccines is conducted in a highly regulated and controlled environment.

All vaccine manufacturers are subject to national and international regulatory control and must comply with specifications for Good Manufacturing Practices (GMP). These requirements vary between countries, but the fundamentals are common .

Manufacturing is conducted in an aseptic environment and closely monitored by quality control measures. Vaccines also require a strict cold chain to maintain their stability. Under most circumstances vaccines are shipped and stored under refrigeration . (Table 2-9)

Table (2-9) Vaccine manufacturing steps



Regulatory process for vaccines under development

Because of their biological nature and because they are largely administered to healthy individuals, the entire vaccine development and manufacturing process is regulated.

Before vaccines are licensed, the three successive phases of clinical development must be approved by a national regulatory authority and may only proceed from one phase to the next upon approval of the national regulator.

The regulator has the authority to refuse or withdraw a product license if the manufacturer is not compliant with current regulations.(Figure2-106)

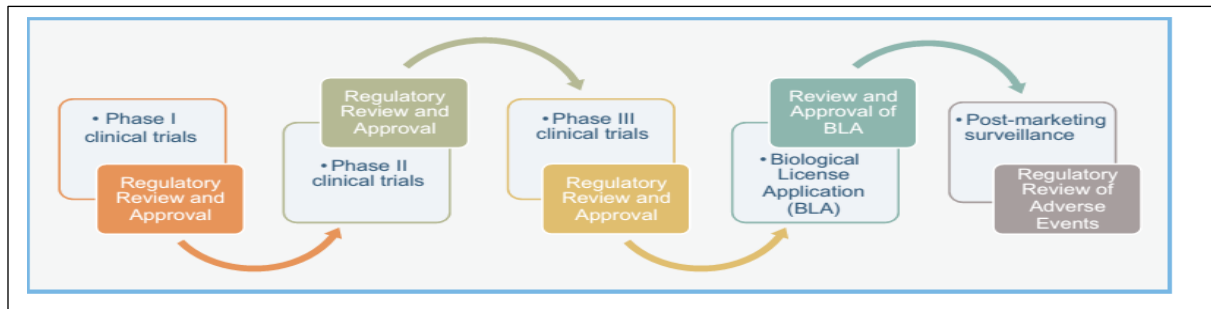


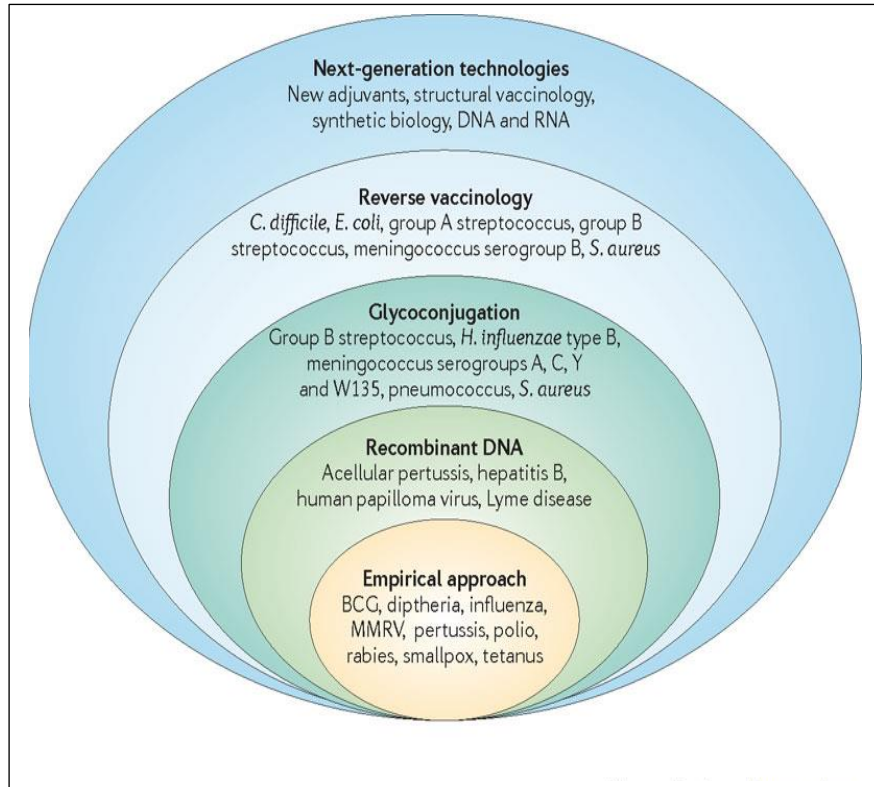
Figure (2-106) Regular steps for vaccine under development

Technologies for vaccine development.

Since the times of Pasteur, vaccines have been developed using empirical approaches consisting mostly of killed or live-attenuated microorganisms, partially purified components of pathogens (subunit vaccines), detoxified toxins or polysaccharides.

These vaccines have been very successful in eliminating many devastating diseases.

During the past 30 years, subsequent waves of new technologies have made possible vaccines that were impossible with the empirical approaches. These include recombinant DNA technology, glycoconjugation, reverse vaccinology and many emerging next-generation technologies, such as novel adjuvants, synthetic biology and structure-based vaccine design (structural vaccinology)(Figure2-107)



Figure(2-107) Technology for vaccine development

Vaccine efficacy

Vaccine efficacy varies according to the type of vaccine and the manner in which the vaccine antigen is processed by the immune system.

Vaccine efficacy may also vary between different populations. However, in general, the efficacy of licensed vaccines ranges from above 70% to almost 100.

In other words, vaccines could be expected to reduce the attack rates in the vaccinated population by 70–100% compared to the attack rates in the unvaccinated population.

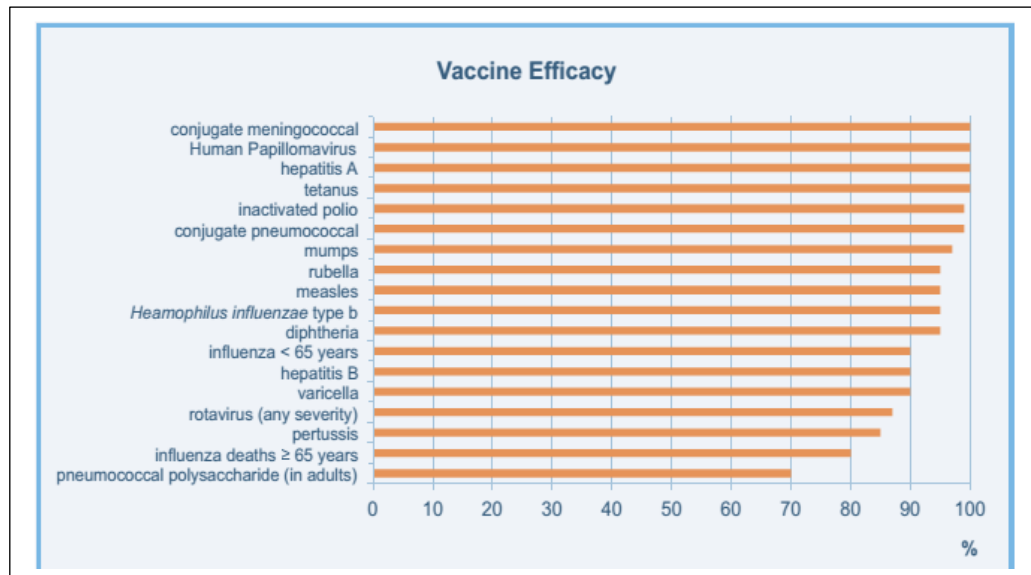
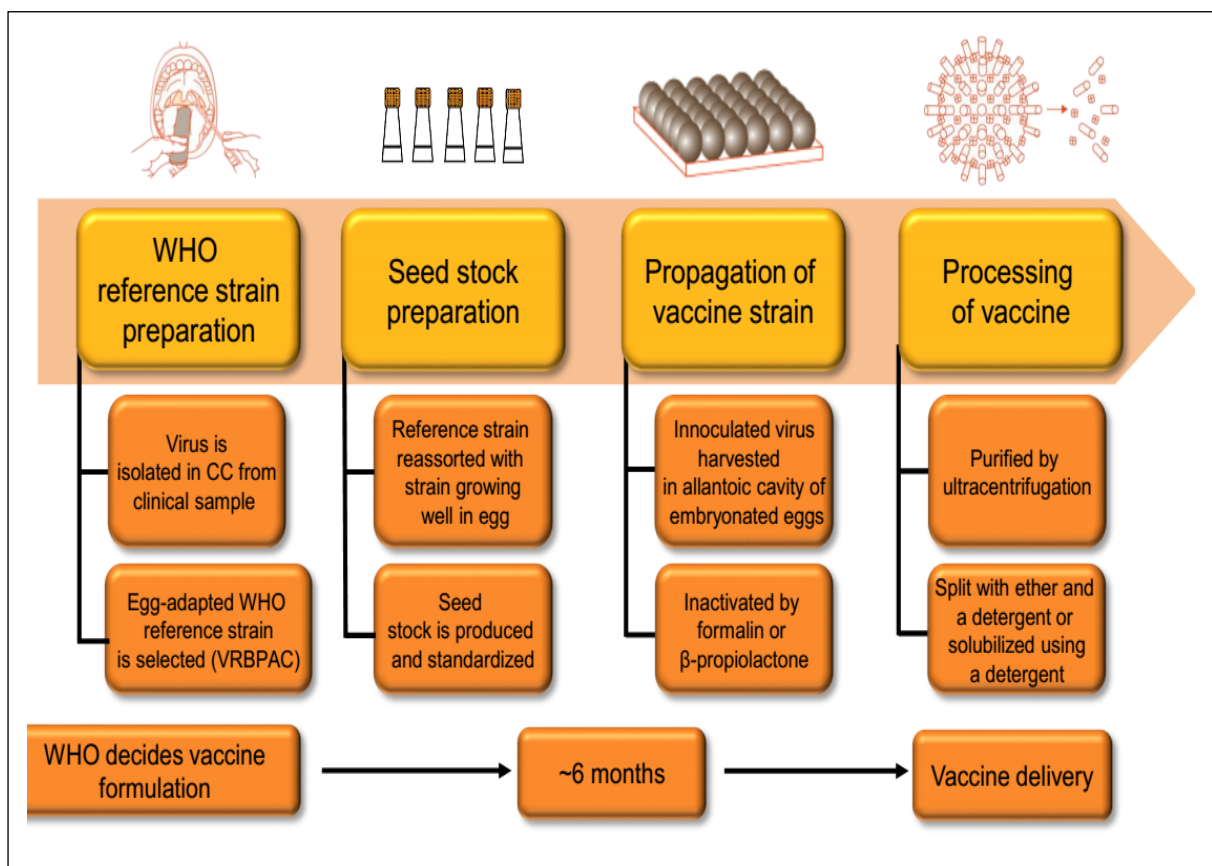


Figure (2-108) Vaccine efficacy

Production of Licensed US Influenza Vaccines: by Growing Viral Isolates in Embryonated Chicken Eggs



Figure(2-109) Influenza Vaccines production steps

Gardasil

A genetically engineered vaccine

Gardasil, a genetically engineered vaccine, prevents cervical cancer by blocking infection with the two viruses that together cause about 70 percent of cervical cancers. HPV 16 and 18, both sexually transmitted viruses, are two of the 100-plus types of human papilloma virus.(Figure2-110)



Figure2-110)labelling for Gardasil

Vaccines and bananas

Bananas have potential to become the world's first edible vaccine due to Agrobacterium. An edible vaccine doesn't need sterile syringes, costly refrigeration, or multiple injections. According to the World Health Organization (WHO), more than 2 million children die worldwide each year from diarrhea that can be prevented easily with vaccines.(Figure 2-111)



Figure(2-111)An edible vaccine

Vaccine trails in Alzheimer's disease

The most promising areas of Alzheimer's disease research involves vaccine-based therapies which stimulate the body to produce antibodies to amyloid-beta protein and remove it from the brain.

(Figure 2-112)



Figure(2-112)Vaccine trails Alzheimer

The field of parasitology is often associated with tropical areas; however, many parasitic organisms that infect humans are worldwide in distribution and occur with some frequency in the temperate zones.

Also, an increase in the number of compromised patients, particularly those who are immunodeficient or immunosuppressed, has led to increased interest in the field of parasitology. These individuals are greatly at risk for certain parasitic infections. Parasites of humans are classified into six major divisions:

1. Protozoa (amebae, flagellates, ciliates, sporozoans, coccidia, microsporidia)
2. Nematoda or roundworms
3. Platyhelminthes, or flatworms (cestodes, trematodes)
4. Pentastomids, or tongue worms
5. Acanthocephala, or thorny-headed worms
6. Arthropoda (e.g., insects, spiders, mites, ticks)

The Parasites to be considered:

1)Protozoa:

A-Intestinal:

1- Amebae (intestinal):

Entamoeba histolytica

Entamoeba dispar

Entamoeba coli

Entamoeba hartmanni

Endolimax nana

Iodamoeba bütschlii

Blastocystis hominis

2- Flagellates (intestinal):

Giardia lamblia†

Chilomastix mesnili

Dientamoeba fragilis

Pentatrichomonas hominis

3- Ciliates (intestinal):

Balantidium coli

4- Coccidia, Microsporidia (intestinal):

Cryptosporidium spp.

Cyclospora cayetanensis

Isospora (Cystoisospora) belli

Sarcocystis hominis

Sarcocystis suihominis

5- Microsporidia (intestinal):

Enterocytozoon bieneusi

Encephalitozoon spp.

B- Blood and Tissue Protozoa (Sporozoa, Flagellates):

1-Sporozoa (Malaria and Babesiosis)

Plasmodium vivax

Plasmodium ovale

Plasmodium malariae

Plasmodium falciparum

Plasmodium knowlesi

Babesia spp.

2-Flagellates (Leishmaniae, Trypanosomes)

Leishmania tropica complex

Leishmania mexicana complex

Leishmania braziliensis complex

Leishmania donovani complex

Leishmania peruviana

Trypanosoma brucei gambiense

Trypanosoma brucei rhodesiense

Trypanosoma cruzi

Trypanosoma rangeli

C- Other Body Sites: Amebae, Flagellates, Coccidia.

1-Amebae

Naegleria fowleri

Acanthamoeba spp.

Balamuthia mandrillaris

2-Flagellates

Trichomonas vaginalis

Trichomonas tenax

3-Coccidia (Other Body Sites)

Toxoplasma gondii

2) Nematoda or round worms:

A-Intestinal Nematodes (Roundworms):

Helminths

Nematodes

Ascaris lumbricoides

Enterobius vermicularis (pinworm)

Strongyloides stercoralis (threadworm)

Trichostrongylus spp.

Trichuris trichiura (whipworm)

Capillaria philippinensis (hookworms)

Ancylostoma duodenale (Old World)

Necator americanus (New World)

B-Tissue Nematodes (Roundworms):

Helminths

Trichinella spiralis

Visceral larva migrans (*Toxocara canis* or *Toxocara cati*)

Ocular larva migrans (*Toxocara canis* or *Toxocara cati*)

Cutaneous larva migrans (*Ancylostoma braziliense* or

Ancylostoma caninum)

Dracunculus medinensis

Parastrongylus (*Angiostrongylus cantonensis*)

Parastrongylus (*Angiostrongylus costaricensis*)

Gnathostoma spinigerum)

C-Blood and Tissue (Filarial) Nematodes:

Nematodes

Wuchereria bancrofti

Brugia malayi

Brugia timori

Loa loa

Onchocerca volvulus

Mansonella ozzardi

Mansonella streptocerca

Mansonella perstans

3)Platyhelminthes or flatworms:(Cestodes, Trematodes)

3.1.Cestodes:

A-Intestinal Cestodes (Tapeworms):

Diphyllobothrium latum

Dipylidium caninum

Hymenolepis nana

Hymenolepis diminuta

Taenia solium

Taenia saginata

B-Tissue Cestodes(Tapeworms):

Tissue (Larval Forms)

Taenia solium

Echinococcus granulosus

Echinococcus multilocularis

Taenia multiceps

Spirometra mansonioides

3.2. Trematodes:

A-Intestinal Trematodes

Helminths Trematodes (Flukes) Intestinal Like:

Fasciolopsis buski

Heterophyes heterophyes

Metagonimus yokogawai

B-Liver and Lung Trematodes

Clonorchis (Opisthorchis) sinensis

Opisthorchis viverrini

Fasciola hepatica

Paragonimus westermani

Paragonimus mexicanus

C-Blood Trematodes

Schistosoma mansoni

Schistosoma haematobium

Schistosoma japonicum

Schistosoma intercalatum

Schistosoma mekongi

Protozoa:

The protozoa are unicellular eukaryotic organisms, most of which are microscopic. They have a number of specialized organelles that are responsible for life functions and that allow further division of the group into classes. Most protozoa multiply by binary fission and are ubiquitous worldwide.

The clinically relevant intestinal protozoa are generally considered to be *Entamoeba histolytica*, *Blastocystis hominis*, *Giardia lamblia*, *Dientamoeba fragilis*, *Balantidium coli*, *Isospora (Cystoisospora) belli*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, and the microsporidia.

(Table 1):Description of the More Common Groups of Human Parasites.

Parasite Group	Description
Flagellates	Trypanosomatid protozoa; morphologic forms are identified based on the position,

(trypanosomes	length, and attachment site of the flagella. At some time in their life cycle, these protozoa have the trypomastigote form with the typical undulating membrane and free flagellum at the anterior end. Transmission is typically through an insect vector. Some organisms cause African sleeping sickness (e.g., <i>Trypanosoma brucei gambiense</i>, <i>T. b. rhodesiense</i>). The etiologic agent of American trypanosomiasis is <i>T. cruzi</i>, which has amastigote and trypomastigote stages in the mammalian host and an epimastigote form in the arthropod host
Nematodes, intestinal	Helminthic parasites; roundworms. Nematodes have separate sexes, are elongate-cylindrical and bilaterally symmetrical with a triradiate symmetry at the anterior end. Nematodes have an outer cuticle layer, no circular muscles, and a pseudocoel that contains all systems (digestive, excretory, nervous, reproductive). Transmission is by ingestion of eggs or by skin penetration of larval forms from the soil. Examples: <i>Ascaris</i>, <i>Enterobius</i>, <i>Trichuris</i>, and <i>Strongyloides</i> spp. and hookworm.
Nematodes, tissue	Helminthic parasites; roundworms. Many of these organisms are rarely seen in the United States; however, some are important and are found worldwide. Diagnosis may be difficult if the only specimens are obtained through biopsy and/or autopsy, and interpretation must be based on examination of histologic preparations. Examples: <i>Trichinella</i> spp., visceral larva migrans (VLM), ocular larva migrans (OLM), cutaneous larva migrans (CLM).
Nematodes, filarial	Helminthic round worms. Transmission is via arthropods. Adult worms tend to live in the tissues or lymphatics of the vertebrate host. The diagnosis is made on the basis of recovery and identification of the larval worms (microfilariae) in the blood, other body fluids, or skin. Examples: <i>Wuchereria</i>, <i>Brugia</i>, <i>Loa</i>, and <i>Onchocerca</i> spp
Cestodes, intestinal	Helminthic tapeworms. Adult tapeworm consists of a chain of egg-producing units called <i>proglottids</i>,

	which develop from the neck region of the attachment organ (scolex). Food is absorbed through the worm's integument. The intermediate host contains the larval forms that are acquired through ingestion of the adult tapeworm eggs. Transmission is through the ingestion of larval forms in poorly cooked or raw meat or freshwater fish. Examples: <i>Dipylidium caninum</i> (infection is acquired by accidental ingestion of dog fleas). <i>Hymenolepis nana</i> and <i>H. diminuta</i> are transmitted via ingestion of certain arthropods (fleas, beetles). Also, <i>H. nana</i> can be transmitted through egg ingestion (life cycle can bypass the intermediate beetle host). Humans can serve as both the intermediate and definitive hosts in <i>H. nana</i> and <i>Taenia solium</i> infections
Cestodes, tissue	Tissue tapeworms. Transmission is through ingestion of certain tapeworm eggs or accidental contact with certain larval forms, leading to tissue infection. Humans serve as the accidental intermediate host. Examples: <i>Taenia solium</i>, <i>Echinococcus granulosus</i>, and several other species.
Trematodes, intestinal	Flatworms that are exclusively parasitic. Except for the schistosomes (blood flukes), flukes are hermaphroditic. They may be flattened; most have oral and ventral suckers. Transmission: Intestinal trematodes require a freshwater snail to serve as an intermediate host; these infections are food borne (freshwater fish, mollusks, or plants). Example: <i>Fasciolopsis buski</i>, the giant intestinal fluke.
Trematodes, liver, lung	Transmission: Liver and lung trematodes require a freshwater snail to serve as an intermediate host; these infections are food borne (freshwater fish, crayfish or crabs, or plants). Examples: Public health concerns include cholangiocarcinoma associated with <i>Clonorchis</i> and <i>Opisthorchis</i> infections, severe liver disease associated with <i>Fasciola</i> infections, and misdiagnosis of tuberculosis in individuals infected with <i>Paragonimus</i> spp.
Trematodes, blood	Schistosomes; sexes are separate. Males are characterized by an infolded body that forms the

	gynecophoral canal in which the female worm is held during copulation and oviposition. Transmission: Infection is acquired by skin penetration by the cercarial forms that are released from freshwater snails. The adult worms reside in the blood vessels over the small intestine, large intestine, or bladder. Examples: <i>Schistosoma mansoni</i>, <i>S. haematobium</i>, and <i>S. japonicum</i>

Amebae:

Amebae, includes the organisms capable of movement by means of cytoplasmic protrusions called **pseudopodia**. This group includes free-living organisms, in addition to nonpathogenic and pathogenic organisms found in the intestinal tract and other areas of the body. (see Table 1).

Occasionally, when fresh stool material is examined as a direct wet mount, motile trophozoites may be seen, as well as other, nonparasitic structure.

Entamoeba histolytica:

General Characteristics:

Living trophozoites (motile feeding stage) of *E. histolytica* vary in size from about 12 to 60 μm in diameter. Organisms recovered from diarrheic or dysenteric stools generally are larger than those in formed stool from an asymptomatic individual. The motility has been described as rapid and unidirectional. Although this characteristic motility is often described, amebiasis rarely is diagnosed on the basis of motility seen in a direct mount. The cytoplasm is differentiated into a clear outer ectoplasm and

a more granular inner endoplasm. *E. histolytica* has directional and progressive motility, whereas the other amebae tend to move more slowly and at random. However, motility is rarely seen even in a fresh wet mount from a patient with diarrhea or dysentery. The cytoplasm is generally more finely granular, and the presence of red blood cells (RBCs) in the cytoplasm is considered diagnostic for *E. histolytica* (Figure1).

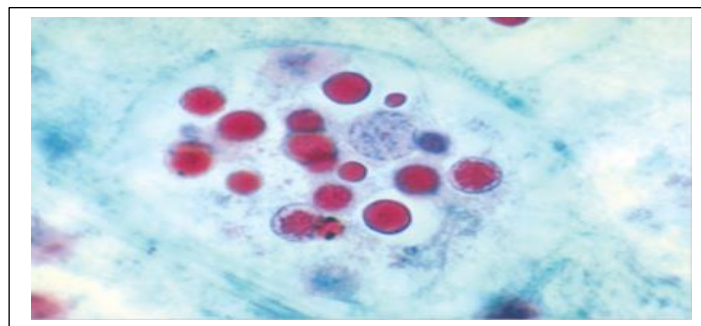


Figure 1 *Entamoeba histolytica* trophozoite containing ingested red blood cells.

Permanent stained smears demonstrate accurate morphology compared with other techniques. When the organism is examined on a permanent stained smear (trichrome or iron-hematoxylin stain), the morphologic features of the mature cyst (metacyst) (see Figure 2,3),

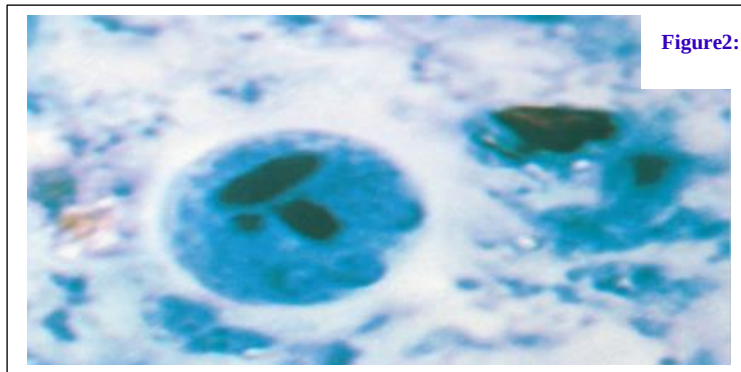


Figure 2: *Entamoeba histolytica*/*Entamoeba dispar* cyst.

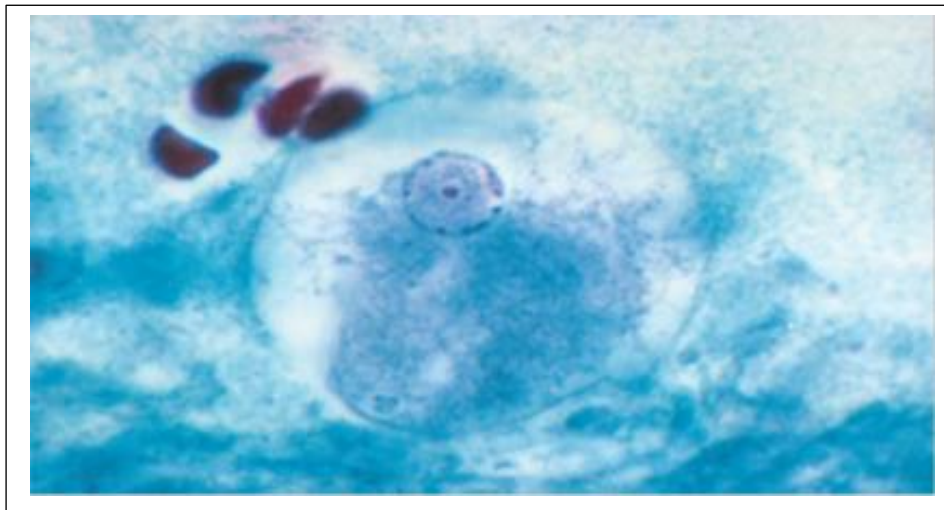


Figure 3: *Entamoeba dispar* trophozoite; no ingested red blood cells are present.

nuclear division occurs, with the production of four nuclei. Often chromatoids may be absent in the mature cyst. Cyst morphology does not differentiate *E. histolytica* from *E. dispar*. Cyst formation occurs only in the intestinal tract; once the stool has left the body, cyst formation does not occur. The one-, two-, and four-nucleated cysts are infective and represent the mode of transmission from one host to another.

Epidemiology:

Amebiasis is caused by infection with the true pathogen, *Entamoeba histolytica*. Recent evidence from molecular studies confirms the differentiation of pathogenic *E. histolytica* and nonpathogenic *E. dispar* as two distinct species. *E. histolytica* is considered the etiologic agent of amebic colitis and extraintestinal abscesses (amebic liver abscess), whereas nonpathogenic *E. dispar* produces no intestinal symptoms and is not invasive in humans.

Infection is acquired through the fecal-oral route from infective cysts contained in the feces. These cysts can be ingested in contaminated food or drink or contracted from fomites or various sexual practices that could include accidental ingestion of fecal organisms.

The infection occurs worldwide, particularly in areas with poor sanitation. It is estimated that *E. histolytica* infection kills more than 100,000 people each year.

Pathogenesis and Spectrum of Disease:

The pathogenesis of *E. histolytica* is related to the organism's ability to directly lyse host cells and cause tissue destruction.

Amebic lesions show evidence of cell lysis, tissue necrosis, and damage to the extracellular matrix. Evidence indicates that *E. histolytica* trophozoites interact with the host through a series of steps: adhesion to the target cell, phagocytosis, and cytopathic effect.

Numerous other parasite factors also play a role. From the perspective of the host, *E. histolytica* induces both humoral and cellular immune responses; cell-mediated immunity is the major human host defense against this complement-resistant cytolytic protozoan.

The presentations of disease are seen with invasion of the intestinal mucosa or dissemination to other organs (most often the liver) or both. However, it is estimated that a small proportion (2% to 8%) of infected individuals have invasive disease beyond the lumen of the bowel. Also, organisms may be spontaneously eliminated with

no disease symptoms. Blood flow from the mesenteric veins surrounding the intestine returns blood, via the portal vein, to the liver, most commonly the upper right lobe. Amebae in the submucosa can be carried by the bloodstream to the liver. The onset of symptoms may be gradual or sudden; upper right abdominal pain and fever (38° to 39°C) are the most consistent findings. Although the liver may be enlarged and tender, liver function tests may be normal or slightly abnormal (jaundice is rare). The abscess can be visualized radiologically, sonically, or by radionuclear scan; most patients have a single abscess in the right lobe of the liver. The most common complication is rupture of the abscess into the pleural space. An abscess also can extend into the peritoneum and through the skin. Hematogenous spread to the brain, lung, pericardium, and other sites is possible (figure 4).

Laboratory Diagnosis:

Routine Methods: The standard O&P examination is the recommended procedure for recovery and identification of *E. histolytica* in stool specimens. Microscopic examination of a direct saline wet mount may reveal motile trophozoites, which may contain RBCs. However, trophozoites with RBCs are found only in a limited number of cases. In many patients who do not present with acute dysentery, trophozoites may be present but do not contain RBCs, and the organisms may be pathogenic *E. histolytica* or nonpathogenic *E. dispar*.

An asymptomatic individual may have few trophozoites and possibly only cysts in the stool. Although the concentration technique is helpful for demonstrating cysts, the most important technique for the recovery and identification of protozoan organisms is the permanent stained smear (normally stained with trichrome or iron-hematoxylin). A minimum of three specimens collected over not more than 10 days may be required for identification. Sigmoidoscopy specimens may be very helpful for identifying organisms. At least six areas of the mucosa should be sampled. Smears from these areas should be examined after permanent staining. However, these specimens are not considered a substitute for the recommended minimum of three stool specimens submitted for O&P examination (direct, concentration, and permanent stained smear).

Liver aspirate material is rarely examined, and often the specimen was not collected properly. Aspirated material must be aliquoted into several different containers as it is removed from the abscess; amebae may be found only in the last portion of the aspirated material, theoretically material from the abscess wall, not necrotic debris from the abscess center.

Antigen Detection: A number of enzyme immunoassay reagents are commercially available, and their specificity and sensitivity provide excellent options for the clinical laboratory. These tests can differentiate the *E. histolytica*/ *E. dispar* group from the rest of the *Entamoeba* species, such as nonpathogenic *Entamoeba coli* or *Entamoeba hartmanni*. Other test reagents can distinguish between *E. histolytica* and *E. dispar*. These kits require fresh or frozen stool;

Antibody Detection. Serologic testing for intestinal disease is rarely recommended unless the patient has true dysentery; even in these cases, the titer (e.g., indirect hemagglutination) may be low and thus difficult to interpret. A definitive diagnosis of intestinal amebiasis should not be made without demonstrating the presence of the organisms. In patients suspected of having extraintestinal disease, serologic tests are diagnostically more effective. Indirect hemagglutination and indirect fluorescent antibody tests have been reported positive with titers greater

than or equal to 1 : 256 and greater than or equal to 1 : 200, respectively, in almost 100% of cases of amebic liver abscess. In the absence of STAT serologic tests for amebiasis (tests with very short turnaround times for results), the decision on diagnosis must be made on clinical grounds and on the basis of results of other diagnostic tests, such as scans.

Histology. A histologic diagnosis of amebiasis can be made when the trophozoites in the tissue are identified. Organisms must be differentiated from host cells. Periodic acid-Schiff (PAS) staining often is used to help locate the organisms, which appear bright pink with a green-blue background (depending on the counterstain used).

Nucleic Acid-Based Techniques : Nucleic acid-based amplification methods, including polymerase chain reaction, have been developed for the identification of *E. histolytica*. Stool specimens, however, may contain inhibitors that would prevent accurate detection using amplification methods. These tests are not widely used, because they require more technical expertise and currently have not proven to be more sensitive than antigen-based immunoassays.

Therapy:

Two classes of drugs are used in the treatment of amebic infections: luminal amebicides, such as iodoquinol or diloxanide furoate, and tissue amebicides, such as metronidazole, chloroquine, or dehydroemetine. Because of the differences in drug efficacy, it is important that the laboratory report indicates whether cysts, trophozoites, or both are present in the stool specimen.

Prevention:

Humans are the reservoir host for *E. histolytica*, and infection can be transmitted to other humans, primates, dogs, cats, and possibly pigs. Accidental consumption of sewage-contaminated water provides another route of infection. Amebiasis is considered a zoonotic waterborne infection. The cyst stages are resistant to environmental conditions and can remain viable in the soil for 8 days at 28° to 34°C, for 40 days at 2° to 6°C, and for 60 days at 0°C. Cysts normally are removed by sand filtration or destroyed by 200 ppm of iodine, 5% to 10% acetic acid,

or boiling. However, an asymptomatic carrier who is a food handler generally is thought to play the most important role in transmission. Proper disposal of contaminated feces is considered the most important preventive measure. Although vaccines have been discussed as a possibility for eliminating human disease, nothing currently is available.

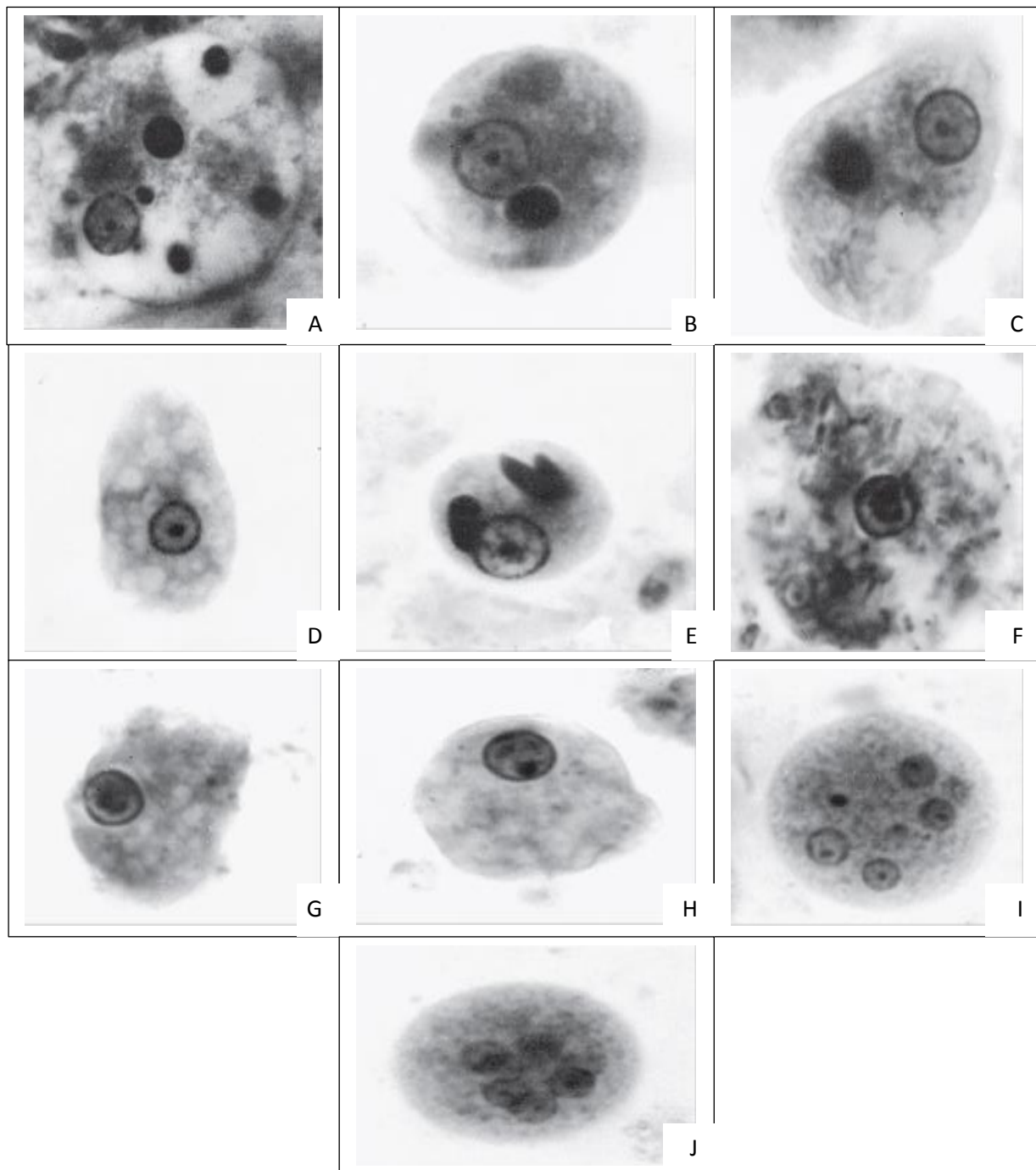


Figure 4: A to C, Trophozoites of *Entamoeba histolytica* (note ingested red blood cells). D, Trophozoite of *E. histolytica*/*E. dispar*. E, Early cyst of *E. histolytica*/*E. dispar*. F to H, Trophozoites of *Entamoeba coli*. I and J, Cysts of *E. coli*.

Entamoeba coli*:*General Characteristics**

The life cycle of *E. coli* is identical to that of *E. dispar*. After digestion of infective cysts, the organisms excyst in the intestinal tract and produce trophozoites. Cyst formation occurs as the gut contents move through the intestinal tract; the excreted cysts are the infective form that is transmitted to humans and some animals. *E. coli* trophozoites are somewhat larger than those of *E. histolytica* and *E. dispar* and range from 15 to 50 μm in Diameter.

Motility is sluggish with broad, short pseudopods. In wet preparations, differentiating nonpathogenic *E. coli* from pathogenic *E. histolytica* is almost impossible. On the permanent stained smear viewed at a higher magnification, the cytoplasm is granular with vacuoles containing bacteria, yeasts, and other food materials. The nucleus has a large blotlike karyosome that may be eccentric rather than centrally located. The chromatin on the nuclear membrane tends to be clumped and irregular. Although rare, if RBCs are present in the intestinal tract, *E. coli* may ingest them rather than bacteria.

Early cysts often contain chromatoidal bars, which tend to be splinter shaped and irregular. Eventually, the nuclei divide until the mature cyst, containing eight nuclei, is formed (see Figures 4).

In rare cases, the number of nuclei reaches 16. The cysts measure 10 to 35 μm in diameter, and as they mature, the chromatoidal bars disappear. When the cyst of *E. coli* matures, it becomes more refractive to fixation; therefore, the cyst may be seen on the wet preparation but not on the permanent stained smear. Occasionally, on trichrome smears, the cysts appear distorted and somewhat pink (Figure 5,6).

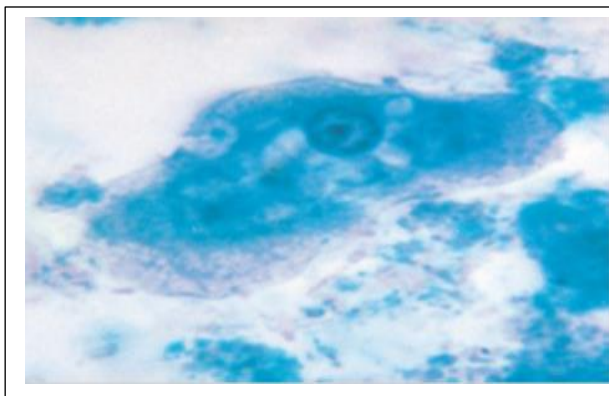


Figure 5: *Entamoeba coli* trophozoite.

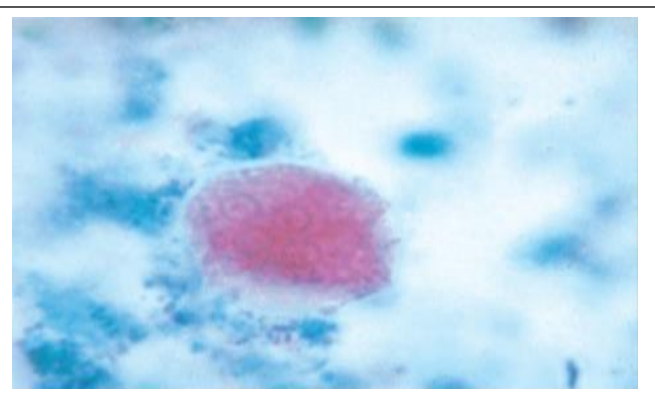


Figure 6 *Entamoeba coli* cyst (trichrome stain) (poor preservation; typical appearance of some *E. coli* cysts).

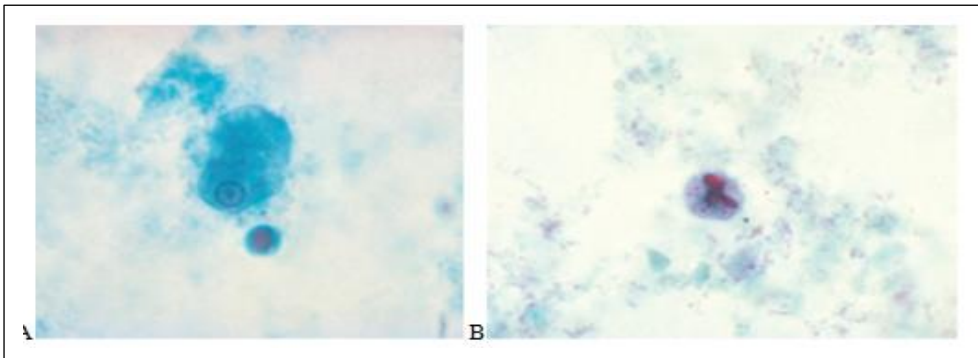


Figure 7 **A**, *Entamoeba hartmanni* trophozoite. **B**, *E. hartmanni* cyst.

Epidemiology:

Transmission occurs through the ingestion of mature cysts from contaminated food or water. The organism is readily acquired, and in some warmer climates or areas with primitive hygienic conditions, the colonization rate can be quite high.

Pathogenesis and Spectrum of Disease:

E. coli are considered nonpathogenic and do not cause disease.

Laboratory Diagnosis:

Unless the mature cyst with eight nuclei is seen, the morphologies of *E. histolytica*, *E. dispar*, *E. moshkovskii*, and *E. coli* are similar in the trophozoite and immature cyst stages. *E. moshkovskii* is typically a free-living amoeba isolated in river or stream sediment and rarely infects humans. Definitive identification relies on examination of permanent stained smears.

Therapy:

Specific treatment is not recommended for the nonpathogen *E. coli*. Correct differentiation among the species is critical to good patient care. Because the amoebae are acquired through fecal-oral contamination, pathogens and nonpathogens can be found in the same patient. If few *E. histolytica*/*E. dispar* organisms are present among many *E. coli* organisms, extended microscopic examination and/or the use of species-specific immunoassay testing may be required to make the correct

Identification

Prevention:

Prevention depends on adequate disposal of human excreta and improved personal hygiene, preventive measures that apply to most of the intestinal protozoa.

***Entamoeba hartmanni*:**

General Characteristics:

The life cycle of *E. hartmanni* is similar to that of *E. dispar*, with differences in size (Figures 7 and 8). In wet preparations, *E. hartmanni* trophozoites range in size from 4 to 12 μm in diameter, and cysts range in size from 5 to 10 μm in diameter. On the permanent stained smear, the cysts, primarily, tend to shrink as a result of dehydration; therefore, the sizes of all the organisms, including pathogenic *E. histolytica*, may be somewhat smaller (1 to 1.5 μm) than the wet preparation measurements. Trophozoites do not ingest RBCs, and the motility is usually less rapid. The morphologic characteristics of *E. hartmanni* are very similar to those of *E. histolytica*, with two exceptions. Frequently, *E. hartmanni* cysts may contain only one or two nuclei, even though the mature cyst contains four nuclei. Mature cysts of *E. hartmanni* also retain their chromatoidal bars, a characteristic not usually seen in *E. histolytica*/*E. dispar*. *E. hartmanni*'s chromatoidal bars are similar to those of *E. histolytica* and *E. dispar* but smaller and more numerous. At the species level, differentiation between *E. hartmanni* and *E. histolytica*/*E. dispar* depends on size; therefore, laboratories are required to use calibrated microscopes that are checked periodically for accuracy.

Epidemiology:

Transmission occurs through the ingestion of mature cysts from contaminated food or water. If accurate identifications have been recorded, the colonization rate tends to match that of *E. histolytica*.

Pathogenesis and Spectrum of Disease:

E. hartmanni is considered nonpathogenic and does not cause disease.

Laboratory Diagnosis:

Unless the trophozoites and cysts match the size requirements, they are unlikely to be *E. hartmanni*. Definitive identification relies on examination of permanent stained smears and measurements made with the calibrated microscope.

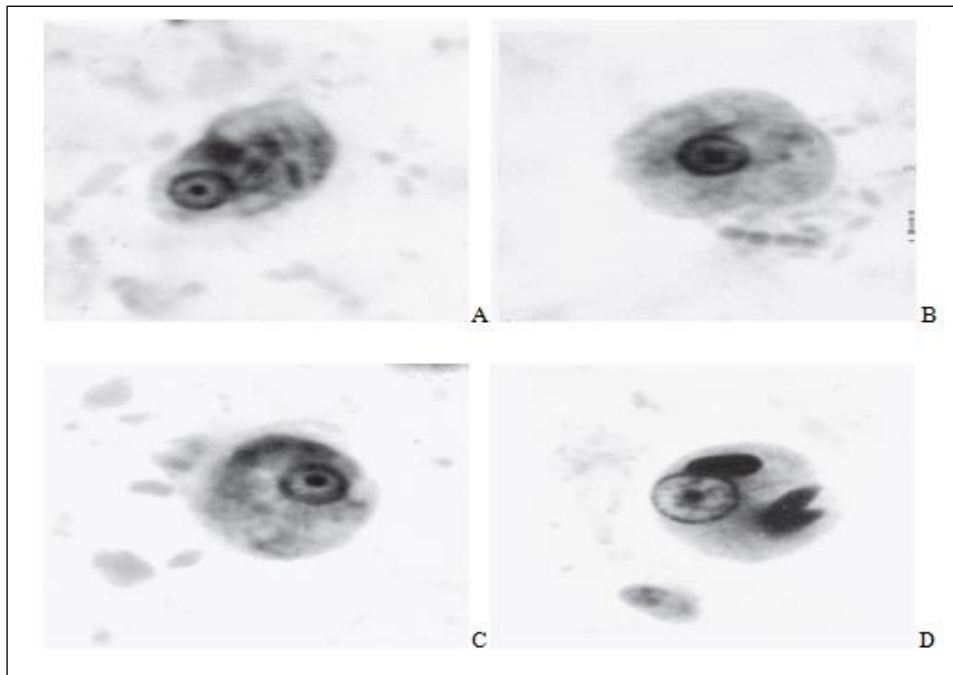


Figure 8 A to C, Trophozoites of *Entamoeba hartmanni*. D and E, Cysts of *E. hartmanni*.

ENDOLIMAX NANA:

General Characteristics:

Endolimax nana, one of the smaller nonpathogenic amebae, has a worldwide distribution and is seen as frequently as *E. coli*. *E. nana* has the same life cycle stages as *E. dispar* and the other nonpathogenic amebae. The trophozoite usually measures 6 to 12 μm in diameter (normal range, 8 to 10 μm) .

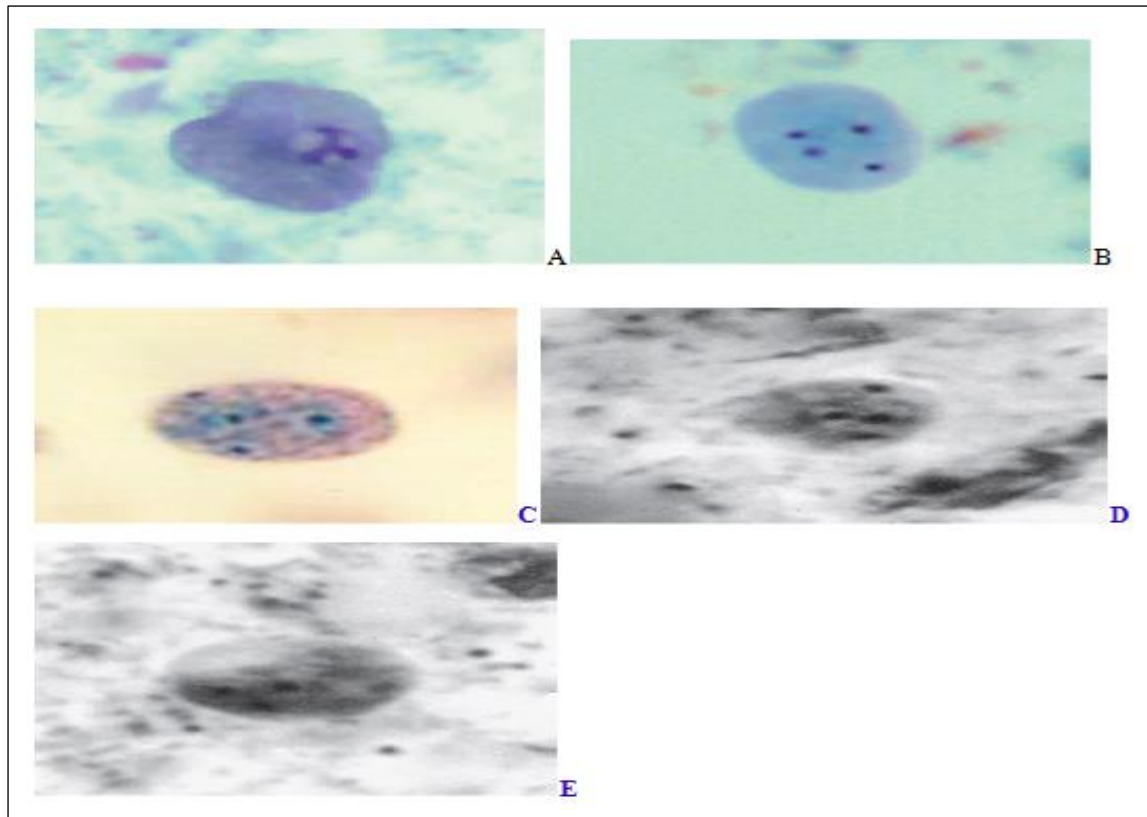


Figure 9 A to C, Trophozoites of *Endolimax nana*. D and E, Cysts of *E. nana*.

Although rarely seen, motility is sluggish and nonprogressive with blunt, hyaline pseudopods. In the permanent stained smear, normally no peripheral chromatin is seen on the nuclear membrane, and the karyosome is large, with either a central or an eccentric location in the nucleus (see Figures 9 and 10). *E. nana* shows more nuclear variation than any of the other amebae, and occasionally *E. nana* can mimic *D. fragilis* or *E. hartmanni*. The cytoplasm may have small vacuoles containing ingested debris or bacteria, but it also may appear relatively clean.

Cysts usually measure 5 to 10 μm in diameter (normal range, 6 to 8 μm). Cysts as large as 14 μm have been seen. The cyst is usually oval to round, with the mature cyst containing four nuclei. The nuclei typically have no peripheral chromatin and are somewhat evenly distributed in the cyst. Occasionally, very small, slightly curved chromatoidal bars are present. The two-nucleated stage is not commonly seen, and frequently both trophozoites and cysts are present in clinical specimens.

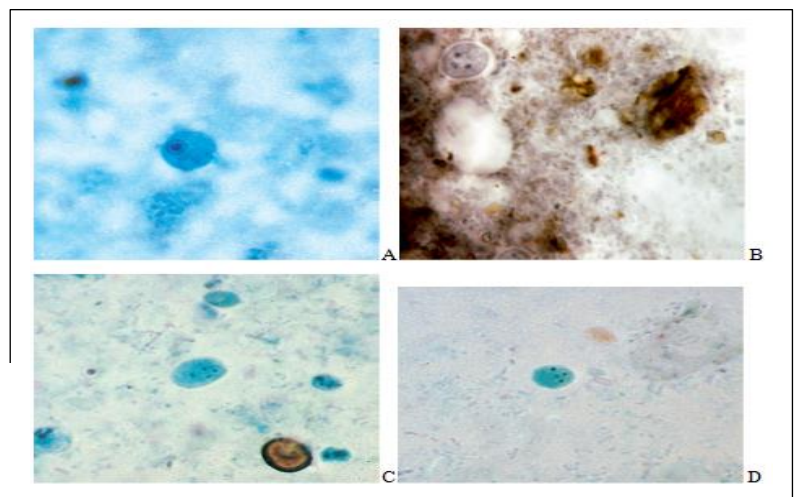


Figure 10 A, *Endolimax nana* trophozoite. B, *E. nana* cyst, iodine stain. C, *E. nana* cyst. D, *E. nana* cyst (Travers, Sioux Falls, S.D.)

Arranged by Sarah Mohssen

Epidemiology:

Transmission occurs through the ingestion of mature cysts from contaminated food or water. The cysts of *E. nana* are less resistant to desiccation than those of *E. coli*. *E. nana* is also found in warm, moist climates and in other areas with a low standard of personal hygiene and poor sanitary conditions. Pathogenesis and Spectrum of Disease *E. nana* is considered nonpathogenic and does not cause disease.

Laboratory Diagnosis:

Although cysts sometimes can be seen in a wet preparation, definitive identification of *E. nana* relies on examination of permanent stained smears.

Iodamoeba bütschlii

General Characteristics:

Iodamoeba bütschlii, one of the nonpathogenic amebae, has a worldwide distribution. Generally, the acquisition rate for this organism is not as high as that for *E. coli* and *E. nana*. The life cycle stages of *I. bütschlii* are exactly the same as those of *E. nana*. The trophozoite varies from 8 to 20 μm in diameter and has fairly active motility in a fresh stool preparation. The cytoplasm is granular, containing numerous vacuoles with ingested debris and bacteria. The cytoplasm is more vacuolated than in *E. nana* trophozoites. The nucleus has a large karyosome, which can be either centrally located or eccentric (Figures 11 and 12).

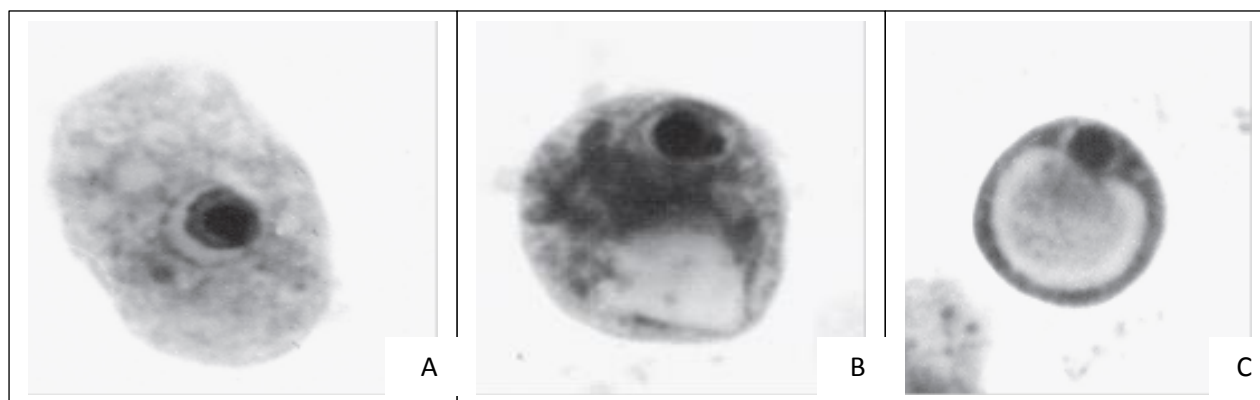


Figure 11 A, Trophozoites of *Iodamoeba bütschlii*. B and C, Cysts of *I. bütschlii*

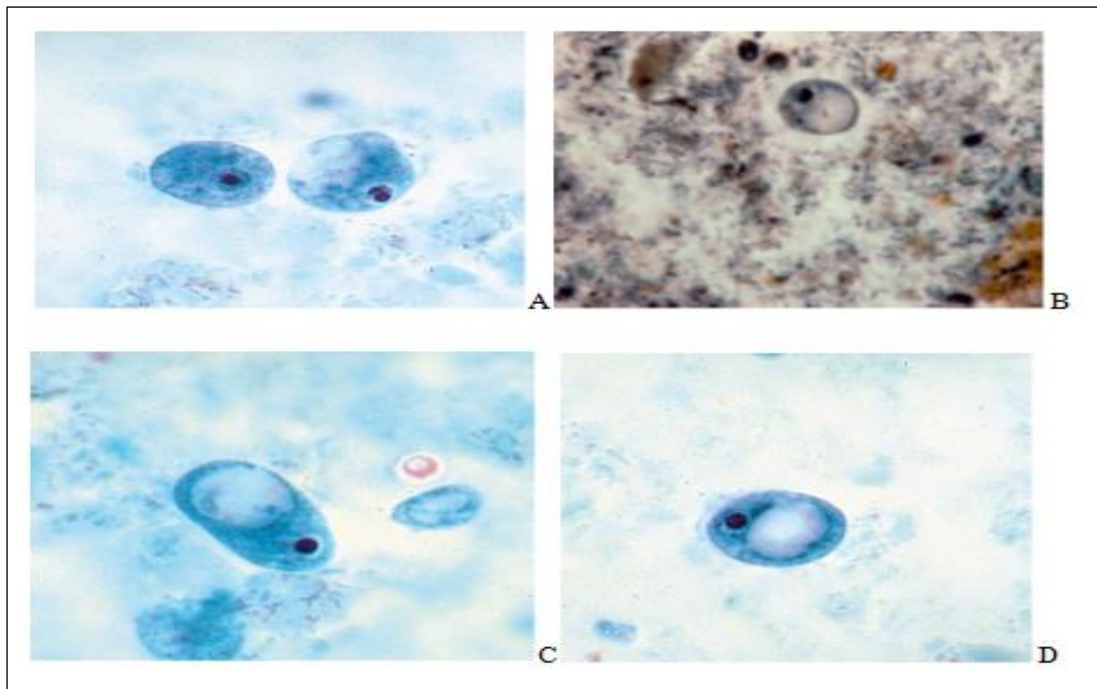


Figure 12 A, *Iodamoeba bütschlii* trophozoites. B, *I. bütschlii* cyst. C, *I. bütschlii* cyst. D, *I. bütschlii* cyst.

On the permanent stained smear, the nucleus may appear to have a halo, and chromatin granules fan out around the karyosome. If the granules are on one side, the nucleus may appear to have a “basket nucleus” arrangement of chromatin, more commonly seen in the cyst stage. The trophozoites of *I. bütschlii* and *E. nana* may appear similar and are difficult to differentiate at the species level, even on the permanent stained smear. Both organisms are considered nonpathogenic. *E. nana* is recovered in clinical specimens much more frequently than is *I. bütschlii*. *I. bütschlii* cysts are round to oval.

The glycogen vacuole is so large that occasionally the cyst collapses on itself. Because nuclear multiplication does not occur in the cyst form, the mature cyst contains a single nucleus. The cysts measure approximately 5 to 20 μm in diameter and are rarely confused with those of other amebae (see Figures 11 and 12).

Epidemiology:

Transmission of *I. bütschlii* occurs through the ingestion of mature cysts from contaminated food or water. This organism is also found in warm, moist climates and in other areas with a low standard of personal hygiene and poor sanitary conditions.

Pathogeneis and Spectrum of Disease:

I. bütschlii is considered nonpathogenic and does not cause disease.

Laboratory Diagnosis

Although *I. bütschlii* cysts sometimes can be seen in a wet preparation, definitive identification relies on the examination of permanent stained smears. Therapy Specific treatment is not recommended for *I. bütschlii*.

Prevention (*E. hartmanni*, *E. nana*, *I. bütschlii*):

Prevention depends on adequate disposal of human excreta and improved personal hygiene, preventive measures that apply to most of the intestinal protozoa.

Blastocystis hominis:

General Characteristics:

Now generally considered a causative agent of intestinal disease. The current recommendation is to report the presence of *B. hominis* and quantitate from the permanent stained smear (i.e., rare, few, moderate, many, packed); this information may be valuable in helping to assess the pathogenicity of the organism in the individual

Patient/ *B. hominis* consists of four major forms. The cyst form is the most recently described form of the life cycle stages. Thick-walled cysts are thought to be responsible for external transmission through the fecal-oral route; thin-walled cysts are thought to cause autoinfection. Cysts can vary in shape but are mostly ovoid or spherical. The central vacuole form (also referred to as the **central body form**) is the most common form found in clinical stool samples. The large central vacuole can occupy most of the cellular volume. The amoeboid form is rarely seen. The granular form can be seen in cultures of *B. hominis*.

Epidemiology:

Infection with *B. hominis* is acquired by the fecal-oral

route from infective forms contained in the feces. The organisms can be ingested in contaminated food and drink or acquired from fomites or through various sexual practices that may include accidental ingestion of fecal organisms. As with *E. histolytica*, flies and cockroaches can be responsible for mechanical transmission. Human-to-human and animal-to-human transmission are probably more common than suspected. *B. hominis* is a common intestinal parasite of humans and animals, with a worldwide distribution. Depending on the geographic location, it may be detected in 1% to 40% of fecal specimens. *B. hominis* may be the most common parasite found in the intestinal tract. Pathogenesis and Spectrum of Disease *B. hominis* can cause diarrhea, cramps, nausea, fever, vomiting, abdominal pain, and urticaria and may require therapy. A possible relationship between *B. hominis* and intestinal obstruction and perhaps even infective arthritis has been suggested. In patients with other underlying conditions, the symptoms may be more pronounced. The incidence of this organism appears to be higher than suspected in stools submitted for parasite examination. In symptomatic patients in whom no other etiologic agent has been identified, *B. hominis* should certainly be considered the possible pathogen. It has been suggested that proteases of genetic subtype 3 could be considered a virulence factor responsible for protein degradation and subsequent pathogenesis.

Laboratory Diagnosis:

Routine Methods. Routine stool examinations are very effective in recovering and identifying *B. hominis*; the permanent stained smear is the procedure of choice, because examination of wet preparations may not easily reveal the organism. If the fresh stool is rinsed in water before fixation (for the concentration method), *B. hominis* organisms, other than the cysts, are destroyed, and a false-negative report may result. The organisms should be quantitated in the report (i.e., rare, few, moderate, or many). It is also important to remember that other possible pathogens should be adequately ruled out before a patient is treated for *B. hominis*.

Antigen Detection. Fecal immunoassays to detect *B.hominis* antigen have been developed but are not yet commercially available. The technique currently used is the enzyme-linked immunosorbent assay (ELISA).

Antibody Detection. ELISA and fluorescent antibody tests have been developed to detect serum antibody to *B. hominis* infections. A strong antibody response is consistent with the ability of this organism to cause symptoms. Also, demonstration of serum antibody production both during and after *B. hominis* symptomatic disease is immunologic evidence for the pathogenic role for this protozoan, although it may take 2 years or longer with chronic infections to develop a serologic response.

Therapy:

Although clinical evidence is limited, studies have been done on the in vitro susceptibility of *B. hominis* to numerous drugs. Currently, metronidazole (Flagyl) appears to be the most appropriate drug. Diiodohydroxyquin (Yodoxin) also has been effective, and dosage schedules for these two drugs are as recommended for other intestinal protozoa. The development of new drug sensitivity assays may improve researchers' ability to evaluate the activities of various drugs against this organism.

Prevention:

Prevention requires improved personal hygiene and sanitary conditions, in addition to proper disposal of fecal material.

FLAGELLATES:

The Mastigophora, or flagellates, have specialized locomotor organelles called **flagella**; these are long, thin, cytoplasmic extensions that may vary in number and position, depending on the species. Different genera of flagellates may live in the intestinal tract, the bloodstream, or various tissues.

Four common species of flagellates are found in the intestinal tract: *Giardia lamblia*, *Dientamoeba fragilis*, *Chilomastix mesnili*, and *Pentatrichomonas hominis* (Figures 48-16 to 48-23). Several other smaller, nonpathogenic flagellates, such as *Enteromonas hominis* and *Retortamonas intestinalis* (see Figure 48-16) *G. lamblia* and *D. fragilis* are the flagellates considered pathogenic. *D. fragilis* has been associated with diarrhea, nausea, vomiting, and other nonspecific intestinal complaints. *Trichomonas vaginalis* is pathogenic but occurs in the urogenital tract. *Trichomonas tenax* is occasionally found in the mouth and may be associated with poor oral hygiene.

Giardia lamblia*:*General Characteristics:**

G. lamblia is the most common cause of intestinal infection worldwide.

Other than *B. hominis*, *G. lamblia* (also called *G. duodenalis* and *G. intestinalis*) is probably the most common protozoan organism identified in individuals in the United States. It causes symptoms ranging from mild diarrhea, flatulence, and vague abdominal pains to acute, severe diarrhea to steatorrhea and a typical malabsorption syndrome. Various documented waterborne and food-borne outbreaks have occurred during the past several years. A number of animals may serve as reservoir hosts for *G. lamblia*. Differentiation of flagellates is based on overall shape, numbers, and arrangements of flagella. Both the trophozoite and cyst stages are included in the life cycle of *G. lamblia*. Trophozoites divide by means of longitudinal binary fission, producing two daughter trophozoites. The organism is found most commonly in the crypts in the duodenum. Trophozoites are the intestinal dwelling stage and attach to the epithelium of the host villi by means of the **ventral disk**. The attachment is substantial and results in disk “impression prints” when the organism detaches from the surface of the epithelium. Trophozoites may remain attached to or may detach from the mucosal surface. Because the epithelial surface sloughs off the tip of the villus every 72 hours, the trophozoites apparently detach at that time. *G. lamblia* trophozoites are teardrop shaped and have been described as “someone looking at you”. Cyst formation takes place as the organisms move down through the jejunum after exposure to biliary secretions. The trophozoites retract the flagella into the axonemes, the cytoplasm becomes condensed, and the cyst wall is secreted (see Figures 13 to 14).

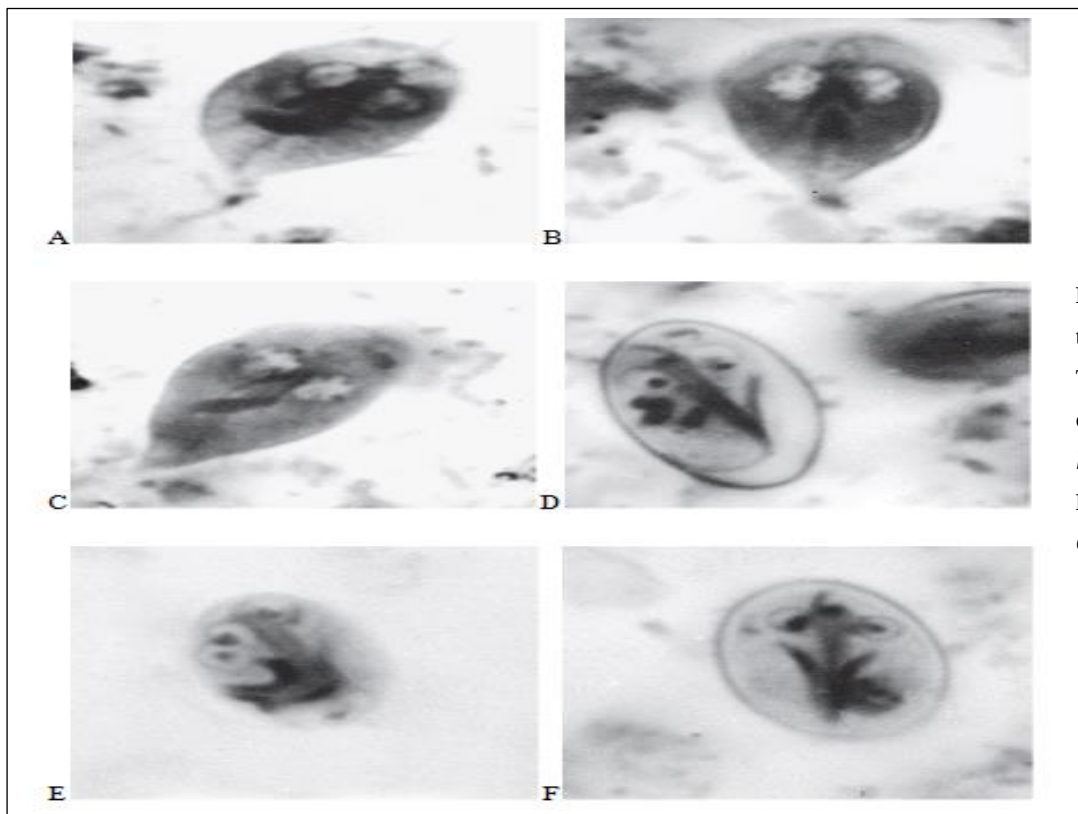


Figure 13 A to C, Trophozoites of *Giardia lamblia*. D to F, Cysts of *G. lamblia*.

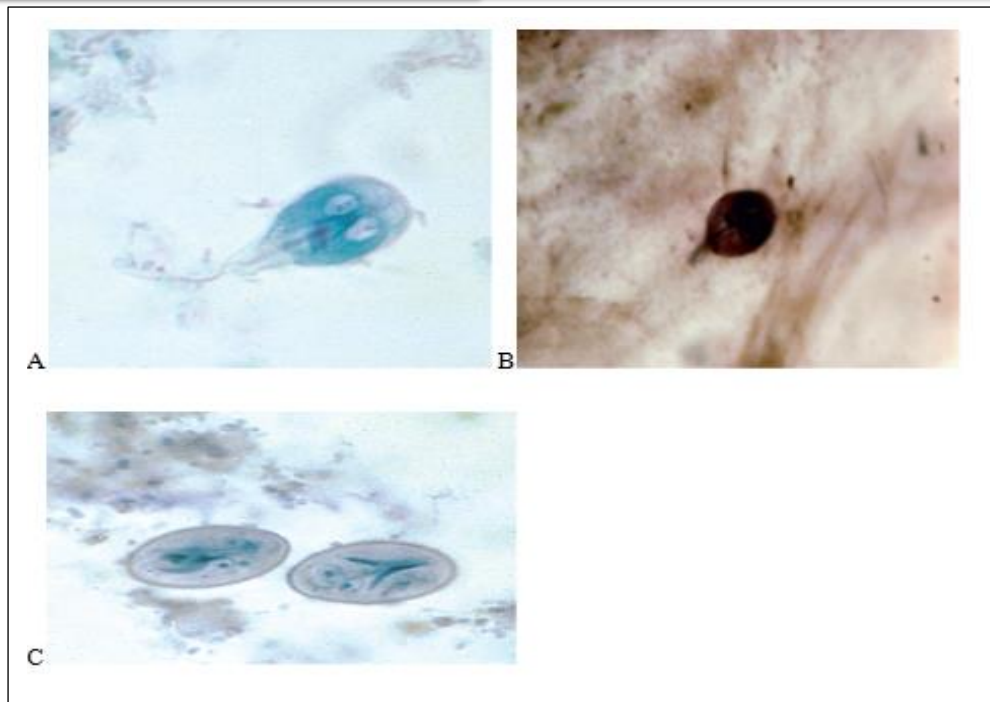


Figure 14 A, *Giardia lamblia* trophozoite. B, *G. lamblia* trophozoite, iodine stain. C, *G. lamblia* cysts

the cyst matures, the internal structures are doubled, so that when excystation occurs, the cytoplasm divides, producing two trophozoites. Excystation occurs in the duodenum or appropriate culture medium.

Epidemiology:

Transmission of *G. lamblia* occurs by ingestion of viable cysts. Although contaminated food or drink may be the source, intimate contact with an infected individual may also result in transmission of the organism. This organism is found more frequently in children or in groups living in close quarters. Outbreaks have been associated with poor sanitation facilities or sanitation breakdowns, as evidenced by infections of travelers and campers. Limited information is available on seasonal variations in giardiasis. Some data suggest an association with the cooler, wetter months of the year, which may implicate environmental conditions as advantageous to cyst survival.

Certain occupations may place an individual at risk for infection, such as sewage and irrigation workers, who may be exposed to infective cysts. In situations in which young children are grouped together, such as in nursery schools, an increased incidence of exposure and subsequent infection of both children and staff members may be seen. A high incidence of giardiasis occurs in patients with immunodeficiency syndromes, particularly in those with common variable hypogammaglobulinemia.

Giardiasis is the most common cause of diarrhea in these patients and may be associated with mild to severe villus atrophy. An estimated 200 million people in Asia, Africa, and Latin America have symptomatic infections. In the United States, approximately 20,000 cases are reported yearly. However, an estimated 2 million cases may occur annually.

Pathogenesis and Spectrum of Disease:

The incubation period for giardiasis ranges from approximately 12 to 20 days. Giardiasis may not be recognized as the cause, because the infection mimics acute viral enteritis, bacillary dysentery, bacterial or other food poisonings, acute intestinal amebiasis, or “traveler’s diarrhea” (toxigenic *Escherichia coli*). However, the type of diarrhea plus the lack of blood, mucus, and cellular exudate is consistent with giardiasis.

Asymptomatic Infection. Although the parasites in the crypts of the duodenal mucosa may reach very high numbers, they may not cause a pathologic condition. The organisms feed on the mucous secretions and do not penetrate the mucosa. Although organisms have been seen in biopsy material obtained from inside the intestinal mucosa, others have been seen attached to the epithelium.

Intestinal Disease. For unknown reasons, symptomatic patients may have irritation of the mucosal lining, increased mucus secretion, and dehydration. The onset may be accompanied by nausea, anorexia, malaise, low-grade fever, and chills, in addition to a sudden onset of explosive, watery, foul-smelling diarrhea. Other symptoms include epigastric pain, flatulence, and diarrhea with increased amounts of fat and mucus in the stool but no blood. Weight loss often accompanies these symptoms. Although some speculate that the organisms coating the mucosal lining may act to prevent fat absorption, this does not completely explain the prevention of the uptake of other substances normally absorbed at other intestinal levels. Severe malabsorption has also been linked with isolated levothyroxine malabsorption, leading to severe hypothyroidism and secondary impairment of pancreatic function. In both cases, treatment with metronidazole led to complete remission of symptoms. Occasionally the gallbladder is involved, resulting in gallbladder colic and jaundice. *G. lamblia* also has been identified in bronchoalveolar lavage fluid.

Chronic Disease. The acute phase often is followed by a subacute or chronic phase. Symptoms include recurrent, brief episodes of loose, foul-smelling stools and possibly increased distention and foul flatus. Between episodes of mushy stools, the patient may have normal stools or may be constipated. Abdominal discomfort includes marked distention and belching with a rotten-egg taste. Chronic disease must be differentiated from amebiasis; disease caused by other intestinal parasites (e.g., *D. fragilis*, *Cryptosporidium* spp., *Cyclospora cayentanensis*, *Isospora belli*, *Strongyloides stercoralis*); inflammatory bowel disease; and irritable colon. On the basis of symptoms such as upper intestinal discomfort, heartburn, and belching, giardiasis must also be differentiated from duodenal ulcer, hiatal hernia, and gallbladder and pancreatic disease.

Laboratory Diagnosis:

Routine Methods. Routine stool examinations are normally recommended for the recovery and identification of intestinal protozoa. However, in the case of *G. lamblia*, because the organisms are attached securely to the mucosa by means of the sucking disk, a series of five or six stool samples may be examined without recovering the organism. The organisms also tend to be passed in the stool on a cyclic basis. The Entero-Test capsule can be helpful for recovering the organisms, as can the duodenal aspirate. Although cysts often can be identified on the wet stool preparation, many infections may be missed without examination of a permanent stained smear. If material from the string test or mucus from a duodenal aspirate is submitted, it should be examined as a wet preparation for motility; however, motility may be

represented by nothing more than a slight flutter of the flagella, because the organism is caught up in the mucus. After diagnosis, the positive specimen can be preserved as a permanent stain.

Antigen Detection. The development of fecal immunoassays to detect *Giardia* antigen in stool has dramatically improved the sensitivity seen with the routine O&P examination. The ELISA has been used to detect *Giardia* antigen in feces. Fluorescent methods with monoclonal antibodies have also proven extremely sensitive and specific in detecting *G. lamblia* in fecal specimens. Other products are available as a cartridge format that uses an immunochromatographic strip-based detection system for *G. lamblia* and/or *Cryptosporidium* spp. Any antigen detection system should always be reviewed for compatibility with stools submitted in preservatives rather than fresh specimens. Some limitations exist on the use of kits for organisms in the genus *Entamoeba*. However, commercial reagent kits for detecting *Giardia* and *Cryptosporidium* spp. can be used with formalin-based stool preservatives or with fresh or frozen specimens. Many of these cartridge format tests provide an answer within 10 minutes and are equal to or better than other immunoassays with regard to sensitivity and specificity. Many of these newer methods are being used to test patients suspected of having giardiasis or those who may be involved in an outbreak. The detection of antigen in stool or visual identification of organisms by using monoclonal antibody reagents indicates current infection. The value of these detection assays as rapid, reliable immunodiagnostic procedures has been emphasized by the increase in *Giardia* infections and the greater awareness of particular incidences (e.g., nursery school settings). Because the organisms are shed so sporadically, use of a fecal immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of *G. lamblia*; a minimum of two stools should be tested. If the first specimen is negative, it may represent a false negative.

Histology. Trophozoites are detectable in the duodenum and proximal jejunum; however, mucosal invasion generally has been found in areas where necrosis or mechanical trauma was present.

Apparently, patients with giardiasis also have reduced mucosal surface areas compared with control patients.

Nucleic Acid-Based Techniques: Currently, there are no molecular-based assays commercially available for the detection of *G. lamblia*.

Prevention:

The most effective practice for preventing the spread of infection in the child care setting is thorough hand washing by the children, staff members, and visitors. Filtration systems have also been recommended, although they have certain drawbacks, such as clogging.

***Chilomastix mesnili*:**

General Characteristics

The *C. mesnili* trophozoite is pear shaped, measuring 6 to 24 μm long and 4 to 8 μm wide. It has a single nucleus and a distinct oral groove, or cytostome (mouth), close to the nucleus. Flagella are difficult to see without obvious motility in a wet preparation. The morphology can be

seen on the permanent stained smear; the cytostome may be visible in some trophozoites. The cysts are pear or lemon shaped and range from 6 to 10 μm long and 4 to 6 μm wide . They have a single nucleus and a typical curved cytostomal fibril, called the **shepherd's crook**. The cyst's definitive morphology can be seen on a permanent stain.

Epidemiology:

C. mesnili tends to have a cosmopolitan distribution, although it is found more frequently in warm climates. Transmission occurs through ingestion of infective cysts.

Pathogenesis and Spectrum of Disease:

C. mesnili is considered nonpathogenic and does not cause disease.

Laboratory Diagnosis

Although cysts sometimes can be seen in a wet preparation, definitive identification of *C. mesnili* relies on examination of permanent stained smears.

Therapy

Specific treatment is not recommended for *C. mesnili*. Because these nonpathogenic organisms are acquired through fecal-oral contamination.

Prevention:

Prevention depends on adequate disposal of human excreta and improved personal hygiene, preventive measures that apply to most of the intestinal protozoa.

Dientamoeba fragilis

General Characteristics:

D. fragilis was described in 1918. *D. fragilis* tends to be common in some pediatric populations, and the incidence is higher for patients under 20 years of age in some studies. Some speculate that *D. fragilis* may be infrequently recovered and identified; a low incidence or absence from survey studies may be due to poor laboratory techniques and a general lack of knowledge about the organism. The *D. fragilis* trophozoite is characterized as having one nucleus (20% to 40%) or two nuclei (60% to 80%). The nuclear chromatin usually is fragmented into three to five granules, and normally no peripheral chromatin is seen on the nuclear membrane. The cytoplasm is usually vacuolated and may contain ingested debris and some large, uniform granules. The cytoplasm can also appear uniform and clean with few inclusions. Size and shape vary considerably among organisms, even on a single smear (Figure 15)

Pathogenesis and Spectrum of Disease:

D. fragilis has been associated with a wide range of symptoms. Case reports of children infected with *D. fragilis* reveal a number of symptoms, including intermittent diarrhea, abdominal pain, nausea, anorexia,

malaise, fatigue, poor weight gain, and unexplained eosinophilia. The most common symptoms in patients infected with this parasite appear to be intermittent diarrhea and fatigue. In some patients, both the organism and the symptoms persist or reappear until appropriate treatment is initiated.

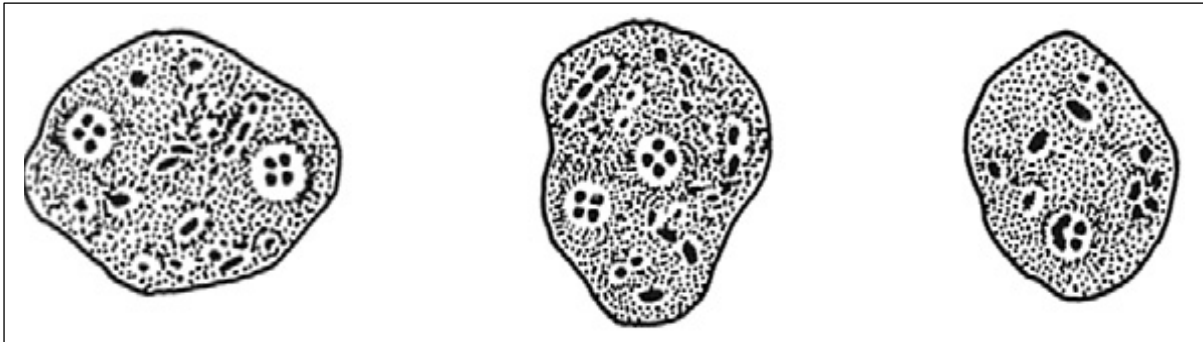


Figure 15 Trophozoites of *Dientamoeba fragilis*

Laboratory Diagnosis:

Routine Methods. Diagnosis of *D. fragilis* infections depends on proper collection and processing techniques (a minimum of three fecal specimens). Although the survival time for this parasite has been reported as 24 to 48 hours in the trophozoite form, the survival time in terms of morphology is limited, and stool specimens must be examined immediately or preserved in a suitable fixative soon after defecation. It is particularly important to examine permanent stained smears of stool with an oil immersion objective ($\times 100$). These trophozoites have been recovered in formed stool; therefore, a permanent stained smear must be prepared for every stool sample submitted for examination. Organisms seen in direct wet mounts may appear as refractile, round forms; the nuclear structure cannot be seen without examination of the permanent stained smear.

Antibody Detection. On indirect immunofluorescence assay, serum samples from patients with confirmed *D. fragilis* infections showed positive titers, and all matched controls had positive titers ranging from 20 to 160. However, these tests are not routinely used, nor are the reagents commercially available.

Therapy:

Clinical improvement has been seen in adults receiving tetracycline, and symptomatic relief has been observed in children receiving diiodohydroxyquin, metronidazole, or tetracycline.

Prevention:

Fecal-oral transmission has not been documented; therefore, it is difficult to speculate about preventive measures. However, if transmission does occur from ingestion of certain helminth eggs, the appropriate hygiene and sanitary measures to prevent contamination with fecal material are appropriate.

***Pentatrichomonas hominis*:**

P. hominis is probably the most commonly identified flagellate, other than *G. lamblia* and *D. fragilis*. *P. hominis* has been recovered from all parts of the world, in both warm and temperate climates, and is considered nonpathogenic and noninvasive. It is not known to have a cyst stage. *P. hominis* trophozoites live in the cecum and feed on bacteria. The trophozoite measures 5 to 15 μm long and 7 to 10 μm wide. It has a pyriform **membrane**, which aid identification of the organism. The undulating membrane extends the entire length of the body, in contrast to that seen in the pathogen *T. vaginalis* (on which the membrane extends halfway down the body).

Epidemiology:

Because *P. hominis* is not known to have a cyst stage, transmission probably occurs in the trophic form. If ingested in a substance such as milk, these organisms apparently can survive passage through the stomach and small intestine in patients with achlorhydria.

Pathogeneis and Spectrum of Disease:

P. hominis is considered nonpathogenic and does not cause disease.

Laboratory Diagnosis:

P. hominis trophozoites can sometimes be seen on a permanent stained smear, but definitive identification can be difficult. However, it is important to report the presence of the organism if seen.

Therapy:

Specific treatment is not recommended for this nonpathogen.

Prevention:

Prevention depends on adequate disposal of human excreta and improved personal hygiene, preventive measures that apply to most of the intestinal protozoa.

CILIATES:

The class Ciliata, or ciliates, includes species that move by means of **cilia**, or short extensions of cytoplasm that cover the surface of the organism. The ciliates also have two different types of nuclei, one **macronucleus** and one or more **micronuclei**. This group includes only one organism that infects humans, *Balantidium coli*, which infects the intestinal tract and may produce severe symptoms.

***Balantidium Coli*:**

General Characteristics:

The life cycle of *B. coli* includes both the trophozoite and cyst stages (Figures 16).

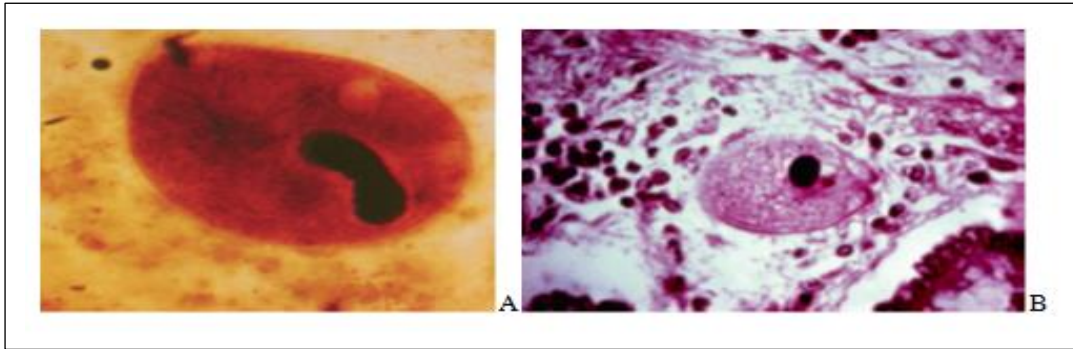


Figure 16 A, *Balantidium coli* trophozoite. B, *B. coli* trophozoite.

The cyst form is the infective stage. After ingestion of the cysts and excystation, trophozoites secrete hyaluronidase, which aids the invasion of the colonic tissue. The trophozoite is quite large, oval, and covered with short cilia. It measures approximately 50 to 150 μm long

and 40 to 70 μm wide. The organism can be seen wet preparation on lower power. The anterior end is somewhat pointed and has a cytostome (primitive mouth opening); in contrast, the posterior end is broadly rounded. The cytoplasm contains many vacuoles with ingested bacteria and debris. The trophozoite has two nuclei: one very large, bean-shaped macronucleus and a smaller, round micronucleus. The organisms live in the large intestine. The trophozoites have a rapid, rotatory, boring motion because of the movement of the cilia. The cyst is formed as the trophozoite moves down the intestine. Nuclear division does not occur in the cyst; therefore, only two nuclei are present, the macronucleus and the micronucleus. The cysts measure 50 to 70 μm in diameter.

Epidemiology:

B. coli is widely distributed in hogs, particularly in warm and temperate climates, and in monkeys in the tropics. Human infection is found in warmer climates, sporadically in cooler areas, and in institutionalized groups with low levels of personal hygiene.

Pathogenesis and Spectrum of Disease:

Some individuals with *B. coli* infection are asymptomatic, whereas others have severe dysentery, similar to that seen in patients with amebiasis. Symptoms include diarrhea or dysentery, tenesmus, nausea, vomiting, anorexia, and headache. Insomnia, muscular weakness, and weight loss also have been reported. Diarrhea may persist for weeks to months, with or without subsequent development of dysentery. Tremendous fluid loss may occur, with diarrhea similar to that seen in cholera or in some coccidial or microsporidial infections. *B. coli* can invade tissue. It may penetrate the mucosa on contact, with cellular infiltration in the area of the developing ulcer. Some of the abscess formations may extend to the muscular layer. The ulcers may vary in shape, and the ulcer bed may be full of pus and necrotic debris. Although the number of cases is small, extraintestinal disease (peritonitis, urinary tract infection, inflammatory vaginitis) has been reported

Laboratory Diagnosis:

Routine stool examinations, particularly wet preparation examinations of fresh and concentrated material, demonstrate the presence of organisms. Organism recognition and identification on a permanent stained smear is usually difficult. These protozoa are large and stain very darkly, which obscures any internal morphology. *B. coli* organisms may be confused with helminth eggs or debris because of their size.

Therapy:

Tetracycline is the drug of choice for treating *B. coli* infection, although it is considered investigational for this infection. Iodoquinol or metronidazole may be used as an alternative. Nitazoxanide, a broad-spectrum antiparasitic drug, may be another alternative.

Prevention

In areas where pigs are raised, the incidence of human infection can be quite high in pig farmers and slaughterhouse workers. Human infection is fairly rare in temperate areas, although infections can develop into an epidemic, particularly in areas of poor environmental sanitation and personal hygiene. This situation has been seen in mental hospitals in the United States. Preventive measures involve increased attention to personal hygiene and sanitation measures, because the mode of transmission is ingestion of infective cysts through contaminated food or water.

Sporozoa (APICOMPLEXA):

All the Apicomplexa are unicellular and have an **apical complex**. These structures can be seen in electron microscopy studies and are used to help classify the various organisms. Genera that develop in the gastrointestinal tract of vertebrates throughout their entire life cycle include Isospora, Cyclospora, and Cryptosporidium. Genera capable of or requiring extraintestinal development are referred to as *cyst-forming coccidia*; they include *Sarcocystis* and *Toxoplasma* spp. The genera that cause disease in humans include Cryptosporidium, Cyclospora, Isospora, Sarcocystis, and Toxoplasma .

Cryptosporidium SPP.:

General Characteristics

Cryptosporidium spp. are intracellular parasites that primarily infect epithelial cells of the stomach, intestine, and biliary ducts. The organism previously called *Cryptosporidium parvum*, thought to be the primary *Cryptosporidium* species infecting humans, now is classified as two species, *C. parvum* (mammals, including humans) and *C. hominis* (primarily humans). Differentiation of these two species based on oocyst morphology is not possible. Currently, more than 20 established *Cryptosporidium* species have been identified in vertebrates, and more than 10 *Cryptosporidium* spp. have been reported in humans. *Cryptosporidium* infections begin with ingestion of viable oocysts. Upon contact with gastric and duodenal fluid, each oocyst releases four sporozoites, which invade.

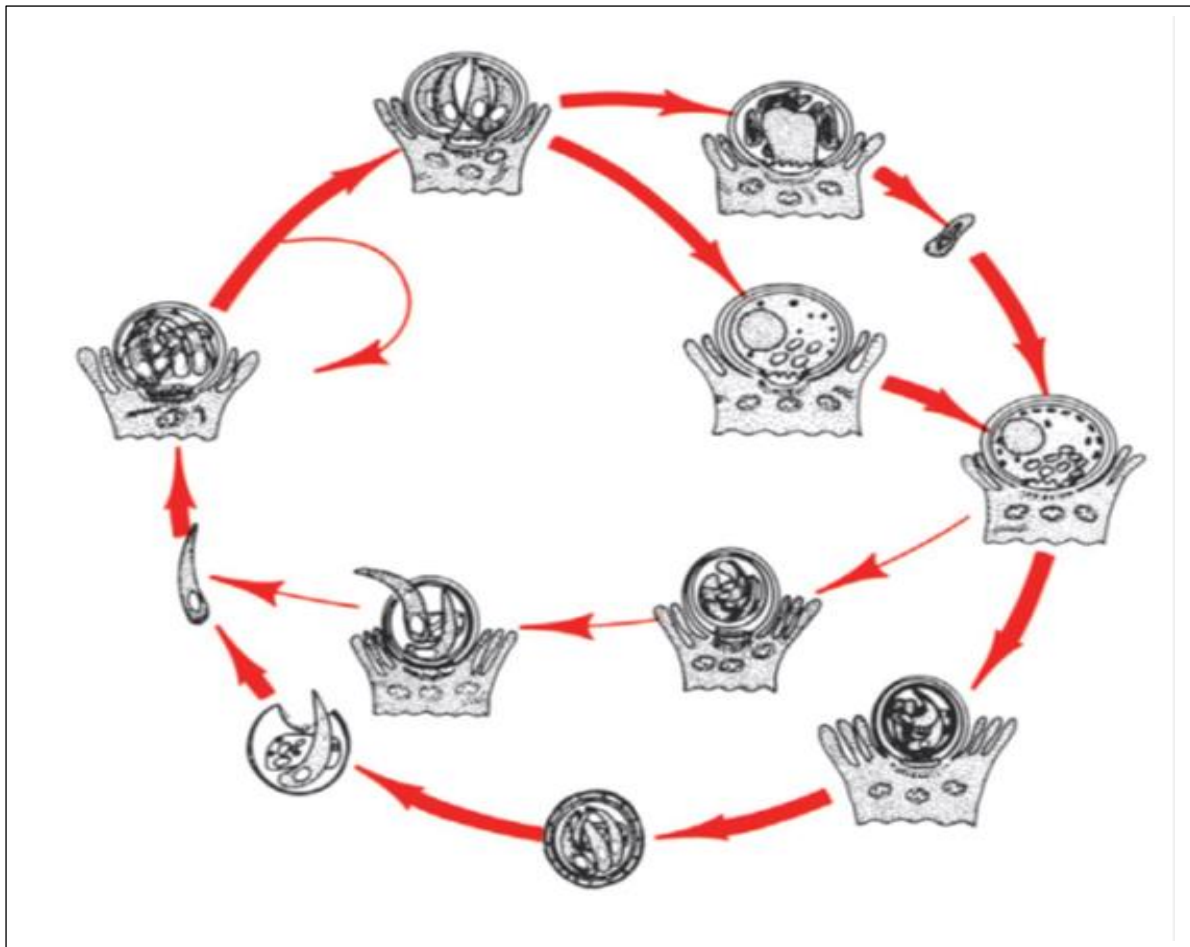


Figure 17 Life cycle of *Cryptosporidium*. (a) Sporulated oocyst infects. (b) Excystation in intestine. (c) Free sporozoite in intestine. (d) Type I meront (six or eight merozoites). (e) Recycling of type I merozoite. (f) Type II meront (four merozoites). (g) Microgametocyte with approximately 16 microgametes. (h) Microgamete fertilizes macrogamete (i) to form zygote (j). Approximately 80% of the zygotes form thick-walled oocysts (k), which sporulate within the host cell. About 20% of the zygotes do not form an oocyst wall; their sporozoites are surrounded only by a unit membrane (l). Sporozoites in “autoinfective,” thin-walled oocysts (l) are released into the intestinal lumen (m) and reinitiate the endogenous cycle (at c).

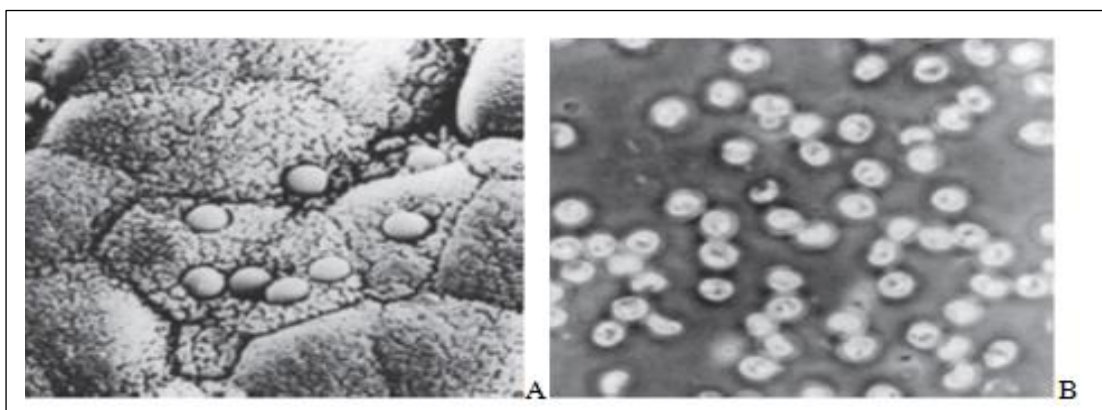


Figure 18 *Cryptosporidium*. A, Oocysts recovered from a Sheather's sugar flotation; organisms measure 4 to 6 μ m. B, Scanning electron microscopy view of organisms at brush border of epithelial cells.

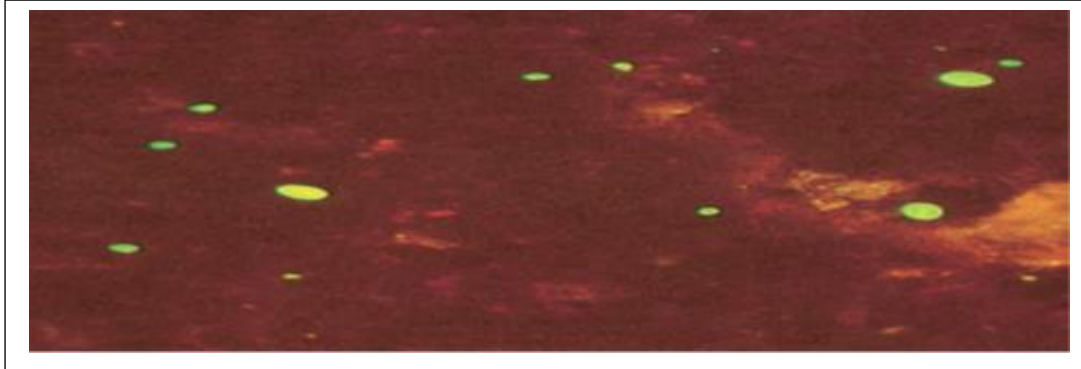


Figure 19 *Cryptosporidium* oocysts and *Giardia* cysts stained with monoclonal antibody–conjugated fluorescent reagent.

the epithelial cells and develop into trophozoites surrounded by a parasitophorous vacuole (layers of endoplasmic reticulum around an intracellular parasite). In the epithelial cells, trophozoites undergo two or three generations of asexual amplification, called **merogony**, leading to the formation of different types of meronts containing four to eight merozoites. The merozoites differentiate into sexually distinct stages in a process called **gametogony**. New **oocysts** are then formed in the epithelial cells in a process called **sporogony**. About 20% of the oocysts are thin walled and may excyst in the digestive tract of the host, leading to the infection of new cells (autoinfection). The remaining 80% of the oocysts are excreted into the environment; are resistant to low temperature, high salinity, and most disinfectants; and can initiate infection in a new host. *Cryptosporidium* oocysts in humans measure 4 to 6 μm in diameter.

Epidemiology:

Humans can acquire cryptosporidiosis through several transmission routes, such as direct contact with infected people or animals or consumption of contaminated water (drinking or recreational) or food. The interval between ingestion of infective oocysts to completion of the life cycle and excretion of new oocysts usually is 4 to 10 days. The only extracellular stage in the *Cryptosporidium* life cycle is the oocysts; these are the environmental stage of the parasite and are immediately infectious when passed in the stool.

Cryptosporidiosis is common in immunocompromised individuals, such as those with AIDS or primary immunodeficiency and cancer and transplant patients undergoing immunosuppressive therapy.

Pathogenesis and Spectrum of Disease:

Immunocompetent Individuals: Clinical symptoms include nausea, low-grade fever, abdominal cramps, anorexia, and five to 10 watery, frothy bowel movements per day, which may be followed by constipation. Some patients may have diarrhea, and others may have few symptoms, particularly later in the course of the infection.

Immunocompromised Individuals. Hemodialysis patients with chronic renal failure and renal transplant patients

with cryptosporidiosis can have chronic, life-threatening diarrhea. In individuals infected with the human immunodeficiency

virus (HIV), cryptosporidiosis increases as the CD4⁺ lymphocyte count falls, *Cryptosporidium* infections are not always confined to the gastrointestinal tract; additional symptoms (respiratory problems, cholecystitis, hepatitis, and pancreatitis) have been associated with extraintestinal infections.

Laboratory Diagnosis:

Oocysts in clinical specimens are difficult to see without special staining techniques, such as the modified acid-fast, Kinyoun's, or Giemsa method, or the newer immunoassay methods. The four sporozoites may be seen in the oocyst wall in some of the organisms, although they are not always visible in freshly passed specimens.

Antigen Detection. Immunoassays are very helpful, because they are a more sensitive method of detecting organisms in stool specimens. A direct fluorescent antigen (FA) procedure with excellent specificity and sensitivity has been developed and results in a significantly increased detection rate over conventional staining and microscopy methods. Some of these reagents, particularly the combination direct FA product used to identify both *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts, are being widely used in water testing and outbreak situations. Most antibodies in commercial direct fluorescent antibody (DFA) kits react with oocysts of almost all *Cryptosporidium* species, making identification to the species level impossible. Enzyme immunoassay (EIA) tests also provide excellent specificity and sensitivity for laboratories using this approach, as do the immunochromatographic cartridge rapid test formats. It is important to remember that if a patient is in the carrier state or undergoing self-cure, the number of oocysts may drop below the sensitivity levels of these kits, producing a false-negative result.

Nucleic Acid-Based Methods. Molecular techniques, especially polymerase chain reaction (PCR) and PCR-related methods, have been used to detect and differentiate *Cryptosporidium* spp., and a few of the PCR assays are commercially available. Several genus-specific PCR-restriction fragment length polymorphism-based genotyping tools have been developed for detecting and differentiating *Cryptosporidium* organisms at the species level. Other genotyping techniques are designed mostly for differentiation of *C. parvum* and *C. hominis* and cannot detect and differentiate other *Cryptosporidium* spp. Or genotypes.

Histology. In the examination of histologic preparations, developmental stages (sporozoites, trophozoites, merozoites, and oocysts) in the life cycle of *Cryptosporidium* spp. can be found at all levels of the intestinal tract, with the jejunum being the most heavily infected site. Routine hematoxylin and eosin staining is sufficient to demonstrate these parasites. Under regular light microscopy, the organisms are visible as small, round structures (about 1 to 3 μm in diameter) aligned along the brush border. They are intracellular but extracytoplasmic and are found in parasitophorous vacuoles. In clean wet mounts, *Cyclospora* organisms are seen as nonrefractile spheres, which are difficult to recognize as parasites. Unless a high number of oocysts are present, they may easily be mistaken for artifacts. They are acidfast variable with the modified acid-fast stain; those that are unstained appear as glassy, wrinkled spheres (wrinkled cellophane). The oocysts are twice the size of those of *Cryptosporidium* spp. and measure 8 to 10 μm in diameter. Because it takes 10 days to 2 weeks for the oocysts to sporulate, no internal structures are visible (sporozoites), as can be seen in *Cryptosporidium* organisms.

Therapy:

Oral or intravenous rehydration and antimotility drugs are used whenever severe diarrhea is associated with cryptosporidiosis. Nitazoxanide is the only drug approved by the U.S. Food and Drug Administration (FDA) for the treatment of cryptosporidiosis in immunocompetent individuals. This drug can shorten the clinical disease and reduce the number of parasites present.

Cyclospora cayetanensis

These organisms are acid-fast variable and have been found in the feces of immunocompetent travelers to developing countries, immunocompetent individuals with no travel history, and patients with AIDS.

The life cycle of *C. cayetanensis* involves only humans as hosts. Oocysts are passed in the feces unsporulated. At room temperature (23° to 25°C), small numbers of oocysts may sporulate within 10 to 12 days. In clean wet mounts, *Cyclospora* organisms are seen as nonrefractile spheres, which are difficult to recognize as parasites. Unless a high number of oocysts are present, they may easily be mistaken for artifacts. They are acidfast variable with the modified acid-fast stain; those that are unstained appear as glassy, wrinkled spheres (wrinkled cellophane). The oocysts are twice the size of those of *Cryptosporidium* spp. and measure 8 to 10 μm in diameter. Because it takes 10 days to 2 weeks for the oocysts to sporulate, no internal structures are visible (sporozoites), as can be seen in *Cryptosporidium* organisms.

Epidemiology:

Transmission of *C. cayetanensis* is thought to be by the fecal-oral route. However, direct person-to-person transmission has not been well documented and may not be a factor, because sporulation takes a number of days. Outbreaks linked to contaminated water and various types of fresh produce (raspberries, basil, baby lettuce leaves, and snow peas) have been reported. Information on reservoir hosts is not well defined; however, in some areas humans appear to be the only host.

Pathogenesis and Spectrum of Disease:

Although some patients are asymptomatic, others report a flulike illness, marked by nausea, vomiting, anorexia, weight loss, and explosive diarrhea lasting 1 to 3 weeks.

The incubation period is not yet known. However, the onset of symptoms after infection generally averages 7 to 8 days, and the symptoms last 2 to 3 weeks. Oocyst shedding in the feces is highly variable and may range from 7 days to several months. Indigenous infections are confined primarily to tropical, subtropical, or warm temperate regions of the world. Outbreaks occur in other areas of the world as a result of contaminated foodstuffs.

In immunocompromised and immunocompetent patients, *C. cayetanensis* infection can be associated with biliary disease. With light and transmission electron microscopy developmental stages have been seen in the gallbladder epithelium of AIDS

Laboratory Diagnosis:

Special Stains. With modified acid-fast stains, the oocysts appear light pink to deep red, and some contain granules or have a bubbly appearance (described as wrinkled cellophane).

Flow Cytometry. Flow cytometry is another diagnostic option. This approach appears to be a useful alternative to microscopy, particularly for screening large numbers of clinical specimens for *Cyclospora* oocysts in an outbreak situation. However, it is not commonly used. Other Diagnostic Methods. Although culture, antigen detection, nucleic acid detection, and serologic tests for antibody have been developed, none of these methods is routinely available for most clinical laboratories.

Therapy:

Patients have been treated symptomatically with antidiarrheal preparations and have obtained some relief; however, the disease appears to be self-limiting within a few weeks. Trimethoprim (TMP-SMX), currently the drug of choice, is given orally twice daily for 7 days. Elimination of parasites, a decrease in diarrhea, and diminished abdominal pain occur within 2 to 3 days after treatment. Patients with AIDS may need higher doses and long-term maintenance treatment. However, more than 40% of patients have a recurrence of symptoms in 1 to 3 months after treatment.

Prevention:

Individuals in endemic areas should wear gloves when gardening to prevent exposure to oocysts of *C. cayetanensis*. Thorough washing of produce may help remove oocysts. Most of the produce items implicated in the transmission of *C. cayetanensis* are consumed raw; thus cooking as a means of prevention is not relevant.

ISOSPORA (CYSTOISOSPORA) BELLI

General Characteristics:

Although isosporiasis is found worldwide, certain tropical areas in the Western Hemisphere have specific locations where endemic infections occur. These organisms infect both adults and children, and intestinal involvement and symptoms are generally transient unless the patient is immunocompromised. *I. belli* has also been implicated in traveler's diarrhea. However, unlike with *Cryptosporidium* spp. and *C. cayetanensis*, large outbreaks of isosporiasis have not been reported. *I. belli* oocysts are passed in the stool. They are long and oval, measuring 20 to 33 μm long by 10 to 19 μm wide. Usually the oocyst contains one immature **sporont**, but two may be present. Continued development occurs outside the body, with the development of two mature **sporocysts**, each containing four **sporozoites**, which can be recovered from the fecal specimen. The sporulated oocyst is the infective stage that excysts in the small intestine, releasing the sporozoites, which penetrate the mucosal cells and initiate the life cycle.

Epidemiology

I. belli oocysts are passed in the feces unsporulated or partially sporulated. Oocysts complete sporulation within 72 hours, although it may take longer, depending on the temperature. The time required for unsporulated oocysts to appear in the feces after ingestion of sporulated oocysts is 9 to 17 days. Oocyst shedding is variable and depends on the immune status of the infected individual. Oocysts can be found for 30 to 50 days in immunocompetent patients, and immunosuppressed patients may continue to shed oocysts for 6 months or

longer. Chronic infections can occur, and oocysts can be shed for months to years. In one particular case, an immunocompetent individual had symptoms for 26 years, and *I. belli* was recovered in stool a number of times over 10 years. *I. belli* is thought to be the only species of *Isospora* that infects humans, and no other reservoir hosts are recognized

for this infection. Transmission occurs through ingestion of water or food contaminated with mature, sporulated oocysts. Sexual transmission by direct oral contact with the anus or perineum also occurs, although this mode of transmission is probably much less common.

The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist; oocysts usually mature within 48 hours after stool passage and are then infectious. Pathogenesis and Spectrum of Disease Symptoms include diarrhea (most common), weight loss, abdominal colic, and fever. Stools (usually six to 10 per day) are watery to soft, foamy, and offensive smelling, suggesting a malabsorption process. Many patients have eosinophilia, recurrences are quite common, and the disease is more severe in infants and young children. Patients who are immunosuppressed, particularly those with AIDS, often present with profuse diarrhea associated with weakness, anorexia, and weight loss. Biopsies reveal an abnormal mucosa with short villi, hypertrophied crypts, and infiltration of the lamina propria with eosinophils, neutrophils, and round cells. Physicians should consider *I. belli* in AIDS patients with diarrhea who have immigrated from or traveled to Latin America, are Hispanics born in the United States, are young adults, or who have not received prophylaxis with TMP-SMX for *Pneumocystis* infection. It has also been recommended that patients with AIDS traveling to Latin America and other developing countries be advised of the waterborne and food-borne transmission of *I. belli* and that chemoprophylaxis should be considered.

Extraintestinal infections in patients with AIDS have been reported. At autopsy, microscopic findings associated with *I. belli* infection were seen in the lymph nodes and walls of the small and large intestines, mesenteric and mediastinal lymph nodes, lymphatic channels, liver, and spleen. *I. belli* infections in the gallbladder epithelium and endometrial epithelium have also been reported, and oocysts have been recovered in bile specimens.

Laboratory Diagnosis:

Examination of fresh material, either as the direct smear or as concentrated material, is recommended rather than the permanent stained smear. The oocysts are very pale and transparent and can easily be overlooked. The light level should be reduced, and additional contrast should be obtained with the microscope for optimal examination conditions. On the permanent stained smear, the organisms may take up excess stain and resemble helminth eggs or artifacts.

It is possible to have a positive biopsy specimen but not recover the oocysts in the stool because of the small numbers of organisms present. The oocysts are acid-fast and can also be demonstrated by using auramine rhodamine stains. Organisms tentatively identified by using auramine rhodamine stains should be confirmed by wet smear examination or acid-fast stains, particularly if the stool contains other cells or excess artifact material

(more normal stool consistency). Currently, there are no commercially available nucleic acid-based methods for the detection of *I. belli*. However, PCR assays have been developed for the detection of the organism in stool samples.

Histology:

Developmental stages of *I. belli* have been reported for intestinal biopsy specimens of the duodenum, jejunum, and occasionally ileum. Intestinal development tends to occur in epithelial cells, although developing stages are occasionally reported from the lamina propria or submucosa. Extraintestinal infections in patients with AIDS have been reported; the organisms become dormant as cysts in a variety of tissues, including the intestine, mesenteric lymph nodes, liver, and spleen;

these cysts are called *unizuite cysts*. In histologic sections, these cysts are thick walled and measure $12-22 \times 8-10 \mu\text{m}$, and each contains a single dormant sporozoite/merozoite of about $8-10 \times 5 \mu\text{m}$. As immunity declines, these cysts can reactivate patent infections.

Therapy:

The drug of choice to treat *I. belli* infection is trimethoprim-sulfamethoxazole, which is given two to four times a day for 10 to 14 days. With this approach, the parasites are

eliminated, the diarrhea stops, and the abdominal pain decreases within a few days.

Prevention:

Because transmission occurs through the infective oocysts, prevention includes improved personal hygiene measures and sanitary conditions to eliminate possible fecal-oral transmission from contaminated food, water, and possibly environmental surfaces.

***Sarcocystis* spp.:**

General Characteristics:

Two well-described *Sarcocystis* species include *S. bovis* (cattle) and *S. suis* (pigs). (Some publications refer to *S. bovis* as *S. hominis*.) When uncooked meat from these infected animals is ingested by humans, gamogony (fission resulting in the production of sporozoan gametes) can occur in the intestinal cells, with eventual production of the sporocysts in stool. *Sarcocystis* spp. have an obligatory two-host life cycle. Intermediate hosts (herbivores and omnivores) become infected through ingestion of sporocysts excreted in the feces of the definitive hosts (carnivores and omnivores).

The definitive hosts become infected through ingestion of mature cysts found in the muscles of the intermediate hosts. In some intermediate hosts, such as cattle and sheep, all adult animals may be infected. Extraintestinal human sarcocystosis is rare, with a much lower incidence than is seen with the intestinal infection. Humans who have ingested meat containing the mature sarcocysts serve as the definitive hosts. Fever, severe diarrhea,

abdominal pain, and weight loss have been reported in immunocompromised hosts, although the number of patients with these symptoms has been quite small.

The sporocysts found in the stool are broadly oval and slightly tapered at the ends. They measure 9 to 16 μm long and contain four mature sporozoites and the residual body. Normally, the oocyst contains two sporocysts (similar to *I. belli*); however, in *Sarcocystis* infections, the sporocysts are released from the oocyst and normally are seen singly. These sporocysts tend to be larger than *Cryptosporidium* oocysts that contain four sporozoites, so they look totally different. The oocysts are fully sporulated when passed in the stool.

Pathogenesis and Spectrum of Disease:

When humans (intermediate host) ingest oocysts from other animal stool sources, the sarcocysts that develop in human muscle are 7 to 16 μm long and cause few, if any, problems. Basically, no inflammatory response to these organisms occurs in the muscle, and no evidence of pathogenicity is seen. Patients demonstrate symptoms related to the disintegration of the sarcocysts and death of intracystic bradyzoites. Painful muscle swellings measuring 1 to 3 cm in diameter are associated with erythema of the overlying skin; these occur periodically and last 2 days to 2 weeks. Symptoms also include fever, diffuse myalgia, muscle tenderness, weakness, eosinophilia, and bronchospasm. Different types of skeletal and cardiac muscle sarcocysts have been found in humans. No specific therapy is required for this type of infection. Corticosteroids can reduce allergic inflammatory reactions.

Infections in humans can manifest primarily as intestinal disease if infected meat is ingested or as muscular disease if sporocysts are ingested. Intestinal disease occurs within a few hours after consumption of infected meat and is characterized by nausea, abdominal pain, and diarrhea. However, in both situations patients may be infected and asymptomatic.

Laboratory Diagnosis:

A presumptive diagnosis of intestinal disease may be based on the patient's symptoms, particularly with documented ingestion of raw or poorly cooked meat. Confirmation of the diagnosis may depend on finding human fecal specimens containing sporocysts, which are passed in the stool 11 to 18 days after ingestion of beef or pork.

Sporocysts of the two *Sarcocystis* species are very difficult to differentiate. A muscle biopsy is appropriate for suspected symptomatic intramuscular infection in a patient with a history of travel to or residence in a tropical location. Sarcocysts in biopsy specimens can be identified by microscopy on routine histologic sections stained with hematoxylin and eosin. Most sarcocysts in humans have been found in skeletal and cardiac muscle; however, muscles in the larynx, pharynx, and upper esophagus have also been involved. No molecular assays are currently available for the detection of sarcocystis in humans. However, several amplification methods have been used to detect sarcocystis in intermediate hosts.

Therapy:

No known treatment or prophylaxis is available for intestinal infection, myositis, vasculitis, or related lesions caused by human sarcocystosis. Supportive therapy for patients with severe diarrhea is indicated. It is also unclear whether immunosuppressives are effective at reducing the inflammatory reactions seen in vasculitis or myositis. Without more definitive data, no course of therapy currently can be recommended.

Prevention:

Cooking meat to an internal temperature higher than 67°C kills *Toxoplasma gondii* tissue cysts in meat; this temperature should also kill *Sarcocystis* tissue cysts in meat.

Preventing cattle, buffalos, and swine from consuming human feces shedding infective oocysts also prevents animal infection. Most cases of human muscular *Sarcocystis*

infection have been reported from the Far East. When humans are intermediate hosts, preventive measures involve careful disposal of animal feces that may contain the infective sporocysts. This may be impossible in wilderness areas, where wild animals may serve as reservoir hosts for many *Sarcocystis* spp.

Microsporidia:

Microsporidia are obligate intracellular, spore-forming parasites. More than 140 microsporidial genera and 1200 species have been identified. To date, seven genera (*Anncaliia*, *Encephalitozoon*, *Enterocytozoon*, *Nosema*, *Pleistophora*, *Vittaforma*, and *Trachipleistophora*) and unclassified microsporidia (*Microsporidium*) have been identified as causing human infections.

Although the microsporidia are true eukaryotes, they also have molecular and cytologic characteristics of prokaryotes. Microsporidia evolved from the fungi and are most closely related to the Zygomycetes. Features shared with fungi include the presence of chitin and trehalose, similarities in cell cycles, and certain gene organizations.

Microsporidia are now considered highly derived fungi that underwent genetic and functional losses, resulting in one of the smallest eukaryotic genomes known.

However, the life cycle of microsporidia is unique and unlike that of any fungal species. At this point, clinical and diagnostic issues and responsibilities may remain with the parasitologists, and we may be in a transition stage, similar to that seen with *Pneumocystis jirovecii* as it was moved from the parasites to fungi in terms of classification status.

General characteristics:

Human microsporidial infections have been documented worldwide. The spore, the only life cycle stage able to survive outside the host cell, is the infective stage (see Figures 20).

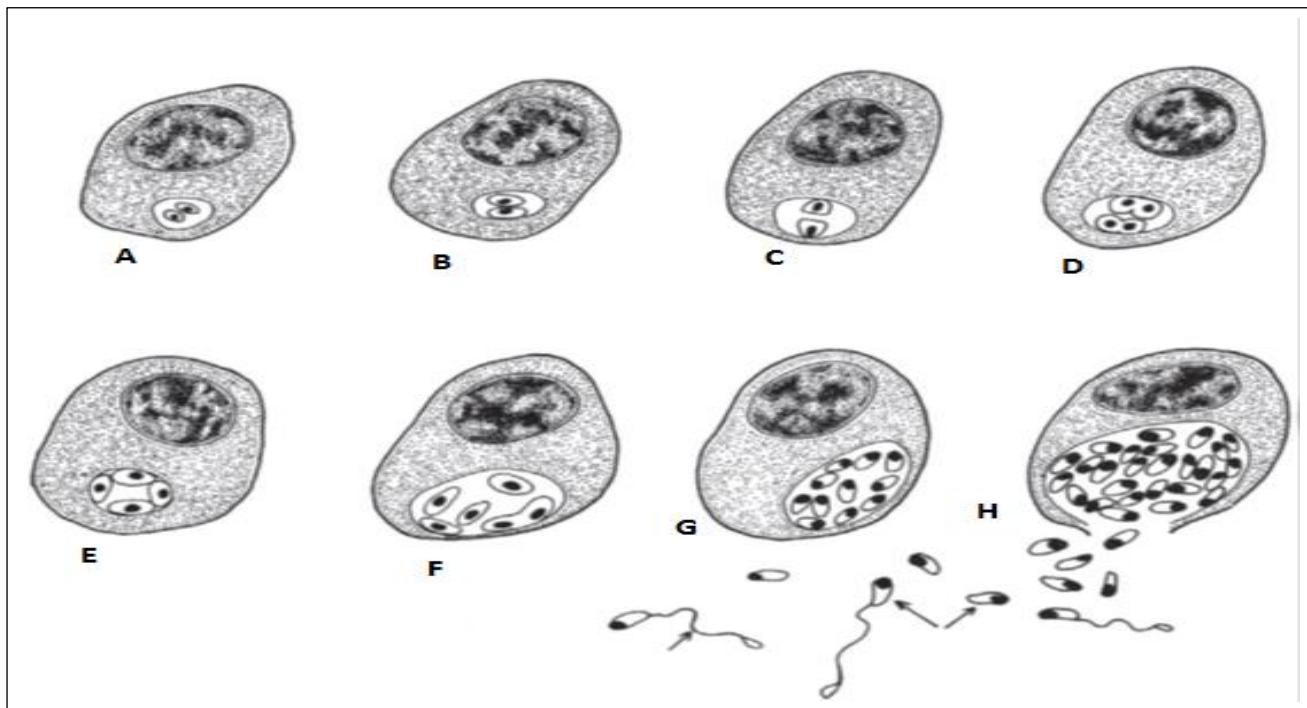


Figure 20 Life cycle diagram of the microsporidia. **A to G**, Asexual development of sporoblasts. **H**, Release of spores.

Infection occurs with ingestion or inhalation of the infective spores, from which the infective sporoplasm (spore protoplasm) enters the host cell through the **polar tubule**. The microsporidia multiply extensively in the host cell cytoplasm; the life cycle includes repeated divisions by binary fission (merogony) or multiple fission (schizogony) and spore production (sporogony). Both merogony and sporogony

can occur in the same cell at the same time. During sporogony a thick spore wall is formed, providing environmental protection for the spore. Microsporidial spores measure 0.7 to about 4 μm in diameter. Mature spores contain a tubular extrusion apparatus (polar tube or tubule) for injecting infective spore contents (sporoplasm) into the host cell.

Epidemiology:

Transmission possibilities include human-to-human and animal-to-human routes. Many questions relating to reservoir hosts and possible congenital infections are still unanswered. Primary infection occurs through inhalation or ingestion of spores from environmental sources or by zoonotic transmission. The presence of *Encephalitozoon intestinalis* has been confirmed in tertiary sewage effluent, surface water, and groundwater;

Enterocytozoon bieneusi has been confirmed in surface water; and *Vittaforma corneae* has been confirmed in tertiary effluent.

This study represents the first confirmation, to the species level, of human-pathogenic microsporidia in water, indicating that these parasites are probably waterborne pathogens. Ingestion of the environmentally highly resistant spores is probably the normal mode of

transmission. *E. bieneusi*, an intestinal pathogen, serves as an example of infection potential. The spores are released into the intestinal lumen and are passed in the stool. These spores are environmentally resistant and can be ingested by other hosts. Zoonotic transmission of microsporidia infecting humans has not been verified but appears likely, because many microsporidial species can infect both humans and animals.

Pathogenesis and spectrum of disease:

Microsporidia were recognized as causing disease in animals as early as the 1920s but were not recognized as agents of human disease until the AIDS pandemic began in the mid-1980s. Before then, several earlier human cases had been reported but were thought to be very unusual.

***Enterocytozoon bieneusi*:**

A number of cases of *E. bieneusi* infection have been reported in patients with AIDS. Chronic intractable diarrhea, fever, malaise, and weight loss are symptoms of *E. bieneusi* infections, and these symptoms mimic those seen with cryptosporidiosis or isosporiasis. Often these patients have four to eight watery, nonbloody stools each day, accompanied by nausea and anorexia. Dehydration and D-xylose and fat malabsorption also may develop. These patients tend to be severely immunodeficient, with a CD4 count almost always below 200 cells/mm³ and often below 100 cells/mm³. Mixed infections with *E. bieneusi* and *E. intestinalis* have also been reported. *E. bieneusi* infection has been implicated in AIDS-related sclerosing cholangitis. However, demonstration of *E. bieneusi* spores in extraepithelial tissues does not always appear to be associated with subsequent development of systemic infection.

E. bieneusi spores have been identified in sputum and bronchoalveolar lavage fluid in addition to stool specimens. *E. bieneusi* can colonize the respiratory tract, and clinical specimens from these specimens may reveal the presence of spores. Multiorgan microsporidiosis caused by *E. bieneusi* has been diagnosed in patients infected with HIV; organisms have been recovered in stools, duodenal biopsy specimens, nasal discharge, and sputum. Infection with *E. bieneusi* has also been reported in immunocompetent individuals; symptoms were selflimited, and diarrheal disease resolved within 2 weeks. *E. bieneusi* may be more commonly associated with sporadic diarrheal disease than was previously suspected, and the immune system may play a role in the control of this intestinal infection. It is also quite possible that *E. bieneusi* may persist as an asymptomatic infection in immunocompetent individuals.

Encephalitozoon spp.:

Both *Encephalitozoon cuniculi* and *Encephalitozoon hellem* have been isolated from human infections. The spectrum disease in patients with AIDS, organ transplant recipients, and otherwise immunocompromised patients includes keratoconjunctivitis, intraocular infection, sinusitis, bronchiolitis, pneumonitis, nephritis, ureteritis, cystitis, prostatitis, urethritis, hepatitis, sclerosing cholangitis, peritonitis, diarrhea, and encephalitis. Clinical manifestations may vary, ranging from an asymptomatic carrier state to organ failure.

Encephalitozoon (Septata) intestinalis:

Encephalitozoon (Septata) intestinalis infects primarily small intestinal enterocytes, but infection does not remain confined to epithelial cells. *E. intestinalis* is also found in lamina propria macrophages, fibroblasts, and endothelial cells. Dissemination to the kidneys, lower airways, and biliary tract appears to occur through infected macrophages.

Fortunately, these infections tend to respond to therapy with albendazole, unlike infections caused by *E. bieneusi*.

Other Microsporidia

Different microsporidial species have been isolated from immunocompetent individuals who presented with keratoconjunctivitis, severe keratitis, or corneal ulcers. Also, keratoconjunctivitis has been found in an immunocompetent contact lens wearer.

In immunocompromised patients, myositis has been seen in infections caused by *Pleistophora* sp., *Pleistophora ronneafiei*, *Trichomonas hominis*, *Anncaliia vesicularum*, and *Anncaliia algerae*. *Trachipleistophora anthropophthera* has been identified at autopsy in cerebral, cardiac, renal, pancreatic, thyroid, hepatic, splenic, lymphoid, and bone marrow tissue of patients with AIDS. Disseminated infection caused by *Anncaliia connori* was found at autopsy in a 4-month-old athymic male infant.

Laboratory diagnosis:

The most commonly used stains are chromotrope-based stains (modified trichrome) and chemofluorescent optical brightening agents, including calcofluor white and other chemofluorescent stains. Regardless of the staining technique selected, the use of positive control material is highly recommended. Detection of the small microsporidial spores requires adequate illumination and magnification (i.e., magnification using the oil immersion objective [×100] for a total magnification of ×1000).

Microsporidia do not tend to stain predictably, if at all, in tissues. However, spores occasionally can be seen very well with use of the periodic acid-Schiff (PAS) stain, silver stains, or acid-fast stains. Modified Gram stains also have proved sensitive. The spore has a small, PAS-positive posterior body; the spore coat stains with silver, and the spores are acid-fast variable. Tissue examination by electronmicroscopy (EM) techniques is still considered the best approach for differentiation of genera; however, this option is not available to all

laboratories, and the sensitivity of EM may not be equal to that of other methods when examining stool or urine.

Therapy:

Albendazole therapy can result in clinical cure of HIV-associated infection with *Encephalitozoon* spp., along with elimination of spore shedding. Albendazole is not effective for *Enterocytozoon* infections, although clinical improvement occurs in some patients. Oral purified fumagillin appears to eradicate *Enterocytozoon bieneusi* in many patients, but serious adverse events and parasitic relapse have been seen. Antiretroviral combination therapy results in complete clinical response with elimination of intestinal microsporidia.

Prevention:

The presence of infective spores in human clinical specimens suggests that taking precautions when handling body fluids and following personal hygiene measures, such as hand washing, may be important in preventing primary infections in the health care setting.

Blood and Tissue Protozoa:

Sporozoa, Flagellates (Blood, Tissue):

Sporozoa (Malaria and Babesiosis):

Plasmodium vivax

Plasmodium ovale

Plasmodium malariae

Plasmodium falciparum

Plasmodium knowlesi

Babesia spp.

Flagellates (Leishmaniae, Trypanosomes):

Leishmania tropica complex

Leishmania mexicana complex

Leishmania braziliensis complex

Leishmania donovani complex

Leishmania peruviana

Trypanosoma brucei gambiense

Trypanosoma brucei rhodesiense

Trypanosoma cruzi

Trypanosoma rangeli

Plasmodium spp.:

Malaria has been well documented as an ancient disease in Egyptian and Chinese writing beginning in 2700 bc. By 200 bc, malaria was identified in Rome, spread throughout Europe during the twelfth century, and arrived in England by the fourteenth century. By the early 1800s malaria was found worldwide.

Malaria has played a tremendous role in world history, influencing the outcome of wars, the movement of populations, and the development and decline of various nations. Before the American Civil War, malaria was found as far north as southern Canada; but it was no longer endemic within the United States by the 1950s. It is estimated that more than 500 million individuals worldwide are infected with *Plasmodium* spp., and as many as 2.7 million people a year, most of whom are children, die from the infection. Malaria is endemic in more than 90 countries with a population of 2400 million people, representing 40% of the world's population. At least 90% of deaths caused by malaria occur in Africa.

The vector for malaria is the female anopheline mosquito. When the vector takes a blood meal, sporozoites contained in the salivary glands of the mosquito are discharged into the puncture wound. Within an hour, these infective sporozoites are carried via the blood to the liver, where they penetrate hepatocytes and begin to grow, initiating the **preerythrocytic** or **primary exoerythrocytic** cycle. The sporozoites become round or oval and begin dividing repeatedly. **Schizogony** results in large numbers of exoerythrocytic merozoites. Once these merozoites leave the liver, they invade the red blood cells (RBCs), initiating the **erythrocytic cycle**. A dormant schizogony may occur in *P. vivax* and *P. ovale* organisms, which remain quiescent in the liver. These resting stages have been termed **hypnozoites** and lead to a true relapse, often within 1 year or up to more than 5 years later.

Delayed schizogony does not occur in *P. falciparum*, *P. malariae*, or *P. knowlesi*. Once the RBCs and reticulocytes have been invaded, the parasites grow and feed on hemoglobin. Within the RBC, the merozoite (or young trophozoite) is vacuolated, ring shaped, more or less ameboid, and uninucleate. The excess protein and heme present from the metabolism of hemoglobin combine to form malarial pigment. Once the nucleus begins to divide, the trophozoite is called a developing schizont. The mature schizont contains merozoites (whose number depends on the species), which are released into the bloodstream. Many of the merozoites are destroyed by the immune system, but others invade RBCs and initiate a new cycle of erythrocytic schizogony. After several erythrocytic generations, some of the merozoites begin to undergo development into the male and female gametocytes. Although malaria is often associated with travelers to endemic areas, other situations resulting in infection include blood transfusions, use of contaminated hypodermic needles, bone marrow transplantation, congenital infection, and transmission within the United States by indigenous mosquitoes that acquired the parasites from imported infections.

PLASMODIUM VIVAX (BENIGN TERTIAN MALARIA):

General Characteristics:

P. vivax infects only the reticulocytes; thus, the parasitemia is limited to approximately 2% to 5% of the available RBCs (Figures 21).

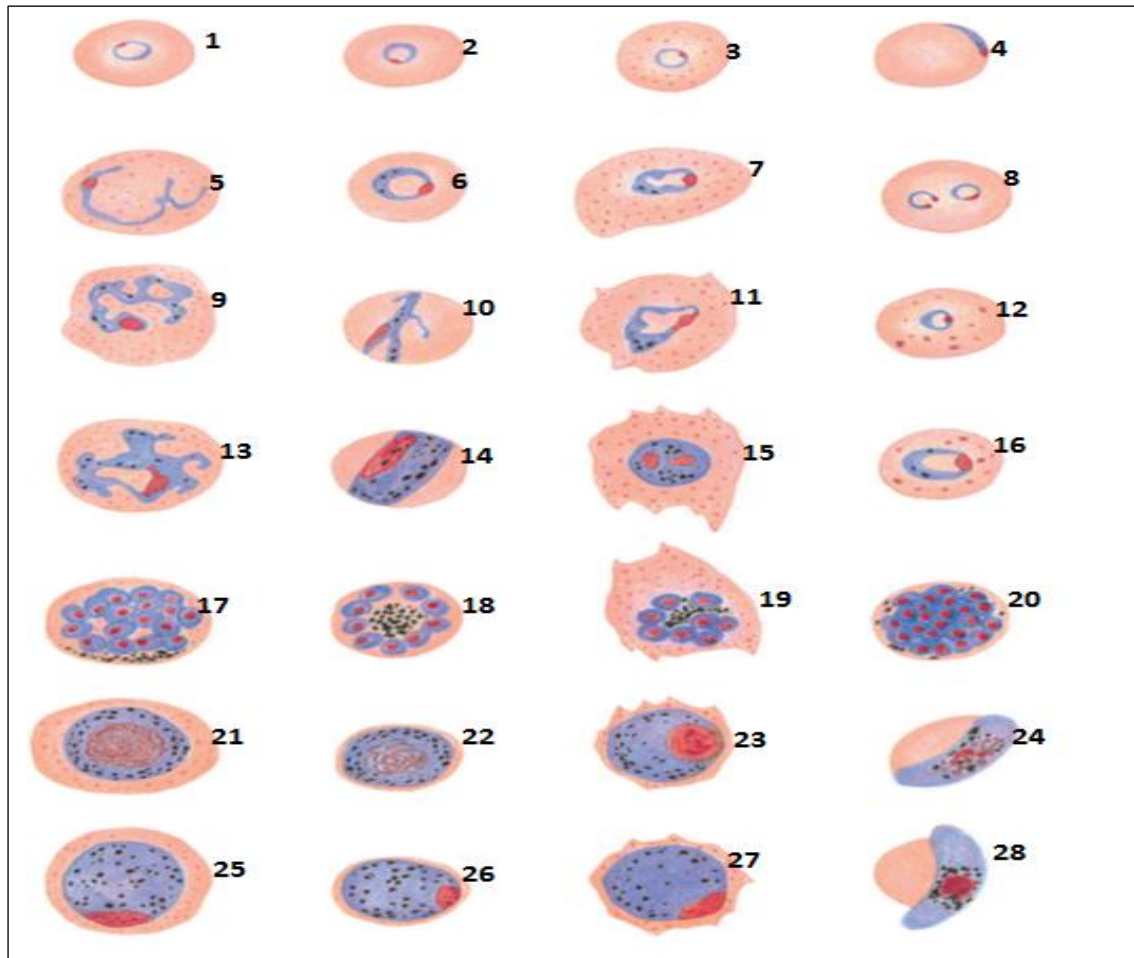


Figure 21 The morphology of malaria parasites. *Plasmodium vivax*: 1, Early trophozoite (ring form). 2, Late trophozoite with Schuffner's dots (note enlarged red blood cell). 3, Late trophozoite with ameboid cytoplasm (very typical of *P. vivax*). 4, Late trophozoite with ameboid cytoplasm. 5, Mature schizont with merozoites (18) and clumped pigment. 6, Microgametocyte with dispersed chromatin. 7, Macrogametocyte with compact chromatin. *Plasmodium malariae*: 1, Early trophozoite (ring form). 2, Early trophozoite with thick cytoplasm. 3, Early trophozoite (band form). 4, Late trophozoite (band form) with heavy pigment. 5, Mature schizont with merozoites (9) arranged in rosette. 6, Microgametocyte with dispersed chromatin. 7, Macrogametocyte with compact chromatin. *Plasmodium ovale*: 1, Early trophozoite (ring form) with Schuffner's dots. 2, Early trophozoite (note enlarged red blood cell). 3, Late trophozoite in red blood cell with fimbriated edges. 4, Developing schizont with irregularly shaped red blood cell. 5, Mature schizont with merozoites (8) arranged irregularly. 6, Microgametocyte with dispersed chromatin. 7, Macrogametocyte with compact chromatin. *Plasmodium falciparum*: 1, Early trophozoite (accolé or appliqué form). 2, Early trophozoite (one ring is in headphone configuration/double chromatin dots). 3, Early trophozoite with Maurer's dots. 4, Late trophozoite with larger ring and Maurer's dots. 5, Mature schizont with merozoites (24). 6, Microgametocyte with dispersed chromatin. 7, Macrogametocyte with compact chromatin. **Note:** Without the appliqué form, Schuffner's dots, multiple rings/cell, and other developing stages, differentiation among the species can be difficult. It is obvious that the early rings of all four species can mimic one another very easily. *Remember: One set of negative blood films cannot rule out a malarial infection.*

Splenomegaly occurs during the first few weeks of infection, and the spleen will progress from being soft and palpable to hard, with continued enlargement during a chronic infection. If the infection is treated during the early phases, the spleen will return to its normal size. A secondary or dormant schizogony occurs in *P. vivax* and *P. ovale*, which remain quiescent in the liver. These resting stages have been termed *hypnozoites*. After a few days of irregular periodicity, a regular 48-hour cycle is established. An untreated primary attack may last from 3 weeks to 2 months or longer. Over time, the paroxysms (symptomatic period) become less severe and more irregular in frequency and then cease altogether. In approximately 50% of patients infected with *P. vivax*, relapses occur after weeks, months, or even after 5 years or more. The RBCs tend to be enlarged (young RBCs), there may be Schüffner's dots (exclusively found in *P. vivax* and *P. ovale*) after 8 to 10 hours, the developing rings are ameboid, and the mature schizont contains 12 to 24 merozoites .

Pathogenesis and Spectrum of Disease:

In patients who have never been exposed to malaria, symptoms such as headache, photophobia, muscle aches, anorexia, nausea, and sometimes vomiting may occur before organisms can be detected in the bloodstream. In other patients with prior exposure to the malaria, the parasites can be found in the bloodstream several days before symptoms appear. Severe complications are uncommon in *P. vivax* infections, although coma and sudden death or other symptoms of cerebral involvement have been reported, particularly in patients with varying degrees of primaquine resistance. These patients can exhibit cerebral malaria, renal failure, circulatory collapse, severe anemia, hemoglobinuria, abnormal bleeding, acute respiratory distress syndrome, and jaundice. Acute cerebral malaria involves changes in mental status and if untreated may result in fatality within 3 days.

Plasmodium ovale:**General Characteristics**

Although *P. ovale* and *P. vivax* infections are clinically similar, *P. ovale* malaria is usually less severe, tends to relapse less frequently, and usually ends with spontaneous recovery, often after no more than 6 to 10 paroxysms . Like *P. vivax*, *P. ovale* infects only the reticulocytes, so that the parasitemia is limited to approximately 2% to 5% of the available RBCs. For many years the literature has stated that as with *P. vivax*, a secondary or dormant schizogony occurs in *P. ovale*, which remain quiescent in the liver.

However, newer findings indicate that hypnozoites have never been demonstrated by biologic experiments. After a few days of irregular periodicity, a regular 48-hour cycle is established. Over time, the paroxysms become less severe and more irregular in frequency and then stop altogether. In some patients infected with *P. ovale*, relapses occur after weeks, months, or up to 1 year or more. The RBCs tend to be enlarged (young RBCs), Schüffner's dots (also known as James stippling) are present from the beginning of the cycle, the developing rings are less ameboid than those of *P. vivax*, and the mature schizont contains an average of eight merozoites.

Pathogenesis and Spectrum of Disease:

The incubation period is similar to that for *P. vivax* malaria, but the frequency and severity of the symptoms are much less, with a lower fever and a lack of typical rigors. The geographic range is usually described as being limited to tropical Africa, the Middle East, Papu New Guinea, and Irian Jaya in Indonesia. However, *P. ovale* infections in Southeast Asia may cause benign and relapsing malaria in this area. In both Southeast Asia and Africa, two different types of *P. ovale* circulate in humans. Human infections with variant-type *P. ovale* are associated with a higher level of parasitemia.

Plasmodium malariae* (QUARTAN MALARIA):*General Characteristics**

P. malariae invades primarily the older RBCs, limiting the number of infected cells (Figures 21). The incubation period between infection and symptoms may be much longer than that for *P. vivax* or *P. ovale* malaria, ranging from about 27 to 40 days. A regular periodicity is seen from the beginning, with a more severe paroxysm, including a longer cold stage and more severe symptoms during the hot stage. Collapse during the sweating phase is not uncommon. A regular periodicity of 72 hours is seen from the beginning of the erythrocytic cycle. The infection may end with spontaneous recovery, or there may be a recrudescence or series of recrudescence (recurrence of symptoms) over many years. These patients are left with a latent infection and persisting low-grade parasitemia for many, many years. The RBCs tend to be normal to small (old RBCs), there is no true stippling, the RBCs may have fimbriated edges, the developing rings tend to demonstrate “band” forms, and the mature schizont contains an average of 6 to 12 merozoites.

Pathogenesis and Spectrum of Disease:

Proteinuria is common in *P. malariae* infections and may be associated with clinical signs of nephrotic syndrome. With a chronic infection, kidney problems result from deposition within the glomeruli of circulating antigenantibody complexes. A membrane proliferative type of glomerulonephritis is the most common lesion seen in quartan malaria. Because chronic glomerular disease associated with *P. malariae* infections is usually not reversible with therapy, genetic and environmental factors may play a role in the disease, as well. The patient may have a spontaneous recovery, or there may be a recrudescence or series of recrudescence over many years (>50 years).

In these cases, patients are left with a latent infection and persisting low-grade parasitemia.

Plasmodium falciparum* (MALIGNANT TERTIAN MALARIA):*General Characteristics**

Plasmodium falciparum invades all ages of RBCs, and the number of infected cells may exceed 50% , Schizogony occurs in the spleen, liver, and bone marrow rather than in the circulating blood. Ischemia caused by the obstruction of vessels within these organs by parasitized RBCs will produce various symptoms,

depending on the organ involved. A decrease in the ability of the RBCs to change shape when passing through capillaries or the splenic filter may lead to plugging of the vessels. Also, only *P. falciparum* causes cytoadherence, a feature that is associated with severe malaria. The asexual and sexual forms circulate in the bloodstream during infections by four of the *Plasmodium* species. However, in *P. falciparum* infections, as the parasite grows, the RBC membrane becomes sticky and the cells adhere to the endothelial lining of the capillaries of the internal organs. Thus, only the ring forms and the gametocytes (occasionally mature schizonts) normally appear in the peripheral blood. Periodicity of the cycle will not be established during the early stages, and the presumptive diagnosis may be totally unrelated to a possible malaria infection. If the fever does develop a synchronous cycle, it is usually a cycle of 36 to 48 hours. Because *P. falciparum* infects young and old RBCs, very heavy parasitemia can occur. The RBCs are all sizes; there is no true stippling, but Maurer's dots (coarse granulation in the cytoplasm of RBCs) are sometimes present; often there are multiple rings per RBC and the rings are delicate and often have two dots of chromatin, appliqué or accolé forms (ring forms identified within the marginal regions of the erythrocytes); and the gametocytes are crescent-shaped.

Pathogenesis and Spectrum of Disease:

The onset of a *P. falciparum* malaria attack occurs 8 to 12 days after infection and is characterized by 3 to 4 days of vague symptoms such as aches, pains, headache, fatigue,

anorexia, or nausea. This stage is followed by fever, a more severe headache, and nausea and vomiting, with occasional severe epigastric pain. At the onset of fever, there may be a feeling of chilliness. As with the other *Plasmodium* spp., periodicity of the cycle will not be established during the early stages.

Severe or fatal complications can occur at any time and are related to the obstruction of vessels in the internal organs (liver, intestinal tract, adrenal glands, intravascular hemolysis/black water fever, and kidneys). Blackwater fever is a complication of malaria that is a result of red blood cell lysis, releasing hemoglobin into the bloodstream and urine, causing discoloration. The severity of the complications may not correlate with the peripheral blood parasitemia, particularly in *P. falciparum* infections in a patient who has never been exposed to malaria before (immunologically naïve).

Disseminated intravascular coagulation is a rare complication and is seen with a high parasitemia, pulmonary edema, anemia, and cerebral and renal complications.

Vascular endothelial damage from endotoxins and bound parasitized blood cells may lead to clot formation in small vessels. Cerebral malaria is more common in *P. falciparum* malaria, but can occur in the other species. If the onset is gradual, the patient becomes disoriented or violent or may develop severe headaches and pass into coma. However, some patients, including those with no prior symptoms, may suddenly become comatose. Physical signs of central nervous system involvement vary, and there is no correlation between the severity of the symptoms and the parasitemia.

Extreme fevers, 41.7° C (107° F) or higher, may occur in an uncomplicated malaria attack or in cases of cerebral malaria. Without vigorous therapy, the patient usually dies. Cerebral malaria is considered to be the most serious complication and the major cause of death with *P. falciparum*; it occurs in up to 10% of all *P. falciparum* patients admitted to the hospital and is responsible for 80% of fatal cases.

Plasmodium knowlesi

General Characteristics:

P. knowlesi invades all ages of RBCs, and the number of infected cells can be significantly more than seen in *P. vivax*, *P. ovale*, and *P. malariae*. *P. knowlesi* infection should be considered in patients with a travel history to forested areas of Southeast Asia, especially if *P. malariae* is diagnosed, unusual forms are seen with microscopy, or if a mixed infection with *P. falciparum*/*P. malariae* is diagnosed. Because the disease is potentially fatal, proper identification to the species level is critical.

The early blood stages of *P. knowlesi* resemble those of *P. falciparum*, whereas the mature blood stages and gametocytes resemble those of *P. malariae*.

Unfortunately, these infections are often misdiagnosed as the relatively benign *P. malariae*; however, infections with *P. knowlesi* can be fatal. The RBCs are all sizes, there is no true stippling (fine, granular, blue stippling in RBCs stained with Wright's stain or red when using eosin hematoxylin as seen in Figure 21 (*P. vivax* photo, third from the top), often there are multiple rings per RBC (there may be 2 to 3 rings), the rings are delicate and often have 2 to 3 dots of chromatin, band forms are typically seen with the developing trophozoites, and the mature schizont contains 16 merozoites. The early stages mimic *P. falciparum*, whereas the later stages mimic *P. malariae*. Because of different levels of parasitemia, low organism densities, and confusion among various morphologic criteria for identification, detection of mixed infections can be quite difficult. Even if a mixed infection is suspected, identification to the species level may not be possible using routine microscopy methods. However, using polymerase chain reaction (PCR) methods, it is likely that higher detection and identification rates of chronic and mixed malarial infections will be possible.

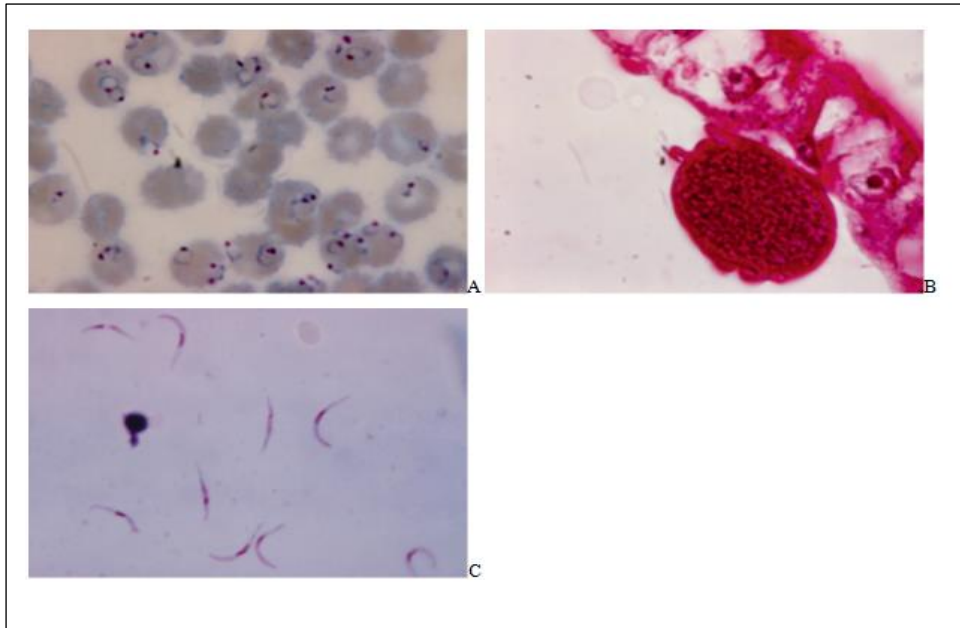


Figure 22 *Plasmodium falciparum*. **A**, Ring forms; **B**, oocyte; and **C**, sporozoites.

Pathogenesis and Spectrum of Disease:

Patients exhibit chills, minor headaches, and daily lowgrade fever. Patients who have been diagnosed with high numbers of *P. malariae* organisms by microscopy should receive intensive management as appropriate for severe *P. falciparum* malaria, assuming the infection is actually caused by *P. knowlesi*. Overall, these infections can be as severe as those caused by *P. falciparum*, with fatal.

Laboratory diagnosis (ALL SPECIES):

Examination of a single blood specimen is not sufficient to exclude the diagnosis of malaria, especially when the patient has received partial prophylaxis or therapy and has a low number of organisms in the blood. Patients with a relapse case or an early primary case may also have few organisms in the blood smear. Regardless of the presence or absence of any fever periodicity, both thick (Figure 23) and thin blood films should be prepared immediately, and at least 200 to 300 oil immersion fields should be examined on both films before a negative report is issued. If the initial specimen is negative, additional blood specimens should be examined over a 36-hour time frame.

Although Giemsa stain is recommended for all parasitic blood work, the organisms can also be seen with other blood stains, such as Wright's stain. Using any of the blood stains, the white blood cells (WBCs) serve as the built-in quality control; if the WBCs look good, any parasites present will also look good. compares the multinucleated stages (schizont) of *Plasmodium malariae* and *Plasmodium vivax*. Fluorescent nucleic acid stains, such as acridine orange, may also be used to identify organisms in infected RBCs. However, this may be more difficult to interpret because of the presence of white blood cell nuclei or RBC Howell-Jolly bodies.

Serologic Methods:

Several rapid malaria tests (RMTs) are now commercially available.

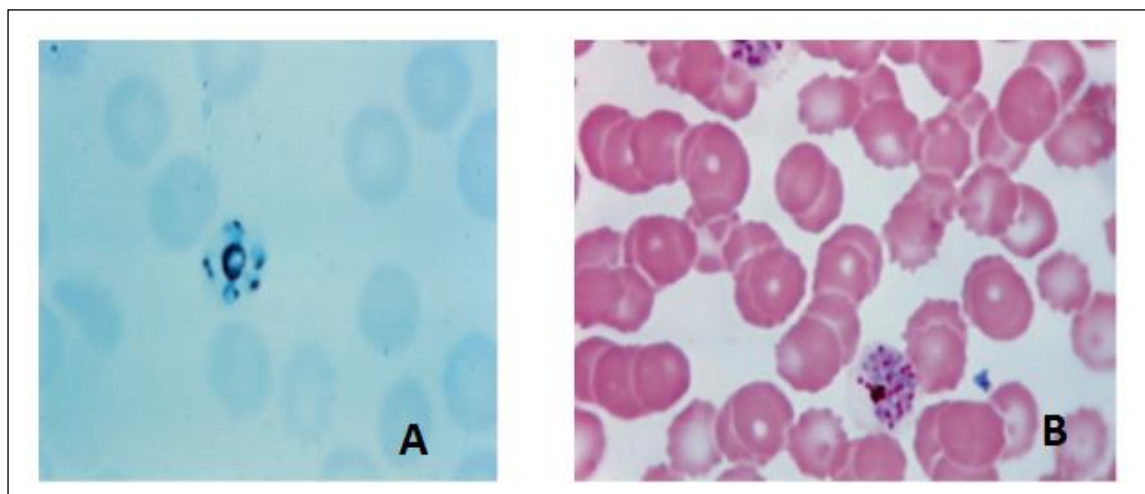


Figure 23 A, *Plasmodium malariae* schizont. B, *Plasmodium vivax* schizont

Molecular Diagnostics:

Other methods include direct detection of the five species by using a specific DNA probe after PCR amplification of target DNA sequences.

Therapy:

Antimalarial drugs are classified according to the stage of malaria against which they are targeted. These drugs are referred to as tissue schizonticides (which kill tissue schizonts), blood schizonticides (which kill blood schizonts), gametocytocides (which kill gametocytes), and sporonticides (which prevent formation of sporozoites within the mosquito). It is important for the clinician to know the species of *Plasmodium* involved in the infection, the estimated parasitemia, and the geographic and patient travel history to assess the possibility of drug resistance related to the organism and geographic area.

***Babesia* spp.:**

The genus *Babesia* includes approximately 100 species transmitted by ticks of the genus *Ixodes*. In addition to humans, these blood parasites infect a variety of wild and domestic animals.

General characteristics:

Organism Although the life cycle of *Babesia* spp. is similar to that of *Plasmodium* spp., no exoerythrocytic stage has been described; also, sporozoites injected by the bite of an infected tick invade erythrocytes directly. Once inside the erythrocytes, the trophozoites reproduce by binary fission rather than schizogony. Once the tick begins to take a blood meal; the sporozoites are injected into the host with the tick's saliva.

The trophozoites of *Babesia* can mimic *P. falciparum* rings; however, there are differences that can help differentiate the two organisms (Figure 24). *Babesia* trophozoites vary in size from 1 to 5 μm ; the smallest are smaller than *P. falciparum* rings. Also, ring forms outside of the RBCs and two to three rings per RBC are much more common in *Babesia*. The ring forms of *Babesia* tend to be very pleomorphic and range in size, even within a single RBC. The diagnostic tetrads, the Maltese Cross, though not seen in every specimen or species, may be present (see Figure 24).

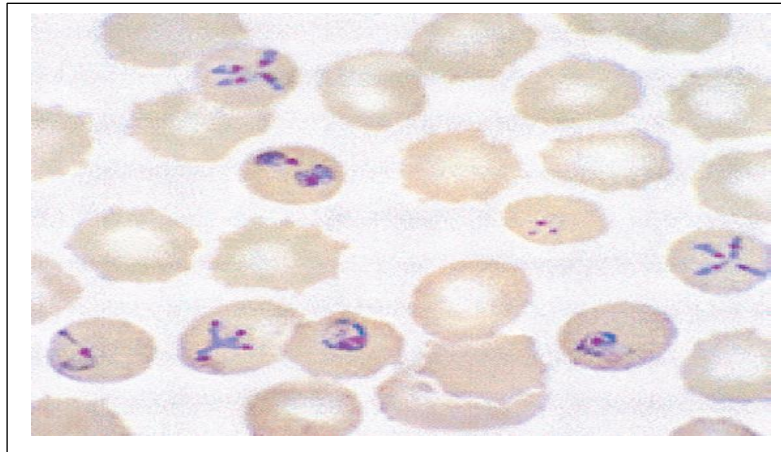


Figure 24 *Babesia* in red blood cells.

Pathogenesis and spectrum of disease:

Babesiosis is clinically similar to malaria, and symptoms include high fever, myalgias, malaise, fatigue, hepatosplenomegaly, and anemia.

Laboratory diagnosis:

Examination of thick and thin stained blood films is the most direct approach to diagnosis.

Molecular Diagnostics:

Although rare, molecular methods such as PCR are available in some laboratories.

Therapy:

Mild cases caused by *B. microti* usually resolve spontaneously, and in more serious cases, treatment with clindamycin and quinine or atovaquone and azithromycin is used.

Prevention:

Personal protective measures, such as long pants, long-sleeved shirts, and insect repellent, may reduce the risk of infection when outdoors in endemic areas for the tick vectors.

***Trypanosoma* spp.:**

Trypanosoma spp. are hemoflagellate protozoa that live in the blood and tissue of the human host (Figures 25).

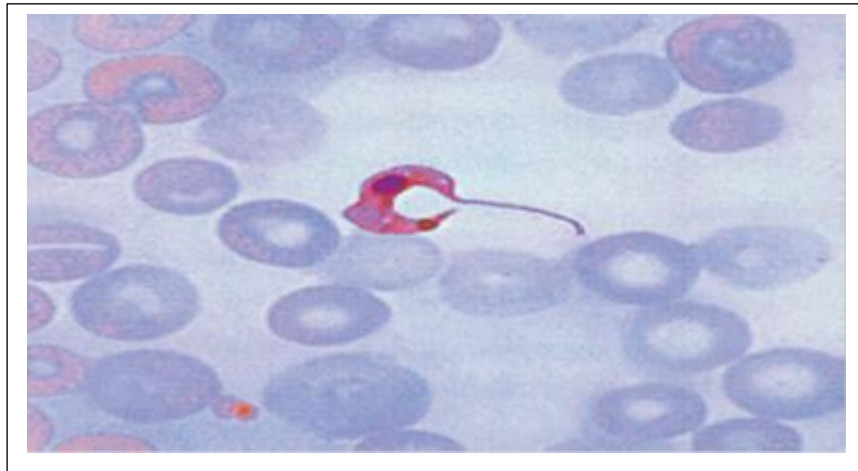


Figure 25 *Trypanosoma cruzi* trypomastigote

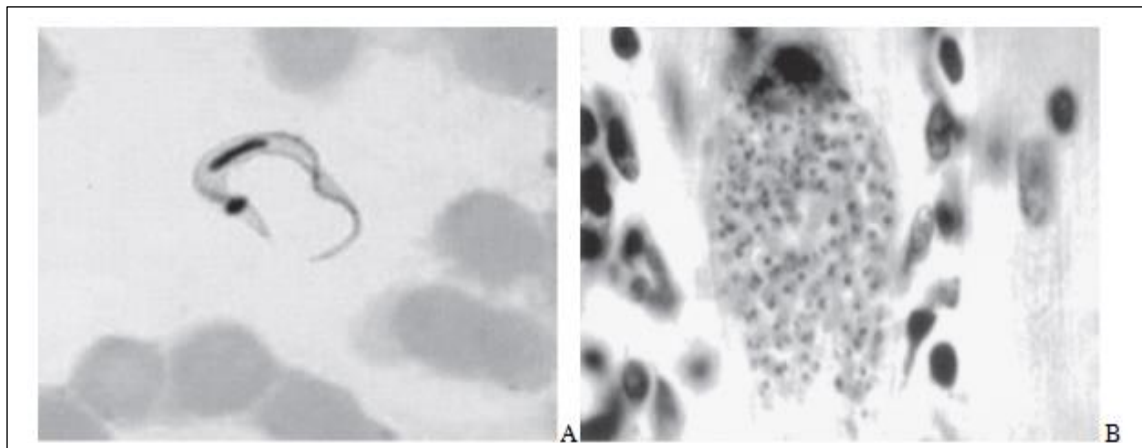


Figure 26 **A**, *Trypanosoma cruzi* in blood film (1600×). **B**, *Trypanosoma cruzi* parasites in cardiac muscle (2500×).

African trypanosomiasis:

The primary area of endemic infection with *T. brucei gambiense* (West African trypanosomiasis) **coincides** with the vector tsetse fly belt through the heart of Africa, where 300,000 to 500,000 people may be infected in Western and Central Africa. *T. brucei rhodesiense* (which causes Rhodesian trypanosomiasis or East African sleeping sickness) is more limited in distribution than *T. brucei gambiense*, being found only in central East Africa, where the disease has been responsible for some of the most serious obstacles to economic and social development of Africa. Within this area, the tsetse flies prefer animal blood, which therefore limits the raising of livestock. The infection in humans has a greater morbidity and mortality than does *T. brucei gambiense* infection, and game animals, such as the bushbuck, and cattle are natural reservoir hosts.

A unique feature of African trypanosomes is their ability to change the antigenic surface coat of the outer membrane of the trypomastigote, helping to evade the host immune response. The trypomastigote surface is

covered with a dense coat of variant surface glycoprotein (VSG). There are approximately 100 to 1000 genes in the genome, responsible for encoding as many as 1000 different VSGs. More than 100 serotypes have been detected in a single infection. It is postulated that the trypomastigote changes its antigenic coat about every 5 to 7 days (antigenic variation). This change is responsible for successive waves of parasitemia every 7 to 14 days and allows the parasite to evade the host humoral immune response.

Each time the antigenic coat changes, the host does not recognize the organism and must mount a new immunologic response. The sustained high immunoglobulin M (IgM) levels are a result of the parasite producing variable antigen types, and in an immunocompetent host, the absence of elevated IgM levels in serum rules out trypanosomiasis.

General Characteristics:

Trypanosomal forms are ingested by the tsetse fly (*Glossina* spp.) when a blood meal is taken. The organisms multiply in the lumen of the midgut and hindgut of the fly. After approximately 2 weeks, the organisms migrate back to salivary glands where the organisms attach to the epithelial cells of the salivary ducts and then transform to their epimastigote forms. Multiplication continues within the salivary gland, and metacyclic (infective) forms develop from the epimastigotes in 2 to 5 days.

While feeding, the fly introduces the metacyclic trypanosomal forms into the next victim in saliva injected into the puncture wound. The entire developmental cycle in the fly takes about 3 weeks, and once infected, the tsetse fly remains infected for life.

In fresh blood, the trypanosomes move rapidly among the red blood cells. An undulating membrane and flagellum may be seen with slower moving organisms. The trypomastigote forms are 14 to 33 μm long and 1.5 to 3.5 μm wide. With a blood stain, the granular cytoplasm stains pale blue. The centrally located nucleus stains reddish. At the posterior end of the organism is the kinetoplast, which also stains reddish, and the remaining intracytoplasmic flagellum (axoneme), which may not be noticeable. The flagellum arises from the kinetoplast, as does the undulating membrane.

The flagellum runs along the edge of the undulating membrane until the undulating membrane merges with the trypanosome body at the anterior end of the organism. At this point, the flagellum becomes free to extend beyond the body.

Pathogenesis and Spectrum of Disease:

Trypanosoma brucei gambiense. African trypanosomiasis caused by *T. brucei gambiense* (West African sleeping sickness) has a long, mild, chronic course that ends in death with central nervous system (CNS) involvement after several years' duration. This is unlike the disease caused by *T. brucei rhodesiense* (East African sleeping sickness), which has a short course and ends fatally within 1 year. After the host has been bitten by an infected tsetse fly, a nodule or chancre at the site may develop after a few days. Usually, this primary lesion will resolve spontaneously within 1 to 2 weeks, and is rarely seen in patients living in an endemic area. Trypomastigotes may

be detected in fluid aspirated from the ulcer. The trypomastigotes enter the bloodstream, causing a low-grade parasitemia that may continue for months with the patient remaining asymptomatic. This is considered stage I disease, where the patient can have systemic trypanosomiasis without CNS involvement. During this time, the parasites may be difficult to detect, even by thick blood film examinations. The infection may self-cure during this period without development of symptoms or lymph node invasion. Symptoms may occur months to years after infection. When the lymph nodes are invaded, the first symptoms appear and include remittent, irregular fevers with night sweats. Headaches, malaise, and anorexia may also be present. The febrile periods of up to 1 week alternate with afebrile periods of variable duration. Many trypomastigotes may be found in the circulating blood during fevers, but few are seen during afebrile periods. Lymphadenopathy is a consistent feature of Gambian trypanosomiasis, and the enlarged lymph nodes are soft and painless. In addition to lymph node involvement, the spleen and liver become enlarged. With Gambian trypanosomiasis, the blood lymphatic stage may last for years before the sleeping sickness syndrome occurs. When the organisms finally invade the CNS, the sleeping sickness stage of the infection is initiated (stage II disease). Behavioral and personality changes are seen during CNS invasion. This stage of the disease is characterized by steady progressive meningoencephalitis, apathy, confusion, fatigue, loss of coordination, and somnolence (state of drowsiness). In the terminal phase of the disease, the patient becomes emaciated and progresses to profound coma and death, usually from secondary infection. Thus, the typical signs of true sleeping sickness are seen in patients with Gambian disease.

Trypanosoma brucei rhodesiense. *T. brucei rhodesiense* produces a more rapid, fulminating disease than does *T. brucei gambiense*. Fever, severe headaches, irritability, extreme fatigue, swollen lymph nodes, and aching muscles and joints are typical symptoms. Progressive confusion, personality changes, slurred speech, seizures, and difficulty in walking and talking occur as the organisms invade the CNS. The early stages of the infection are like those of *T. brucei gambiense* infections. However, CNS invasion occurs early, the disease progresses more rapidly, and death may occur before there is extensive CNS involvement. The incubation period is short, often within 1 to 4 weeks, with trypomastigotes being more numerous and appearing earlier in the blood. Lymph node involvement is less pronounced. Febrile episodes are more frequent, and the patients are more anemic and more likely to develop myocarditis or jaundice. Some patients may develop persistent tachycardia, and death may result from arrhythmia and congestive heart failure. Myocarditis may develop in patients with Gambian trypanosomiasis but is more common and severe with the Rhodesian form.

Laboratory Diagnosis (All Species):

Routine Methods. Blood can be collected from either finger stick or venipuncture (use EDTA anticoagulant). Multiple thick and thin blood films should be made for examination, and multiple blood examinations should be done before trypanosomiasis is ruled out. Parasites will be found in large numbers in the blood during the febrile period and in small numbers when the patient is afebrile. In addition to thin and thick blood films, a buffy coat concentration method is recommended to detect the parasites. Parasites can be detected on thin blood films with a detection limit at approximately 1 parasite/200 microscopic fields (high dry power magnification, $\times 400$) and thick blood smears when the numbers are greater than 2000/mL, and when they are greater than 100/mL with hematocrit capillary tube concentration.

Antigen Detection. A simple and rapid test, the card

indirect agglutination trypanosomiasis test (TrypTect CIATT), is available, primarily in areas of endemic infection, for the detection of circulating antigens in persons with African trypanosomiasis. The sensitivity of the test (95.8% for *T. brucei gambiense* and 97.7% for *T. brucei rhodesiense*) is significantly higher than those for lymph node puncture, micro hematocrit centrifugation, and cerebrospinal fluid examination (CSF) after single and double centrifugation. Its specificity is excellent, and it has a high positive predictive value.

Antibody Detection. Serologic techniques that have been widely used for epidemiologic screening include indirect fluorescent antibody assays (enzyme-linked immunosorbent assay [ELISA]), the indirect hemagglutination test, and the card agglutination trypanosomiasis test. A major problem in endemic areas is that individuals have elevated antibody levels attributable to exposure to animal trypanosomes that are noninfectious to humans.

Serum and CSF IgM concentrations are of diagnostic value. However, CSF antibody titers should be interpreted with caution because of the lack of reference values and the possibility that the CSF will contain serum as the result of a traumatic tap.

Molecular Diagnostics:

Referral laboratories have used molecular methods to detect infections and differentiate species, but these methods are not routinely used in the field.

Therapy:

All drugs used in the therapy of African trypanosomiasis are toxic and require prolonged administration. Treatment should be started as soon as possible, and the antiparasitic drug selected depends on whether the CNS is infected.

American trypanosomiasis:

American trypanosomiasis (Chagas' disease) is a zoonosis occurring throughout the American continent and involves reduviid bugs/kissing bugs (vectors) living in close association with human reservoirs (dogs, cats, armadillos, opossums, raccoons, and rodents). Transmission to humans depends on the defecation habits of the insect vector.

Humans vary with the geographic area. A very serious problem is disease acquisition through blood transfusion and organ transplantation. A large number of patients with positive serologic results can remain asymptomatic. Patients can present with either acute or chronic disease.

Trypanosoma cruzi:

General Characteristics:

Trypomastigotes are ingested by the bug as it obtains a blood meal. The trypomastigotes transform into epimastigotes that multiply in the posterior portion of the bug's midgut. After 8 to 10 days, trypomastigotes develop from the epimastigotes. Humans contract Chagas' disease when the bug defecates while taking a blood meal and the parasites in the feces are rubbed or scratched into the bite wound or onto mucosal surfaces.

In humans, *T. cruzi* is found in two forms: amastigotes and trypomastigotes. The trypomastigote form is present in the blood and infects the host cells. The amastigote form multiplies within the cell, eventually destroying the cell, and both amastigotes and trypomastigotes are released into the blood.

The trypomastigote is approximately 20 µm long, and it usually assumes a C or U shape in stained blood films. Trypomastigotes occur in the blood in two forms: a long slender form and a short stubby one. The nucleus is situated in the center of the body, with a large oval kinetoplast located at the posterior extremity. A flagellum arises from the kinetoplast and extends along the outer edge of an undulating membrane until it reaches the anterior end of the body, where it projects as a free flagellum. When the trypomastigotes are stained with any of the blood stains, the cytoplasm stains blue and the nucleus, kinetoplast, and flagellum stain red or violet.

When the trypomastigote penetrates a cell, it loses its flagellum and undulating membrane and divides by binary fission to form an amastigote.

The amastigote continues to divide and eventually fills and destroys the infected cell. Both amastigote and trypomastigote forms are released from the cell. The amastigote is indistinguishable from those found in leishmanial infections. It is 2 to 6 µm in diameter and contains a large nucleus and rod-shaped kinetoplast that stains red or violet with blood stains. The cytoplasm stains blue. Only the trypomastigotes are found free in the peripheral blood.

Pathogenesis and Spectrum of Disease:

The clinical stages associated with Chagas' disease are categorized as acute, indeterminate, and chronic. The acute stage represents the initial encounter of the patient with the parasite, whereas the chronic phase is the result of late sequelae. In children under the age of 5, the disease is seen in its acute form, whereas in older children and adults, the disease is milder and is commonly diagnosed in the subacute or chronic form. The incubation period in humans is about 7 to 14 days but is somewhat longer in some patients.

Acute symptoms occur 2 to 3 weeks after infection and include high fevers, enlarged spleen and liver, myalgia, erythematous rash, acute myocarditis, lymphadenopathy, keratitis, and subcutaneous edema of the face, legs, and feet. There may be symptoms of CN involvement, which carry a very poor prognosis. Myocarditis is confirmed by electrocardiograph changes, tachycardia, chest pain, and weakness. Amastigotes proliferate within the cardiac muscle cells and destroy the cells, leading to conduction defects and a loss of heart contractility (see Figure 26).

Death may occur due to myocardial insufficiency or cardiac arrest. In infants and very young children, swelling of the brain can develop, causing death.

The chronic stage may be initially asymptomatic (indeterminate stage), and even though parasites are rarely seen in blood films, transmission by blood transfusion is a serious problem in endemic areas. Chronic Chagas' disease may develop years after undetected infection or after the diagnosis of acute disease. Approximately 30% of patients may develop chronic Chagas' disease, including cardiac changes and enlargement of the colon and esophagus. Megacolon results in constipation, abdominal pain, and the inability to discharge feces. There may be acute obstruction leading to perforation, septicemia, and death. However, the most frequent clinical signs of chronic Chagas' disease involve the heart, where enlargement of the heart and conduction changes are commonly seen.

Laboratory Diagnosis:

Routine Methods. Trypomastigotes may be detected in blood by using thick and thin blood films or the buffy coat concentration technique. Any of the blood stains can be used for both amastigote and trypomastigote stages.

Molecular Diagnostics. Referral laboratories have used molecular methods to detect infections with as few as one trypomastigote in 20 mL of blood, but these methods are not routinely used in the field.

Antigen Detection. Immunoassays have been used to detect antigens in urine and sera in patients with congenital infections and those with chronic Chagas' disease.

Antigen detection can also be valuable for early diagnosis and for diagnosis of chronic cases in patients with conflicting serologic test results.

Antibody Detection. Serologic tests for antibody detection include complement fixation, indirect fluorescent antibody, indirect hemagglutination tests, and ELISA.

Histology. In tissue, amastigotes can be differentiated from fungal organisms because they will not stain positive with periodic acid-Schiff, mucicarmine, or silver stains.

Therapy:

Nifurtimox (Lampit) and benznidazole (Radamil) reduce the severity of acute Chagas' disease. In some cases, surgery has been successfully used to treat cases of chagasic heart disease, megaesophagus, and megacolon.

Leishmania spp.:

Leishmaniasis is caused by more than 20 species of the protozoan genus *Leishmania*, with a disease spectrum ranging from self-healing cutaneous lesions to debilitating mucocutaneous infections, subclinical viscerotropic dissemination, and fatal visceral involvement. Published disease burden estimates place leishmaniasis second in mortality and fourth in morbidity among all tropical diseases.

General characteristics:

The parasite has two distinct phases in its life cycle: amastigote and promastigote (Figure 27). The amastigote form is an intracellular parasite in the cells of the reticuloendothelial system and is oval, measuring 1.5 to 5 μm , and contains a nucleus and kinetoplast. *Leishmania* spp. exist as the amastigote in humans and as the promastigote in the insect host. As the vector takes a blood meal, promastigotes are introduced into the human host. Depending on the species, the parasites then move from the bite site to the organs within the reticuloendothelial system (bone marrow, spleen, liver) or to the macrophages of the skin or mucous membranes. Depending on the species involved, infection with *Leishmania* spp. can result in cutaneous, diffuse cutaneous, mucocutaneous, or visceral disease. In endemic areas with leishmaniasis, co-infection with human immunodeficiency virus (HIV)-positive patients is common. If co-infected patients are severely immunocompromised, up to 25% will die shortly after being diagnosed. The use of highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of these co-infected patients.

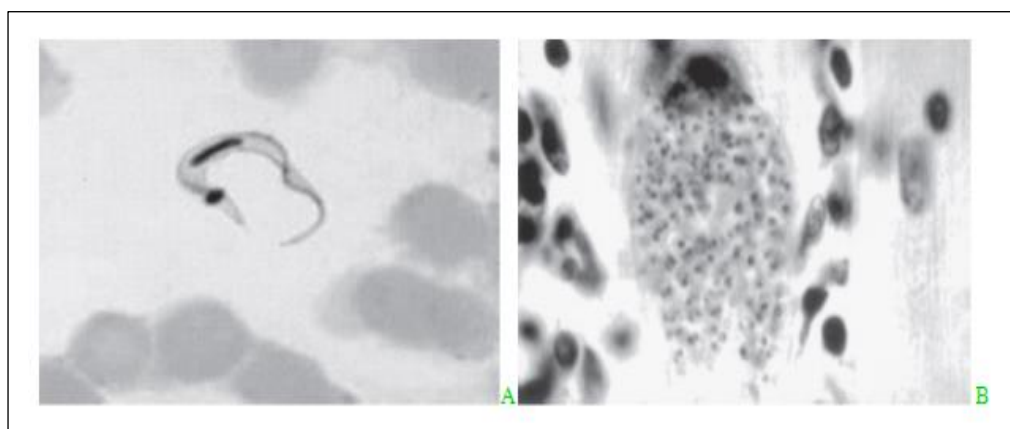


Figure 27, *Leishmania donovani* parasites in Kupffer cells of liver (2000 $\times$). **B,** *Leishmania* sp.

Pathogenesis and spectrum of disease:

The first sign of cutaneous disease is a lesion (generally a firm, painless papule) at the bite site. Although a single lesion may appear insignificant, multiple lesions or disfiguring facial lesions may be devastating for the patient.

Usually, the lesions will have a similar appearance and will progress at the same speed. The original lesion may remain as a flattened plaque or may progress to a shallow ulcer. As the ulcer enlarges, it produces exudate and often becomes secondarily infected with bacteria or other organisms.

In mucocutaneous leishmaniasis, the primary lesions are similar to those found in cutaneous leishmaniasis. Untreated primary lesions may develop into the mucocutaneous form in up to 80% of the cases. Dissemination to the nasal or oral mucosa may occur from the active primary lesion or may occur years later after the original lesion has healed. These mucosal lesions do not heal spontaneously, and secondary bacterial infections are common and may be fatal. Also, untreated visceral leishmaniasis will lead to death; secondary bacterial and viral infections are also common in these patients. The incubation period ranges from 10 days to 2 years, usually being 2 to 4 months. Common symptoms include fever, anorexia, malaise, weight loss, and, frequently, diarrhea.

Clinical signs include nontender enlarged liver and spleen, swollen lymph nodes, and occasional acute abdominal pain. Darkening of facial, hand, foot, and abdominal skin (kala-azar) is often seen in light-skinned persons in India. Death may occur after a few weeks or after 2 to 3 years in chronic cases. The majority of infected individuals will be asymptomatic or have very few or minor symptoms that will resolve without therapy. Since 1990, an increase in leishmaniasis in organ transplant recipients has been documented. Most of these cases have been visceral leishmaniasis.

Laboratory diagnosis:

After the cutaneous lesion exudate is removed, these lesions should be thoroughly cleaned with 70% alcohol. Specimens can be collected from the margin of the lesion by aspiration, scraping, or punch biopsy or by making a slit with a scalpel blade. Smears can be prepared from the material obtained and stained with any of the blood stains; biopsy specimens should also be submitted for routine histologic examination. Specimens for visceral disease include lymph node aspirates, liver biopsy specimens, bone marrow specimens, and buffy coat preparations of venous blood. Amastigotes with reticuloendothelial cells have been detected in a number of different specimens from HIV-positive patients.

Stained smears can be examined for the presence of the amastigotes. Although the specimens can be cultured using special techniques, these procedures are not routinely available.

PCR methods have excellent sensitivity and specificity for direct detection, for identification of causative species, and for assessment of treatment efficacy; although not routinely available, they can be performed at some reference centers. A rapid immunochromatographic dipstick test using the recombinant K39 antigen has become available for the qualitative detection of total anti-*Leishmania* immunoglobulins.

In patients with severe visceral leishmaniasis (kalaazar), there is a characteristic hypergammaglobulinemia, including both IgG and IgM. In highly suspect patients for the diagnosis of visceral leishmaniasis (assuming they are immunocompetent), if hypergammaglobulinemia is not present, this may be used to rule out the original diagnosis. Although serologic testing is available from some reference centers such as CDC, serologic assays are not very useful for the diagnosis of mucocutaneous and visceral leishmaniasis.

THERAPY:

In simple cutaneous leishmaniasis, lesions usually heal spontaneously, although treatment options include cryotherapy, heat, photodynamic therapy, surgical excision of lesions, and chemotherapy. Standard therapy consists of injections of antimonial compounds; however, relapse is quite common and the patient response varies depending on the *Leishmania* species and type of disease.

Other Protozoa:

Amebae, Flagellates (Other Body Sites):

- Amebae:

Naegleria fowleri
Acanthamoeba spp.
Balamuthia mandrillaris

- Flagellates:

Trichomonas vaginalis
Trichomonas tenax

- Coccidia (Other Body Sites):

Coccidia:

Toxoplasma gondii

Free-living amebae:

Infections caused by small, free-living amebae belonging to the genera *Naegleria*, *Acanthamoeba*, and *Balamuthia* are generally not very well-known or recognized clinically.

Also, methods for laboratory diagnosis are unfamiliar and not routinely offered by most laboratories. However, approximately 310 cases of primary amebic meningoencephalitis (PAM) caused by *Naegleria fowleri* and more than 150 cases of granulomatous amebic encephalitis (GAE) caused by *Acanthamoeba* spp. and *Balamuthia mandrillaris* (including several cases in patients with acquired immunodeficiency syndrome [AIDS]) have been documented. Other infections caused by these organisms result in *Acanthamoeba* keratitis, now numbering more than 750 cases and related primarily to poor lens care in contact lens wearers. Additionally, both *Acanthamoeba* spp. and *B. mandrillaris* can cause cutaneous infections in humans. *Sappinia pedata*, a free-living ameba normally found in soil contaminated with the feces of elk and buffalo, was identified in an excised brain lesion from a 38-year-old immunocompetent man who developed a frontal headache, blurry vision, and loss of consciousness following a sinus infection. Additionally, *Paravahlkampfia francinae*, a new species of the free-living ameba genus *Paravahlkampfia*, was recently isolated from the cerebrospinal fluid (CSF) of a patient with a headache, sore throat, and vomiting, symptoms typical of primary amebic meningoencephalitis (PAM) caused by *Naegleria fowleri* from the environment.

NAEGLERIA FOWLERI:

General characteristics

There are both trophozoite and cyst stages in the life cycle, with the stage present primarily dependent on environmental conditions. Trophozoites can be found in water or moist soil and can be maintained in tissue culture or other artificial media. The amebae may enter the nasal cavity by inhalation or aspiration of water, dust, or aerosols containing the trophozoites or cysts. *N. fowleri* is incapable of survival in clean, chlorinated

water. Following inhalation or aspiration, the organisms then penetrate the nasal mucosa, probably through phagocytosis of the olfactory epithelium cells, and migrate via the olfactory nerves to the brain.

The trophozoites can occur in two forms: ameboid and flagellate (Figure 28). The size ranges from 7 to 35 μm . The diameter of the rounded forms is usually 15 μm . There is a large, central karyosome and no peripheral nuclear chromatin. The cytoplasm is somewhat granular and contains vacuoles. The ameboid form organisms change to the transient, pear-shaped flagellate form when they are transferred from culture or teased from tissue into water and maintained at a temperature of 27° to 37° C. These flagellate forms do not divide, but when the flagella are lost, the ameboid forms resume reproduction. Cysts are generally round, measuring from 7 to 15 μm with a thick double wall.

Pathogenesis and spectrum of disease

Primary amebic meningoencephalitis (PAM) caused by *N. fowleri* is an acute, suppurative infection of the brain

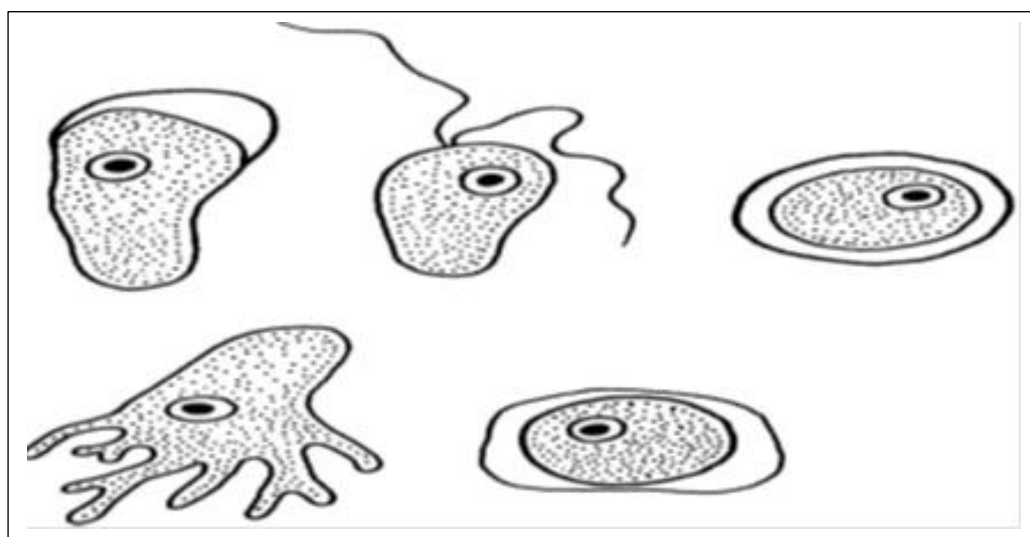


Figure 28 *Naegleria fowleri*, *Acanthamoeba* spp. Diagram of trophozoites and cysts . Flagellate and cyst forms of *Naegleria fowleri*; (lower row) trophozoite and cyst of *Acanthamoeba* spp.

and meninges (Figure 29). With extremely rare exceptions, the disease is rapidly fatal in humans. The period between organism contact and onset of symptoms such as fever, headache, and rhinitis varies from a few days to 2 weeks. Early symptoms include vague upper respiratory tract distress, headache, lethargy, and occasionally olfactory problems. The acute phase includes sore throat; a stuffy, blocked, or discharging nose; and severe headache.

Progressive symptoms include pyrexia, vomiting, and stiffness of the neck. Mental confusion and coma usually occur approximately 3 to 5 days before death, which is usually caused by cardiorespiratory arrest and pulmonary edema.

PAM resembles acute bacterial meningitis, and these conditions may be difficult to differentiate. Unfortunately, if the CSF Gram stain is interpreted incorrectly as a false positive, the resulting antibacterial therapy has no impact on the amebae and the patient will usually die within a few days.

Laboratory diagnosis

Routine Methods

Clinical and laboratory data usually cannot be used to differentiate pyogenic meningitis from PAM. A high index of suspicion is often critical for early diagnosis.

Most cases are associated with exposure to contaminated water through swimming or bathing. There is normally an incubation period of 1 day to 2 weeks, and then a course of 3 to 6 days, most often ending in death. Analysis of the CSF will show decreased glucose and increased protein concentrations. The leukocyte count will range from several hundred to >20,000 cells per mm³. Although Gram stains and bacterial cultures of CSF will be negative, serious patient complications can occur as the result of incorrect therapy if false-positive Gram stains are reported.

A confirmed diagnosis is made by the identification of amebae in the CSF or in biopsy specimens. CSF should be placed on a slide, under a cover slip, and observed for motile trophozoites; smears can be stained with any of the blood stains. It is important not to mistake leukocytes for actual organisms or vice versa. This type of misidentification often occurs when using a counting chamber and the amebae sink to the bottom and round up, hence the recommendation to use just a regular slide and cover slip. Depending on the temperature and lag time between specimen collection and examination, motility may vary.

Slides may be warmed slightly to improve motility. The most important differential characteristic is the spherical nucleus with a large karyosome.

Specimens should never be refrigerated before examination, and CSF should be centrifuged at a slow speed (250× g). If *N. fowleri* is the causative agent, only trophozoites are normally seen, whereas cysts and trophozoites can be seen with *Acanthamoeba* spp.

Other Methods:

Most cases are diagnosed at autopsy; confirmation of tissue findings must include culture and/or special staining with monoclonal reagents in indirect fluorescent antibody procedures.

Therapy:

Although many antimicrobial and antiparasitic drug have been screened for activity against *N. fowleri*, only a few patients have recovered after receiving intrathecal and intravenous injections of amphotericin B or in combination with miconazole.

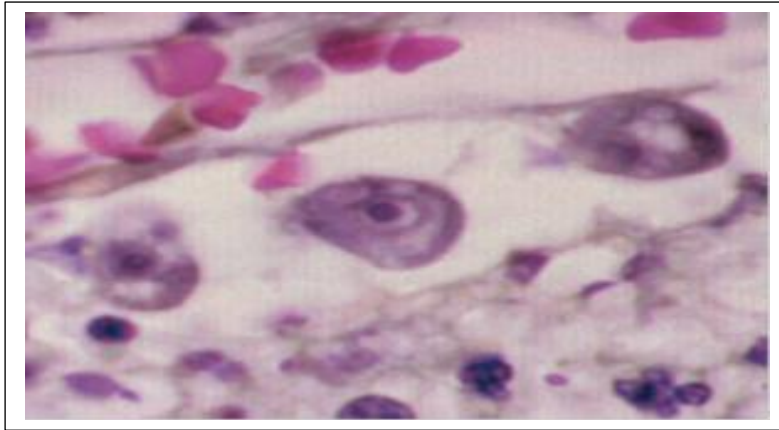


Figure 29 *Naegleria fowleri* in brain tissue. Hematoxylin and eosin stain.

***Acanthamoeba* spp.**

General characteristics

Unlike *N. fowleri*, *Acanthamoeba* spp. do not have a flagellate stage in the life cycle, only the trophozoite and cyst. Several species of *Acanthamoeba* cause granulomatous amebic encephalitis (GAE), primarily in immunosuppressed,

Acanthamoeba spp. also cause amebic keratitis. Motile organisms have spine-like pseudopods; there is a wide organism size range (25 to 40 μm), with the average diameter of the trophozoites being 30 μm . The nucleus has the typical large karyosome, similar to that found in *N. fowleri*. This morphologic characteristic can be seen on a wet preparation.

The cysts are usually round with a single nucleus, also having the large karyosome as in the trophozoite nucleus. The double wall is usually visible, with the slightly wrinkled outer cyst wall and what has been described as a polyhedral inner cyst wall. This cyst morphology is identifiable in organisms cultured on agar plates.

Pathogenesis and spectrum of disease :

GAE (granulomatous amebic encephalitis):

Meningoencephalitis caused by *Acanthamoeba* spp. May present as an acute suppurative inflammation of the brain and meninges similar to *N. fowleri* infection. The incubation period of GAE is unknown; several weeks or months are probably necessary to establish disease. The clinical course tends to be subacute or chronic and is usually associated with trauma or underlying disease, not as a result of swimming. GAE may present with symptoms of confusion, dizziness, drowsiness, nausea, vomiting, headache, lethargy, stiff neck, seizures, and sometimes hemiparesis. Unlike PAM caused by *N. fowleri*, both trophozoites and cysts are found throughout the tissue. Also, dissemination to other tissues such as the liver, kidneys, trachea, and adrenals can occur in immunocompromised individuals; or additional unusual sites also include the ear and necrotic bone from a bone graft of the mandible. Some patients, especially those with AIDS, can develop erythematous nodules, chronic ulcerative skin lesions, or abscesses. Keratitis *Acanthamoeba* spp. also cause keratitis and corneal ulceration. Clinicians need to consider acanthamoebic infection in the differential diagnosis of eye infections that are not responding to bacterial, fungal, or viral therapy.

These infections are often due to direct exposure of the eyes to contaminated materials or solutions. Use of contact lenses is the leading risk factor for keratitis. Conditions that are linked with disease include the use of home-made saline solutions, poor contact lens hygiene, and corneal abrasions. A contact lens can act as a mechanical vector for

transport of amebae present in the storage case onto the cornea. Subsequent multiplication and invasion of the tissue may occur.

Laboratory diagnosis :

- **Routine Methods:**

Tissue stains are effective, and cysts isolated from cultures can be stained with Gomori's silver methenamine, periodic acid-Schiff, and calcofluor white. Identification of *Acanthamoebae* in ocular samples and other tissues can be difficult, even for trained laboratory professionals; in histologic preparations, the organisms appear similar to keratoplasts, as well as neutrophils and monocytes. It has been estimated that up to 70% of clinical *Acanthamoeba* keratitis cases are misdiagnosed as viral keratitis. Also, the average time to diagnosis of keratitis attributable to *Acanthamoeba* infection can average 2.5 weeks longer for non-contact lens wearers than for contact lens users.

- **Other Methods :**

CSF or bronchoalveolar lavage fluid cytospin preparations can be used to look for amebae in patients with GAE or respiratory symptoms.

Organisms have also been found in the adrenal gland, brain, eyes, kidneys, liver, pancreas, skin, spleen, thyroid gland, and uterus.

In infections caused by *Acanthamoeba* spp., periodic acid-Schiff stains the cyst wall red and methenamine silver stains the cyst black.

Therapy :

Disseminated Infections

Trophozoites and cysts of *Acanthamoeba* isolates vary in their sensitivity to antimicrobial agents. They are sensitive in vitro to ketoconazole, pentamidine, hydroxystilbamidine, paromomycin, 5-fluorocytosine, polymyxin, sulfadiazine, trimethoprim-sulfamethoxazole, azithromycin, and extracts of medicinal plants, especially, to combinations of these drugs. In vitro testing confirms strain and species differences in sensitivity.

Balamuthia mandrillaris

Pathogenesis and spectrum of disease

The disease is very similar to GAE caused by *Acanthamoeba* spp. The clinical presentation is subacute or chronic and is usually not associated with swimming in freshwater. No characteristic clinical symptoms, laboratory findings, or radiologic indicators have been found to be diagnostic for GAE. Whether single or multiple, the lesions in the brain involve mainly the cerebral cortex and subcortical white matter. Symptoms include headache, nausea, vomiting, fever, visual disturbances, dysphagia, seizures, and hemiparesis. The clinical course ranges from a few days to several months. In immunocompetent hosts, an inflammatory response occurs; however, with rare exceptions, these patients also tend to die with severe CNS disease.

Laboratory diagnosis :

these organisms can be cultured in mammalian cell cultures using monkey kidney cells ,Although serum antibodies have been identified in infections, laboratory testing is not routinely available.

Therapy :

In vitro studies indicate that *B. mandrillaris* is susceptible to pentamidine isethiocyanate and that patients may benefit from this treatment.

Trichomonas vaginalis:

General characteristics:

Infection is acquired primarily through sexual intercourse, hence the need to diagnose and treat asymptomatic males. The organism is capable of survival for extended periods in a moist environment such as damp towels and underclothes; however, this mode of transmission is thought to be very rare. Trichomoniasis is the primary non-viral sexually transmitted disease worldwide. Infection with *T. vaginalis* has major health consequences for women, including complications in pregnancy, association with cervical cancer, and predisposition to HIV infection.

The life cycle of *T. vaginalis* has a single trophozoite stage, and is very similar in morphology to other trichomonads. The trophozoite is 7 to 23 μm long and 5 to 15 μm wide. The axostyle is usually obvious and protrudes through the bottom of the organism, whereas the undulating membrane ends halfway down the side of the trophozoite. There are a large number of granules evident along the axostyle.

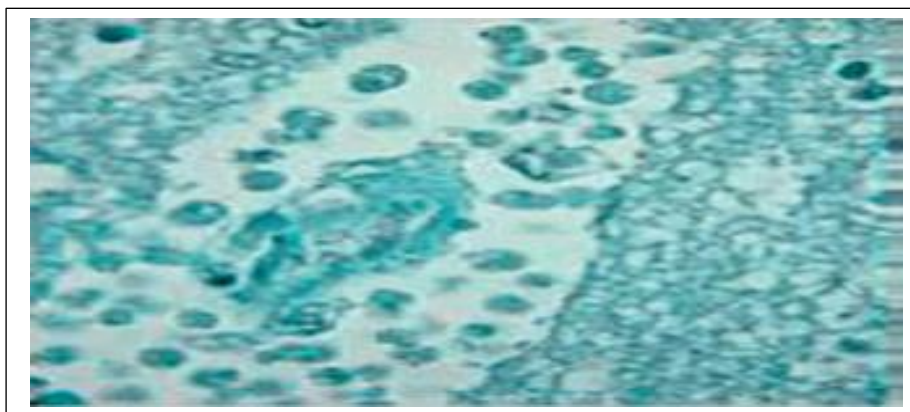


Figure 30 : *Trichomonas*, Giemsa stain

Pathogenesis and spectrum of disease:

Growth of the organism results in inflammation and large numbers of trophozoites in the tissues and the secretions. As the acute infection becomes more chronic, the purulent discharge diminishes, with a decrease in the number of organisms. Symptoms such as vaginal or vulval pruritus and discharge are often sudden and occur during or after menstruation as a result of the increased vaginal acidity. Symptoms include vaginal discharge (42%), odor (50%), and edema or erythema (22% to 37%). Complaints also include dysuria and lower abdominal pain.

From 25% to 50% of infected women may be asymptomatic and have a normal vaginal pH of 3.8 to 4.2 and normal vaginal flora. Even in the carrier form, about 50% of women will become symptomatic during the following 6 months.

Although vaginitis is the most common finding in women with trichomoniasis, other complications include distention of a fallopian tube with pus, endometritis, infertility, low birth weight, and cervical erosion. There is also an increased association with HI transmission and cervical dysplasia.

Dysuria, often the earliest symptom, occurs in about 20% of women with vaginal trichomoniasis. Infected males may be asymptomatic, or the infection may be self-limited, persistent, or result in recurring urethritis. In nonspecific urethritis, *T. vaginalis* has been detected in 10% to 20% of subjects and in 20% to 30% of those whose sexual partners had vaginitis. Once established, the infection persists for an extended period in females but only for about 10 days or less in males. *T. vaginalis* is the cause of 11% of all cases of non-gonococcal urethritis in males.

Respiratory distress has been reported in a full-term, normal male infant with *T. vaginalis* with severe respiratory problems following delivery. A wet preparation of thick, white sputum demonstrated few leukocytes and motile flagellates, which were identified as *T. vaginalis*.

This study supports previous data confirming that the organism may cause neonatal pneumonia.

Laboratory diagnosis:

Humans are the only natural host for *T. vaginalis*, and organisms reside in the vagina and prostate; they usually do not survive outside the urogenital tract. The parasites feed on the mucosal surface of the vagina, where bacteria and leukocytes are abundant. The preferred pH for good parasitic growth in females is slightly alkaline or acidic (6.0 to 6.3 optimal), not the normal pH (3.8 to 4.2) of the healthy vagina. The organisms can also be recovered in urine, in urethral discharge, or after prostatic massage. Often, the organisms are recovered in centrifuged urine sediment from both male and female patients.

Wet mounts:

The identification of *T. vaginalis* is often based on the examination of wet preparations of vaginal and urethral discharges, urine, and prostatic secretions. This examination must be performed within 10 to 20 minutes after sample collection; if not, organisms lose motility and may not be identified. Several specimens may need to be examined for detection of the organisms. The sensitivity associated with wet mount examinations varies between 40% and more than 80%. Often, the percent detection from this procedure is quite low with limited sensitivity and specificity.

Stained Smears:

Giemsa or Papanicolaou stain can be used. However, atypical cellular changes can be misinterpreted, particularly on the Papanicolaou smear. The organisms are routinely missed on Gram stains. The number of false-positive and false-negative results reported on the basis of stained smears strongly suggests that confirmation should be accomplished by observation of motile organisms either from the direct wet mount or from appropriate culture media.

Culture:

A convenient plastic envelope method has been developed, which allows immediate examination and culture in one self-contained system. This system serves as the specimen transport container, the growth chamber during incubation, and the “slide” during microscopy. Once it is inoculated, it requires no opening for examination, and positive growth will occur within 5 days. The sensitivity of this system is reported to be superior to those of other available culture methods.

Antigen Detection:

Several diagnostic tests have been developed, including the XenoStrip-Tv and the OSOM *Trichomonas* Rapid Test both of which are more sensitive than the wet mount.

Molecular Diagnostics:

The use of polymerase chain reaction (PCR) methods has led to improvements in *T. vaginalis* detection; nonviable organisms and cells and target sequences can also be detected.

Therapy:

Metronidazole is recommended for the treatment of urogenital trichomoniasis. Tinidazole has also been used for therapy.

***Pentatrichomonas hominis*:**

Pentatrichomonas hominis derives its name on the basis of the morphologic structure of the trophozoite. The organism has five anterior flagella and a parabasal body. The organism is recovered worldwide and is considered to be nonpathogenic although it has been isolated from patients with diarrhea.

General characteristics:

There is no known cyst stage. The trophozoite resides in the large intestine where it feeds on bacteria. The organism resembles *Trichomonas vaginalis*, measuring 5 to 15 µm in length and 7 to 10 µm in width with an undulating membrane and an axostyle. The organism's undulating membrane extends the entire length of the body, differentiating it from *Trichomonas vaginalis*.

Pathogenesis and spectrum of disease:

Although the organism is considered nonpathogenic, it is the most commonly identified flagellate other than *G. lamblia* and *Dientamoeba fragilis*. The organism may be associated with infections in warm climates.

Laboratory diagnosis:

Similar to *Trichomonas vaginalis*, the wet preparation is commonly used to identify motile trophozoites. However, permanent stained smears provide the greatest sensitivity and specificity.

Therapy:

Because the organism is nonpathogenic, it is important to differentiate it from *Trichomonas vaginalis*. However, if identified, no treatment is necessary.

TOXOPLASMA GONDII:

Toxoplasma gondii is a protozoan parasite that infects most species of warm-blooded animals, including humans. Members of the cat family, Felidae, are the only known definitive hosts for the sexual stages of *T. gondii* and serve as the main reservoirs of infection. Cats become infected with *T. gondii* through carnivorousism or by ingestion of oocysts. After tissue cysts or oocysts are ingested by the cat, organisms are released and invade epithelial cells of the cat small intestine, where they undergo an asexual cycle followed by a sexual cycle with the formation of oocysts, which are excreted in the feces. The uninfected oocyst takes 1 to 5 days after excretion to become infective.

Cats shed oocysts for 1 to 2 weeks and large numbers may be shed, often more than 100,000 per gram of feces. Oocysts survive in the environment for several months to more than 1 year and are resistant to disinfectants, freezing, and drying. However, they are killed by heating to 70° C for 10 minutes. The life cycle in the cat takes approximately 19 to 48 days after infection with the oocysts but only 3 to 10 days after the ingestion of meat infected with cysts (e.g., a mouse).

General characteristics:

There are three infectious stages of *T. gondii*: the tachyzoites (in groups or clones), the bradyzoites (in tissue cysts), and the sporozoites (in oocysts from cat feces).

Tachyzoites rapidly multiply in any cell of the intermediate host and in epithelial cells of the definitive host (cats). Bradyzoites are found within the tissue cysts and usually multiply very slowly; the cyst may contain few to hundreds of organisms, and intramuscular cysts may reach 100 µm in size. The tissue cysts can be found in visceral organs such as the lungs, liver, and kidneys; however, they are more prevalent in the brain, eyes, and skeletal and cardiac muscle. Intact tissue cysts can persist for the life of the host and do not cause an inflammatory response. Tachyzoites are crescent-shaped and are 2 to 3 µm wide by 4 to 8 µm long. One end tends to be more rounded than the other. Giemsa is the stain of choice; the cytoplasm stains pale blue, and the nucleus stains red and is situated toward the broad end of the organism.

Cysts are formed in chronic infections, and the bradyzoites within the cyst wall are strongly periodic acid-Schiff positive. During the acute phase, there may be groups of tachyzoites that appear to be cysts; however, they are not strongly periodic acid-Schiff positive and have been termed pseudocysts.

Pathogenesis and spectrum of disease:

As the tachyzoites actively grow, increase in number, and eventually rupture from the cell, they invade adjacent cells. This process creates additional lesions. Once the cysts are formed, the process becomes quiescent, with little or no multiplication and spread. In the immunocompromised or immunodeficient patient, a cyst rupture Tachyzoites or primary exposure to the organisms often leads to lesions. The organisms can be disseminated via the lymphatic system and the bloodstream to other tissues.

Toxoplasmosis can be categorized into four groups: (1) acquired in the immunocompetent patient; (2) acquired or reactivated in the immunodeficient patient; (3) congenital; and (4) ocular.

Laboratory diagnosis:

The most common method of diagnosis for toxoplasmosis is serologic testing for *T. gondii*– specific antibodies. Other procedures include PCR; examination of biopsy specimens, buffy coat cells, or cerebrospinal fluid; or isolation of the organism in tissue culture or in laboratory animals. The serologic diagnosis of toxoplasmosis a number of additional procedures include enzyme immunoassays, enzyme-linked immunosorbent assays (ELISAs), direct agglutination, an immunosorbent agglutination assay, an indirect immunofluorescence assay (IFA), immunocapture, and immunoblot tests.

Therapy:

Treatment is recommended for the following conditions: clinically active disease, diagnosed congenital toxoplasmosis in newborns, pregnant women with infection during gestation, patients with chorioretinitis, and disease in symptomatic compromised patients. Therapy is also recommended for preventive or suppressive treatment in HIV-infected persons. The currently recommended drugs work primarily against the actively dividing tachyzoite form of *T. gondii* and do not eradicate encysted organisms (bradyzoites).

The most common drug combination used to treat congenital toxoplasmosis consists of pyrimethamine and a sulfonamide, plus folinic acid in the form of leucovorin calcium to protect the bone marrow from the toxic effects of pyrimethamine. Spiramycin is recommended for pregnant women with acute toxoplasmosis when fetal infection has not been confirmed in an attempt to prevent transmission of *T. gondii* from the mother to the fetus.

In immunosuppressed persons with toxoplasmosis, the combination of pyrimethamine and sulfadiazine plus leucovorin is the preferred treatment. Clindamycin, in combination with other antiparasitic medications, is frequently used for the treatment.

Intestinal Nematodes (Roundworms):

Helminths *Nematodes* Intestinal (roundworms):

Ascaris lumbricoides

Enterobius vermicularis (pinworm)

Strongyloides stercoralis (threadworm)

Trichostrongylus spp.

Trichuris trichiura (whipworm)

Capillaria philippinensis (hookworms)

Ancylostoma duodenale (Old World)

Necator americanus (New World)

There are more than 60 species of nematodes known to infect humans. Nematodes are nonsegmented, elongate, cylindrical worms with a well-developed digestive tract and reproductive system. The adult worms

have separate sexes, with the male generally smaller than the female. Most nematodes are diagnosed by finding the characteristic eggs in the stool. The infective stage of the nematodes varies with species; for example, transmission may occur through the ingestion of eggs, whereas others burrow through the skin and migrate to the intestine.

The nematodes have very diverse life cycles providing different routes of transmission as well as disease symptoms.

ASCARIS LUMBRICOIDES:

General characteristics:

Ascaris lumbricoides is the most common and the largest roundworm. The parasite with higher prevalence in the tropical regions. Eggs are ingested and hatch in the duodenum, penetrate the intestinal wall, and migrate to the hepatic portal circulation.

The adult worms live and reproduce in the lumen of the small intestine. The ovum is a thick, oval mammillated (outer protrusions) and embryonated egg. The eggs are passed in the feces and become infective 2 to 6 weeks following deposition, depending on the environment.

Epidemiology:

Transmission is through the fecal-oral route, usually through the ingestion of eggs on contaminated material. *Ascaris* eggs are capable of survival within harsh environmental conditions, including dry or freezing temperatures.

Pathogenesis and spectrum of disease:

Many *A. lumbricoides* infections are asymptomatic. The presentation of symptoms correlates with the length of infection, the number of worms present, and the overall health of the host. Intestinal symptoms range from mild to severe intestinal obstruction. Some patients will develop pulmonary symptoms and present with immune-mediate hypersensitivity pneumonia. The worms may cause an immune condition known as Löffler's syndrome characterized by peripheral eosinophilia.

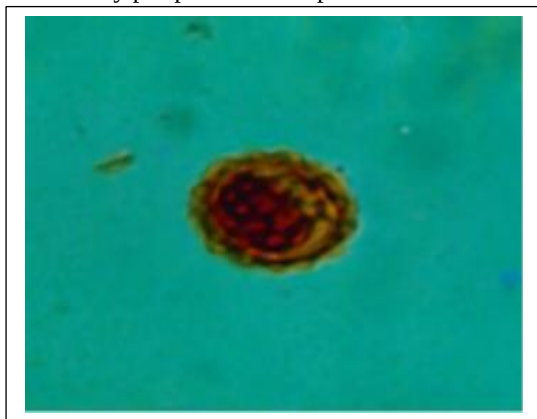


Figure 31 Bile-stained mammillated *A. lumbricoides* ovum.

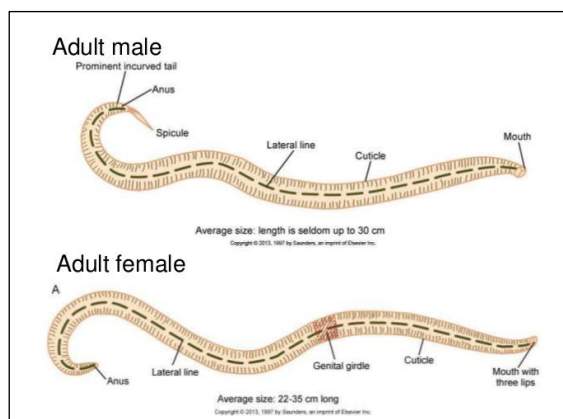


Figure 32 *A. lumbricoides* adult male worm. Note the curved posterior end

Laboratory diagnosis:

Female worms have an extremely high daily output of eggs, making diagnosis relatively easy through the identification of eggs in feces. The large, broadly oval mammillated ova are typically stained brown from bile (Figure 31). Some eggs will be decorticated, or lacking the mammillated outer cover. Infertile eggs may be oval or irregular shaped with a thin shell and containing internal granules. Adult worms may also be identified in feces.

The male is smaller (15 to 31 cm) with a curved posterior end (Figure 32) and contains three well-characterized lips. Larvae may be found in sputum or gastric aspirates as a result of larval migration during development within the human host.

Therapy:

Anthelmintic treatment is recommended for all infections. Preferred therapy includes oral albendazole, mebendazole, or pyrantel pamoate.

Prevention:

Prevention is managed through proper sanitation and good hygiene.

Enterobius vermicularis:

General characteristics:

Enterobius vermicularis (pinworm) is distributed worldwide and commonly identified in group settings of children ages 5 to 10 years. The life cycle is considered direct; transmission occurs from an infected host to another individual. During the night, the mature female worm migrates out of the anus of the infected host and lays eggs in the perianal region. The embryonated eggs will mature and a third-stage larva stage develops, resulting in infectivity within hours. Transmission occurs by ingestion or inhalation of eggs. Reinfection may also occur when the eggs hatch and larvae return to the intestine where they mature.

Epidemiology:

Pinworm is more prevalent in school-age children up to about 14 years of age. Infections are associated with institutional crowding and are familial. Transmission is also associated with an increased rate of reinfection within a group or autoinfection from hatched larvae.

Pathogenesis and spectrum of disease:

Infections with *E. vermicularis* are typically asymptomatic. The most common complaint is perianal pruritus (itching) and resultant restless sleep. Occasionally, the parasite may migrate to other nearby tissues, causing pelvic, cervical, or peritoneal granulomas.

Laboratory diagnosis:

Diagnosis is typically by microscopic identification of the characteristic flat-sided ovum (Figure 33).

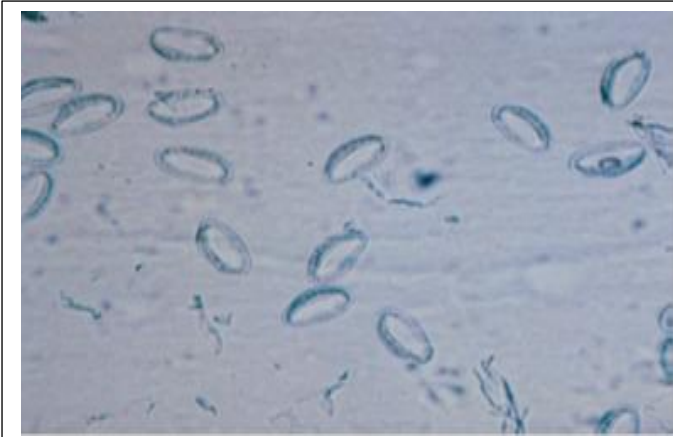


Figure 33: *Enterobius vermicularis* eggs

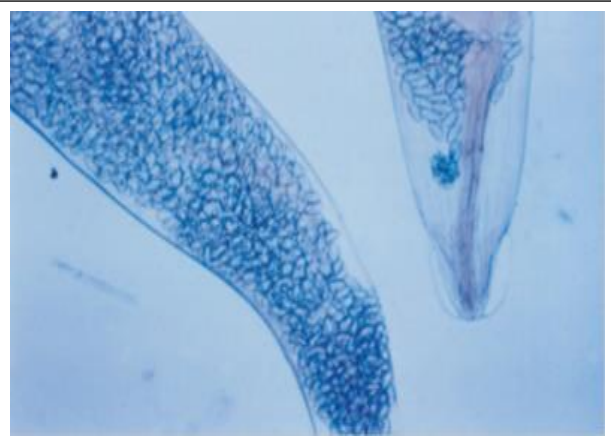


Figure 34: *Enterobius vermicularis* gravid female.

The eggs are collected using a sticky paddle or cellophane tape pressed against the perianal region. Eggs are not typically identified in feces, although they may occasionally be found in a stool specimen. Although adult pinworms may be visible, they can be easily confused with small pieces of thread. The female worm measures 8 to 13 mm long with a pointed “pin” shaped tail. In gravid females, almost the entire body will be filled with eggs (Figure 34). The males measure only 2 to 5 mm in length, die following fertilization, and may be passed in feces.

Therapy:

Anthelmintic therapy is generally effective with one of the following agents: albendazole, mebendazole, pyrantel pamoate, or ivermectin.

Prevention:

Regular good personal hygiene is the major factor for prevention of continued reinfection and autoinfection.

***Strongyloides stercoralis*:**

General characteristics:

Infection with *Strongyloides stercoralis* is less common than other intestinal nematodes.

S. stercoralis, commonly referred to as the threadworm, may inhabit the intestine or exist as a free-living organism in the soil. The life cycle can be classified as direct, indirect (free-living phase), or autoinfective. The filariform (infective larvae) penetrate the skin and migrate via the circulatory system to the heart and lungs. The organism enters the bronchial tree and then is swallowed, where it lives in the digestive tract and matures into an adult worm. In the intestine the filariform larvae may also penetrate the mucosa, resulting in autoinfection.

The female worm produces eggs by parthenogenesis (a form of asexual reproduction where growth and development occur without fertilization), because parasitic adult male worms are nonexistent. Within the indirect life cycle, the rhabditiform (noninfective) larvae develop into mature males and egg-producing females (Figure 35).

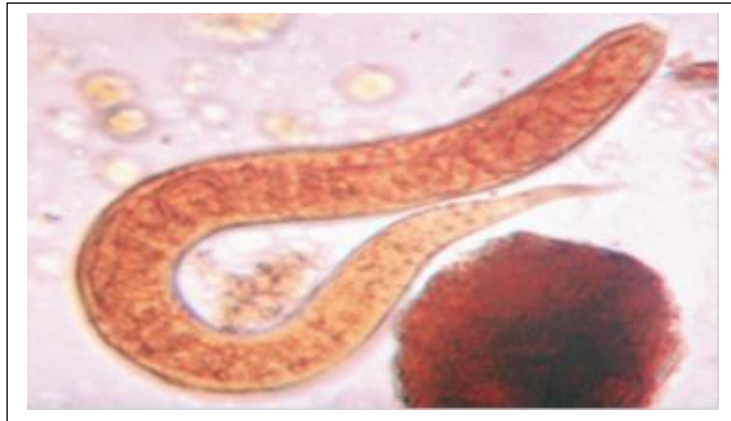


Figure 35 *Strongyloides stercoralis* rhabditiform larva, iodine stain

Epidemiology:

S. stercoralis is transmitted via direct penetration in endemic areas. Person-to-person transmission occurs within institutionalized groups, in day care centers, and among homosexual men.

Pathogenesis and spectrum of disease:

Infections may be asymptomatic or consist of a variety of disseminated strongyloidiasis syndromes. Reinfection is more commonly associated with immunocompromised patients. Acute infections may develop a localized pruritic, erythematous papular rash. Some patients develop a macropapular or urticarial (red and raised) rash on the buttocks, perineum, and thighs. The migration of larvae may cause epigastric pain, nausea, diarrhea, and blood loss. Hyperinfection, an increased worm burden within the lungs and intestines, may occur. Disseminated infections may also result in larvae within the central nervous system, kidneys, and liver. A second species, *Strongyloides fuelleborni*, a primate parasite, has been isolated from humans in Africa and causes a severe life-threatening condition called “swollen belly syndrome.”

Laboratory diagnosis:

The rhabditiform larva is the primary diagnostic stage for strongyloidiasis in humans through microscopic examination of stool. The larvae are 250 to 300 μm long with a short buccal capsule, a large bulb on the esophagus, and a prominent genital primordium. The filariform larvae are larger (up to 500 μm) and have a notched tail with an esophageal to intestinal ratio of 1 : 1. The eggs, which are rarely identified, are segmented with a thin shell. *S. stercoralis* larvae are the most common found in human stool specimens.

Although not available in routine laboratories, realtime polymerase chain reaction (PCR) methods have been developed that amplify the small subunit of the rRNA gene. The assay is used to detect DNA in fecal samples and has a demonstrated sensitivity and specificity of 100%. A high throughput multiplex assay has also been developed that includes primer and probe pairs for *S. stercoralis* as well as other intestinal nematodes and protozoa.

Additional specimens such as sputum, body fluids, and tissues may be used for the diagnosis of hyperinfections.

Therapy:

Ivermectin is the recommended treatment for uncomplicated infections. Albendazole is an alternative.

Prevention:

Immunocompromised individuals and patients taking immunosuppressive medications should avoid contaminated beaches and other areas.

Trichostrongylus spp.:

General characteristics:

Although commonly found in mammals and birds worldwide, approximately 10 different species of *Trichostrongylus* have been found in human infections. The worms are small and live in the mucosa of the small intestine. The adult worm has no visible buccal capsule.

Pathogenesis and spectrum of disease:

Following ingestion of the larvae, the larvae mature and migrate through the lungs. Symptoms are related to the worm burden and the amount of damage within the intestine.

Laboratory diagnosis:

Laboratory diagnosis includes identification of eggs or hatched larvae in the stool. The eggs are oval and resemble hookworm eggs except they are slightly longer and more pointed (Figure 36). Larvae should be differentiated from hookworm and *S. stercoralis*.

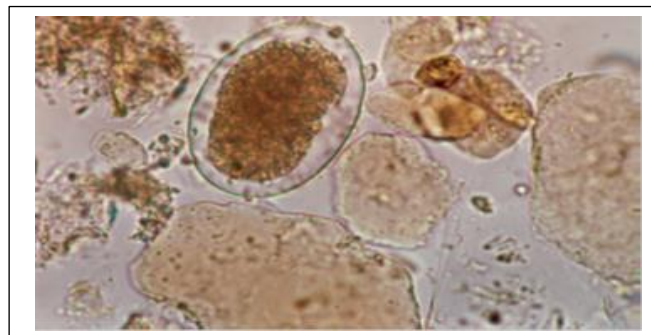


Figure 36 *Trichostrongylus* sp. egg.

Therapy:

Anthelmintic agents are recommended including mebendazole and pyrantel pamoate. Albendazole is the treatment of choice.

Prevention:

Thorough washing of plant material, including cultivated vegetables, before handling or ingestion is recommended.

Trichuris trichiura:

General characteristics:

Trichuris trichiura, whipworm, has a worldwide distribution. Unlike other intestinal nematodes there is no tissue migration phase within the life cycle of *T. trichiura*.

Epidemiology:

Humans are infected by ingestion of the eggs. Larvae are released in the intestine, where they mature into adult worms. Eggs are then passed in the feces and deposited in the soil. The eggs require a warm, moist environment for embryonation in order to become infective to another host.

Pathogenesis and spectrum of disease:

Pathogenesis and severity of the disease are closely related to the worm burden. The lack of symptoms is related to the life cycle that does not include a tissue migration stage, as is seen in other nematode infections. Infections range from mild, very low worm burden, to severe infections with bleeding and weight loss in heavy worm infestations. The characteristic whiplike worm buries its threadlike anterior into the intestinal mucosa and feeds on tissue secretions, causing an inflammatory reaction and peripheral eosinophilia.

Laboratory diagnosis:



Figure 37 *Trichuris trichiura* egg. Note the clearly evident polar hyaline plugs.

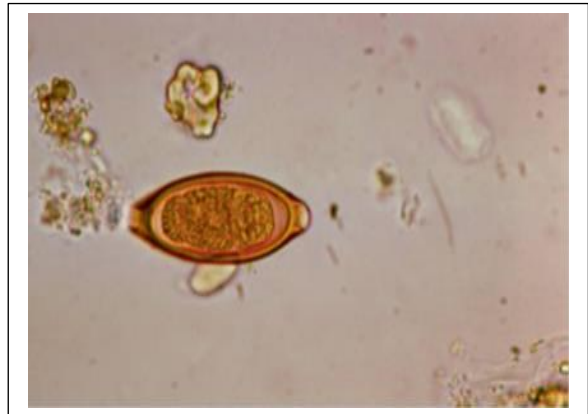


Figure 38 Adult female *Trichuris trichiura*.

Diagnosis is typically from the identification of eggs and rarely the adult worm within the feces. (Figure 37, 38).

Therapy:

Anthelmintics such as albendazole are recommended when necessary.

Prevention:

Prevention includes practicing proper hygiene and sanitation as well as the disposal of dirt or soil contaminated with feces.

Capillaria philippinensis:

General characteristics:

Human infection is thought to occur from the ingestion of uncooked fish harboring infective larvae. The people ingest a large spectrum of raw seafood, including fish, shrimp, crabs, and snails.

Pathogenesis and spectrum of disease

Symptoms vary with the level of worm burden. The larvae are ingested and reside in the small intestine where they burrow into the mucosa. Because of the mechanical insertion into the intestinal wall, patients lose weight rapidly as a result of malabsorption and fluid loss. Long term infections lasting weeks to months may result in death attributable to a severe loss of electrolytes, particularly potassium (hypokalemia), and associated organ

Laboratory diagnosis:

Diagnosis is typically from the identification of eggs, adult worms, or larvae in stool specimens. The eggs resemble those produced by *T. trichiura*. They are somewhat smaller with a thick, striated shell and less prominent polar plugs. Female worms produce the characteristic thick-shelled eggs as well as thin-shelled and free larvae.

Therapy

Anthelmintic agents including albendazole and mebendazole are recommended.

Prevention

Adequate preparation and cooking of seafood, including fish, snails, crabs, and shrimp in endemic areas, are encouraged.

Hookworms:

Hookworms are known to have a worldwide distribution with two species known to infect humans, *Ancylostoma duodenale* (Figure 39) and *Necator americanus*. They are the second most common helminthic infection reported in humans. The eggs and rhabditi- form larvae of the two species are indistinguishable. Differentiation of the species is based on the morphology of the buccal capsule and the adult male copulatory bursa (see Figure 40)

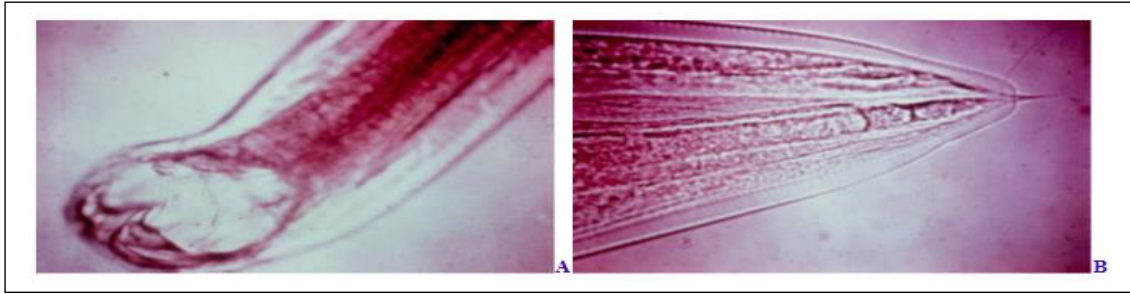


Figure 39: A, *Ancylostoma duodenale* head. B, Tail; note the appearance of the pointed tail

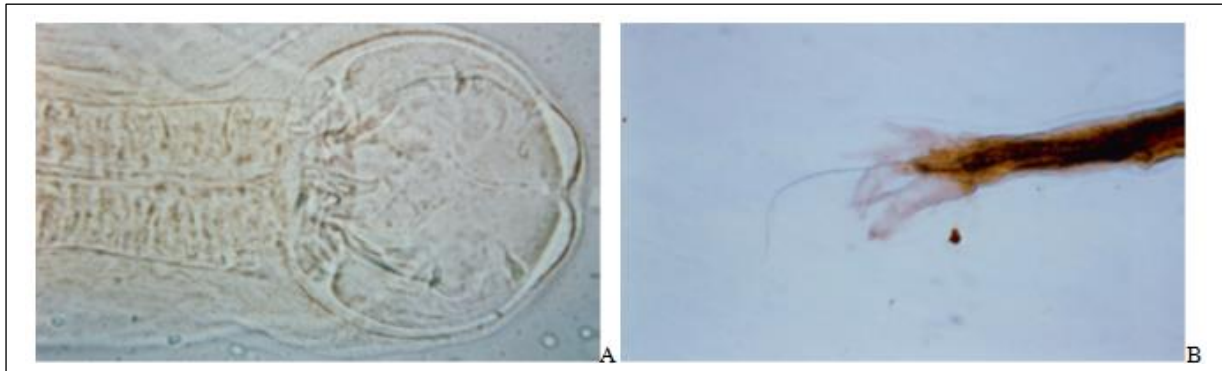


Figure 40 A, *Necator americanus* head; note the clearly evident rounded cutting plates protruding from the head. B, Copulatory bursa.

The parasites, infective filariform larvae, penetrate the skin and enter the circulation where the larvae are capable of breaking through the capillaries and entering the lungs of the host. The larvae migrate up the bronchial tree, over the epiglottis, and are swallowed. Upon entering the digestive system, the hookworms attach to the mucosa of the small intestine. Here they secrete anticoagulants and ingest blood as their source of nourishment.

The worms mature and eggs are passed in the feces and deposited in soil where they mature into rhabditiform larvae. The noninfective rhabditiform larvae will then mature into filariform.

***Ancylostoma duodenale*:**

General characteristics

The adult male tends to be larger than the adult female of *N. americanus*. They attach to the intestinal mucosa by well-developed mouthparts, especially teeth (see Figure 39).

Pathogenesis and spectrum of disease :

A. duodenale is capable of maturation within the intestine without migrating through the lungs of the host.

***Necator americanus*:**

General characteristics

N. americanus, New World hookworm, attach to the intestinal mucosa by well-developed cutting plates (see Figure 40).

Pathogenesis and spectrum of disease

Skin-associated symptoms as described for hookworms Eosinophilia peaks in approximately 2 months in gastrointestinal phase.

Laboratory diagnosis

Hookworms are typically diagnosed by the presence of eggs or rhabditiform larvae found in stool specimens.

The eggs and larvae of the two species are indistinguishable.

The eggs are oval and thin-shelled and contain a clearly visible four- to eight-cell stage embryo. There is a characteristic clear space between the shell and the developing embryo (see figure 40). Recovery and identification of eggs on direct smear or from concentration methods is recommended. Eggs may appear distorted on permanently stained smears. The rhabditiform larvae are typically 250 to 300 μm with a long buccal capsule and an inconspicuous genital primordium (Figures 41 and 42). The larger filariform larvae are approximately 500 μm , with a pointed tail and a esophageal to intestinal ratio of 1 : 4. Both the rhabditiform and filariform larvae must be differentiated from *S. stercoralis*.

Fresh stool stored at room temperature may result in continued maturation and hatching of larvae.

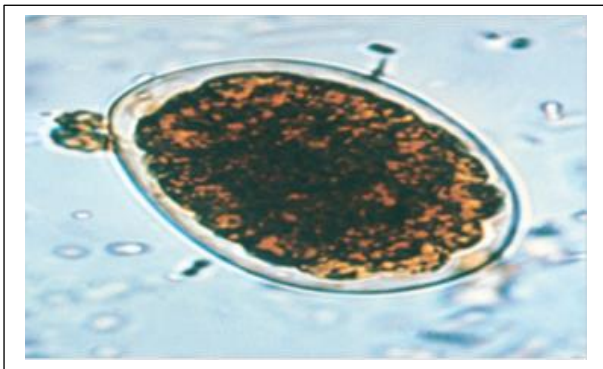


Figure 41: Hookworm egg, iodine stain.

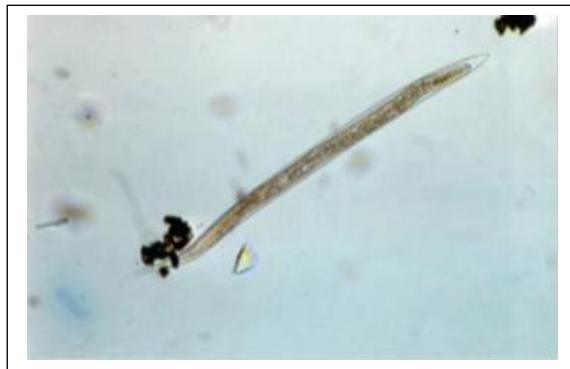


Figure 42 Hookworm rhabditiform larvae.

Therapy:

Anthelmintic agents including albendazole, mebendazole, and pyrantel pamoate are indicated.

Prevention

Avoid contaminated soil and beaches. Wear appropriate footwear such as enclosed shoes in potentially contaminated areas.

Tissue Nematodes (Roundworms):

Helminths *Nematodes (Roundworms)* Tissue:

Trichinella spiralis

Visceral larva migrans (*Toxocara canis* or *Toxocara cati*)

Ocular larva migrans (*Toxocara canis* or *Toxocara cati*)

Cutaneous larva migrans (*Ancylostoma braziliense* or *Ancylostoma caninum*)

Dracunculus medinensis

Parastrongylus (Angiostrongylus) cantonensis

Parastrongylus (Angiostrongylus) costaricensis

Gnathostoma spinigerum

Tissue nematodes have life cycles similar to those of intestinal nematodes, consisting of five distinct stages including adult male and female worms and four larval stages. These organisms are distributed worldwide, predominantly in the tropics and subtropics. The organisms are transmitted by three routes: biting and subsequent blood-feeding arthropods (filarial worms), ingestion of small freshwater crustaceans; and ingestion of contaminated meat. In most cases, adult worms do not multiply and develop within the human host. Clinical symptoms are dependent on the number of infecting parasites, the tissue invaded, and the host's general health and immune response. Diagnosis is typically by the microscopic visualization of the organisms in tissue when appropriate.

Trichinella spiralis:

General characteristics:

The family Trichinellidae contains 11 recognized species including *Trichinella spiralis*, *Trichinella nativa*, *Trichinella nelsoni*, *T. murrelli*, *T. papuae*, *T. zimbabwensis*, *T. pseudospiralis*, and *T. britovi*, all capable of causing trichinosis.

However, *T. spiralis* is the most common human pathogen. The organism is unique in comparison to other helminths in that all stages of development, including the adult and larval stages, occur within a single host.

Epidemiology

Trichinella occurs worldwide with the cycle maintained in several different mammalian species. The mammal serves as the definitive host for the adult worm and the intermediate host for the encysted larvae. Humans acquire the infection by eating undercooked meat that contains the infective encysted larvae. Although this is typically transmitted in pork, human cases have been associated with ingestion of bear, walrus, horsemeat, and other mammals.

The encysted larvae are ingested. When the undercooked meat is digested in the stomach, the larvae are resistant to the gastric pH and pass to the intestine, where they invade the mucosa. In about 1.5 days, the larvae mature and mate, and the female worm begins to release motile larvae. These larvae then migrate to the lymphatic system or mesenteric venules and become distributed throughout the body. The larvae then deposit

in the striated muscle tissue, where they can continue development, coil, and encyst, becoming infective. The larvae encyst in the active striated muscle including the diaphragm, larynx, tongue, jaws, neck, ribs, biceps, and gastrocnemius.. The larvae may remain viable within the cyst for several years. The larvae eventually die and the encysted capsules become calcified.

Pathogenesis and spectrum of disease

Trichinosis is a disease of the muscle caused by infection with the encysted larval form of *Trichinella* spp. (Figure 43). The adult stages reside in the human intestine.

The disease ranges from mild to severe dependent on the number of parasites present. The intestinal stage lasts approximately 1 week and typically includes mild symptoms of nausea, abdominal discomfort, diarrhea, and/or constipation. Diarrhea may last as long as 14 weeks with no apparent muscle involvement. The migration of the larvae results in an intense inflammatory response causing periorbital edema, fever, muscle pain or

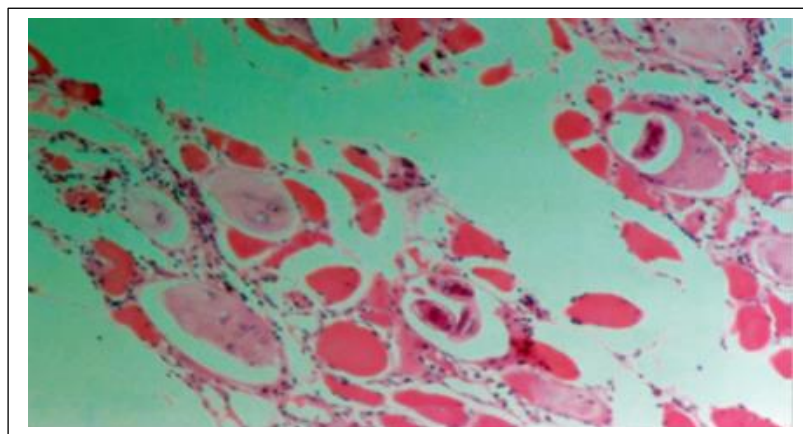


Figure 43: Trichinosis. Encysted larvae within tissue.

tenderness, headache, and myalgia. A marked peripheral eosinophilia is often present. If the parasitic infection is low, eosinophilia may be the only diagnostic sign evident.

Occasionally, splinter hemorrhages may be present below the nails.

In addition to the typical infection of the active striated muscle as previously indicated, occasionally larvae will migrate into the brain, meninges, and myocardium.

However, the larvae will not encyst in these tissues. Brain and meningeal infections will result in neurologic symptoms, and infection of the myocardium may result in myocarditis and dysrhythmias leading to sudden death.

Laboratory diagnosis :

Diagnosis may be difficult, because the symptoms may resemble a variety of flulike illnesses. A thorough patient history is required to assist the physician in diagnosing the condition in a timely fashion. Identification of encysted larvae through muscle biopsy provides definitive diagnosis. However, based on location, some

tissues may be difficult to access and therefore the condition may not be diagnosed until the postmortem examination.

Histologic examination of formalin-fixed or paraffin-embedded tissue may be used to visualize encysted larvae. Occasionally, dependent on the length of infection, calcified larvae may be seen in x-rays.

Serologic diagnosis is sufficient in most cases. Patients will present with a specific antibody response in 3 to 5 weeks following acute illness. A negative serologic test followed by a positive seroconversion is considered definitive diagnosis.

Molecular species-specific polymerase chain reaction (PCR) has been developed.

Therapy :

Thiabendazole is used during the intestinal phase to reduce the number of potentially infective larvae, and although the encysted larvae cannot be removed, albendazole is used to limit the continued pathologic development of the organism. Supportive measures including analgesics and steroids may be administered to lessen the effects of the generalized inflammatory response.

Prevention

Most effective prevention relies on eating only thoroughly cooked meat as well as maintaining good animal husbandry for domestic swine.

TOXOCARA CANIS (VISCERAL LARVA MIGRANS) AND TOXOCARA CATI (OCULAR LARVA MIGRANS):

General characteristics

Toxocara canis (intestinal ascarid of dogs) and *Toxocara cati* (intestinal ascarid of cats) are the cause of a human syndrome resulting from larval migration within the host.

Epidemiology

Toxocariasis is a zoonotic disease with worldwide distribution. Humans become infected with the accidental ingestion of eggs (Figure 44). The definitive hosts, dogs (*T. canis*) and cats (*T. cati*), pass the larvae transplacentally or lactogenically to their offspring and pass unembryonated eggs in the feces. The eggs mature in 10 to 20 days, and then become infective. Once the eggs are ingested, the larvae are released in the small intestine penetrate the mucosa, and migrate to the liver, lungs, or other body sites. The larvae migrate up the respiratory tract and are swallowed, returning to the intestinal tract where they mature into adult worms. The adult worms are unable to mature in a human host and therefore wander throughout the body causing the migratory syndromes.

Pathogenesis and spectrum of disease

Typically the infections are mild but may be severe. Severe life-threatening infections occur when there is involvement in the heart, brain, or other vital organs.

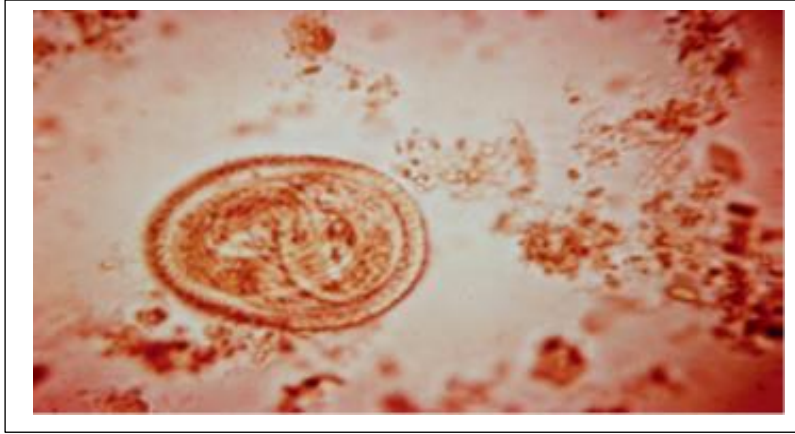


Figure 44: *Toxocara canis* egg. Note the rough appearance on the outer surface of the egg. The egg also contains an infectious L2 larvae.

Disease is frequently found in young children and may persist for long periods with minimal pathologic manifestations. Larvae that remain in the liver or lungs may become encapsulated in fibrous tissue. Visceral (tissue) larva migrans (VLM) may result in a high degree of eosinophilia; however, this may be absent in ocular larva migrans (OLM). Symptoms may include fever, hepatomegaly, hyperglobulinemia, pulmonary infiltration, cough, neurologic symptoms, and endophthalmitis.

OLM may result in the development of a granulomatous reaction in the retina of the eye.

Laboratory diagnosis

Toxocariasis must be differentiated from other migratory helminthic diseases including *A. lumbricoides*, *S. stercoralis*, and *Trichinella* spp. A history of exposure to dogs and cats is of importance when considering an infection with *Toxocara* spp. Because humans are an insufficient host for completion of the organism's life cycle, eggs are not passed in the stool. Diagnosis typically requires biopsy of tissue.

Serologic diagnosis has proven effective, particularly in OLM. Aqueous humor–elevated antibody titer specific for *Toxocara* spp., in comparison to serum levels, is considered diagnostic. Although serologic testing has been useful, it is important to note that antibody titers may vary depending on the location of the infection. A serum titer of 1 : 8 is considered significant for OLM; 1 : 32 is significant for VLM.

Therapy

Effective therapy depends on the location of infection but several anthelmintic medications have been used including thiabendazole, ivermectin, albendazole, and diethylcarbamazine.

Prevention

Small children should be kept out of sandboxes and playgrounds frequented by dogs and cats. In addition, regular deworming of dogs and cat will reduce the spread of infective eggs.

***Ancylostoma braziliense* or *ancylostoma caninum* (cutaneous larva migrans):**

General characteristics

Ancylostoma braziliense and *Ancylostoma caninum* are common hookworms of dogs and cats. The parasites penetrate the skin and cause cutaneous larva migrans (CLM), also referred to as creeping eruption.

Epidemiology

The organisms are found in warm climates within the Southeastern United States. Dogs and cats are the natural definitive host for *Ancylostoma* spp. The infective larvae penetrate the skin of the host and migrate in the circulation. The adult worms reside in the intestine. The eggs are shed in the feces of dogs and cats. The eggs undergo maturation in moist, sandy soil in areas protected from desiccation, such as under shady trees and houses. Children are often infected when playing in sandboxes that have been contaminated with dog and cat feces.

Pathogenesis and spectrum of disease

The infective larvae penetrate the skin of the human host and migrate through the subcutaneous tissue. The host develops pruritic papules at the site of penetration, followed

by serpiginous, vesicular, elevated linear tracks. The larvae will migrate several millimeters each day, forming these continued tracks. The area surrounding the tracks becomes inflamed with marked edema.

The patient may present with a peripheral eosinophilia. Infection is typically self-limiting. As the larvae migrate, the host may scratch and scar the tissue, subjecting the host to potential secondary bacterial infections. The signs and symptoms resemble those of infection with similar insect larvae, *Strongyloides stercoralis*, and other animal hookworms.

Systemic involvement is rare; however, cases of pneumonitis resulting from larvae migration into the lungs have been identified. In addition, gastrointestinal discomfort including abdominal pain, diarrhea, and weight loss has been associated with *Ancylostoma* spp. infections. This condition is referred to as eosinophilic enteritis.

Laboratory diagnosis

Laboratory diagnosis is limited. Evidence of visible tracks and patient history of possible exposure are usually sufficient. The patient may present with a peripheral eosinophilia. In systemic cases, larvae may be recovered from sputum and Charcot-Leyden crystals may be evident.

Therapy

Anthelmintic therapy may include ivermectin or thiabendazole.

***Dracunculus medinensis*:**

General characteristics

Dracunculus medinensis, commonly referred to as the guinea worm, is the cause of a subcutaneous infection known as dracunculiasis. The worm has a characteristic, thick cuticle and a large uterus that fills the body cavity and contains rhabditoid larvae.

Epidemiology

Humans are infected by the ingestion of freshwater from stagnant ponds containing larvae-infected copepods. The copepods are digested in the stomach, releasing the larvae. The larvae penetrate the small intestine and migrate through the thoracic musculature. Both adult male and female worms mature in approximately 2 to 3 months. The gravid female develops in approximately 10 to 14 months, migrating to the lower extremities. The gravid female produces a blister on the skin, and when the host submerges the affected area in water, the blister erupts and releases larvae into the water.

Pathogenesis and spectrum of disease

The blisters formed by the gravid female worm cause burning and itching. Systemic symptoms may include fever, nausea, vomiting, diarrhea, headache, urticarial and eosinophilia. Secondary bacterial infections may occur. In addition, dead worms within the host may be absorbed or may calcify, causing secondary inflammatory symptoms.

Laboratory diagnosis

Diagnosis is by identification of larvae or adult worms.

Therapy

Treatment requires removal of the adult worms. The female worms are attached to a stick and slowly retracted from the host by gradual turning of the stick and removal of the worm. Although anthelmintic medications, such as metronidazole or thiabendazole, are not lethal, they are administered to assist with the retraction of the worms. Analgesics and antimicrobials are administered for discomfort and the prevention of secondary infections.

Parastrongylus cantonensis

(Cerebral angiostrongyliasis):

General characteristics

Parastrongylus cantonensis, previously known as *Angiostrongylus* sp., is a filarial worm commonly referred to as the rat lungworm.

Epidemiology

The adult worms reside in the pulmonary artery and right side of the heart. Eggs shed by the female lodge in the pulmonary capillaries, where the larvae hatch and migrate up the trachea. The larvae are swallowed and passed in the rodent feces. Once released the larvae infect the intermediate host, mollusks. The mollusks are consumed by a variety of paratenic hosts such as shrimp, fish, crabs, or frogs. The rodents then consume the paratenic hosts and the larvae penetrate the intestine, enter the circulation, and migrate to the central nervous system. Following two successive molts, the larvae then reenter the circulation and migrate to the pulmonary artery. Humans are infected by ingestion of either the intermediate or the paratenic host.

Pathogenesis and spectrum of disease

The pathogenesis correlates with the worm burden and the site of infection. The larvae may migrate to the central nervous system, causing meningitis or meningoencephalitis.

Symptoms include headache, fever, eosinophilia, increased cerebrospinal fluid (CSF) protein, and neurologic manifestations. Occasionally, the larvae may migrate to the eye, causing blindness. Most often the disease is self-limiting.

Laboratory diagnosis

Definitive diagnosis relies on histologic identification of the adult female worm. The adult female worm has a distinctive morphologic appearance with spiral, winding, “barber pole” appearing uterus. Highly specific serologic assays are available.

Therapy:

Anthelmintic therapy may be helpful, such as mebendazole. It is important to closely monitor therapy, because the therapy may actually exacerbate the inflammatory response of the host and cause more systemic damage. If larvae are located within the eye, surgical removal is recommended.

Parastrongylus costaricensis

(Abdominal angiostrongyliasis):

General characteristics

Parastrongylus costaricensis is found primarily in the cotton rat and the black rat.

Pathogenesis and spectrum of disease

The life cycle is very similar to that of *Parastrongylus cantonensis*. Human infection is typically by ingestion of salad contaminated with infected slugs or snails. The larvae create inflammatory lesions in the wall of the bowel, resulting in tissue inflammation, necrosis, vomiting, and diarrhea. The patient may experience lower right quadrant abdominal pain similar to that manifested in appendicitis.

Laboratory diagnosis

Histologic identification of larvae or eggs in tissue sections results in definitive diagnosis. Patients often present with leukocytosis and eosinophilia. Radiologic imaging may be useful.

Therapy

Traditional anthelmintic therapy is recommended.

***Gnathostoma spinigerum*.**

General characteristics

Gnathostoma spp., a gastric Spirurida, is found in a variety of mammals worldwide. Dogs and cats serve as the definitive host for *G. spinigerum*.

Epidemiology

The adult worms reside in the stomach of the definitive host where they mate and produce eggs that are passed in the feces. When the feces are deposited in water, the larvae hatch and infect copepods. The larvae mature in the copepods and are then ingested by a variety of hosts including fish, snakes, and frogs. Inside the paratenic host the larvae then migrate to the musculature and encyst until the tissue is ingested by the definitive host. Once in the definitive host, the larvae excyst and penetrate the gastric wall, migrating and maturing in the stomach. Humans act as accidental hosts when they ingest larvae in contaminated fish.

Pathogenesis and spectrum of disease

The worms are incapable of maturation within the human host and migrate aimlessly, causing tissue damage and inflammation. The infection is not typically fatal; however, it depends on the migration pattern and organs infected.

Laboratory diagnosis

The identification of the larvae in tissue is definitive for diagnosis. The head contains four rows of cephalic hooklets. The body is covered with transverse rows of spines that diminish anteriorly to posteriorly.

Therapy

Supportive corticosteroid treatment is recommended. Although anthelmintics are not lethal, they are often recommended. Surgical excision of the larvae is optimal treatment.

Blood and Tissue (Filarial) Nematodes:

Nematodes Blood and Tissues (Filarial Worms):

Wuchereria bancrofti

Brugia malayi

Brugia timori

Loa loa

Onchocerca volvulus

Mansonella ozzardi

Mansonella streptocerca

Mansonella perstans

Blood and tissue filarial nematodes are roundworms that infect humans. These organisms are transmitted via a blood-sucking arthropod vector such as a mosquito, midge, or fly. The filarial nematodes infect the subcutaneous tissues, deep connective tissues, body cavities, and lymphatic system. The life cycles of the

filarial nematodes are complex , The infective larval stage resides in the insect vector with the adult worm stage, which is the pathogenic form in humans. When the arthropod vector feeds on a human blood meal, the infective larvae are injected into the bloodstream. The larvae are motile and migrate to the lymphatic vessels. The infective larvae grow and develop into the adult gravid worm in the human host over a period of months. The male and female adult worms mate in the definitive human host. The female worm produces large numbers of larvae called **microfilariae**.

Depending on the species, the microfilariae may maintain the egg membrane as a sheath or may rupture the egg membrane, resulting in an unsheathed form. These parasites can reside in the host for many years and cause chronic, debilitating conditions and severe inflammatory responses. Identification of the various species is based on the morphology of the microfilaria, the periodicity (defined circadian rhythm), and the location within the human host. Microfilariae morphologic characteristics are important in the identification and include the presence or absence of the sheath and the presence and arrangement of the nuclei in the tail of the worm , A comparison of the morphologic characteristics of the pathogenic filarial worms is depicted in, Diagnosis of infection is based on the identification of the microfilariae in the blood or tissue of the host.

***Wuchereria bancrofti*:**

Epidemiology

W. bancrofti is the most common identified species of filarial worms that infect humans. The mosquito vectors have complex life cycles that include laying eggs and developing larvae on the surface of a water source. When the larvae mature into adult mosquitos, the male and females will swarm in the evening and mate. The female requires feeding on a blood meal in order to reproduce. The mosquito becomes the intermediate host for the microfilaria parasite. Humans are the definitive host and the reservoir for *W. bancrofti*. The parasite has two forms that demonstrate different periodicities. The nocturnal periodic form is found in the peripheral blood during the night between 10 pm and 4 am. The second form is found only in the Pacific Islands and is present in the blood at all times, but more frequently during the day in the afternoon hours.

Pathogenesis and spectrum of disease

Microfilaria clinical disease varies geographically based on the species of nematode causing the infection. The disease may present as acute or asymptomatic for many years. *W. bancrofti* causes bancroftian filariasis and elephantiasis. The adult worm resides in the lymphatic vessels distal to the lymph nodes. The presence of the organisms within the host results in an immunologic response including inflammation, hyperplasia, lymphedema, and hyperplasia.

Lymphedema most often occurs in the lower extremities. Elephantiasis is a crippling condition that results from extended periods of filarial infection. The obstruction of the lymphatic vessels causes fibrosis and proliferation of dermal and connective tissue, resulting in the wrinkled, dry appearance of an “elephant” extremity. Lymphedema may also occur in the arms, female breasts, and scrotum of infected males.

Acute lymphatic filariasis results from worms residing within the lymph nodes. The lymph nodes swell and lymphangitis may appear peripherally from the infected node.

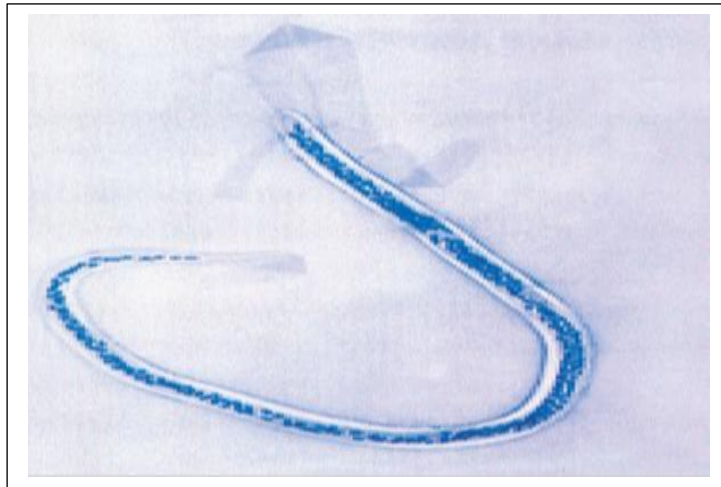


Figure 45:Microfilaria of *Wuchereria bancrofti* in thick blood film

.Laboratory diagnosis

Definitive laboratory diagnosis is based on the identification of the parasites in blood, fluids, or tissue.

Serologic assays that measure antibody response have limited utility in the diagnosis of infections with microfilariae.

The antibodies tend to demonstrate a high cross reactivity with other antibodies made in response to a wide variety of parasitic worm infections. The absence of an antibody reaction would, however, indicate the lack of infection by a microfilaria species. Laboratory detection of *W. bancrofti*-circulating antigens has demonstrated high specificity and sensitivity in detecting parasitic infections. However, the commercial testing formats available are not FDA-approved. Polymerase chain reaction (PCR) amplification is available in reference laboratories for the rapid diagnosis of an infection .

Brugia malayi* and *brugia timori

General characteristics

The *Brugia* spp. are lymphatic filarial parasites resembling *W. bancrofti*

Epidemiology

The organism is transmitted via mosquitos included in the genus *Anopheles* and *Mansonia*.

Pathogenesis and spectrum of disease

As in infections with *W. bancrofti*, two periodic forms exist. The nocturnal form is the most common and located near areas of coastal rice fields, whereas the nonperiodic form is associated with infections in areas near swampy forests. The pathogenesis and spectrum of disease is essentially the same as for *W. bancrofti*, with the exception that involvement of the genital lymphatic vessels is predominantly associated with *W. bancrofti*.

Clinical disease progresses faster following infection with *B. malayi* than with *W. bancrofti*. Microfilariae may appear in the blood in as little as 3 to 4 months. *Brugia* spp. have been implicated in zoonotic infections of dogs, cats, rabbits, and raccoons worldwide. Cases of human infection have occurred in the United States in the northeastern region. Clinical disease is typically asymptomatic but may present with a tender region in the cervical, axillary, or inguinal region. The lymphatic mass may contain either a live or a dead worm. If the worm is no longer viable, the mass may be surrounded by a granulomatous reaction.

Laboratory diagnosis

Definitive diagnosis is generally by the identification of the adult worms in the blood of infected individuals. The adult worms can be distinguished from *W. bancrofti* morphologically. The *B. malayi* microfilariae are sheathed and contain 4 to 5 subterminal and 2 terminal nuclei in the tail. *B. timori* also contains 5 to 8 subterminal and terminal nuclei in the tail, but they are much larger than *B. malayi*. The *B. malayi* sheath will stain bright pink with Giemsa, whereas the *B. timori* sheath does not stain. The microfilariae of *B. timori* tend to be somewhat longer. High-frequency ultrasound has been useful in identifying adult worms in various locations within the patient, such as lymphatic vessels of the legs, inguinal area (groin or lower abdomen), lymph nodes, and female breasts. Nucleic acid-based methods have been developed but are not widely used in clinical laboratories.

Therapy

Diethylcarbamazine (DEC) is the treatment of choice for lymphatic filarial parasites including *W. bancrofti* and *Brugia* spp. Additionally, ivermectin and albendazole may be used. Death of the microfilarial worms may result in an increased hypersensitive reaction requiring the need for treatment with antihistamines to limit the inflammatory symptoms.

Prevention

The use of insect repellent is recommended for travelers in areas where the parasites are endemic. DEC has also been used for prophylactic treatment before travel. Vector control studies in combination with mass drug administration of DEC and ivermectin have successfully decreased the population of the arthropod vectors and decreased filarial infection in the human hosts.

LOA LOA

General characteristics

Loa loa, commonly referred to as the eye worm, is a microfilaria that circulates in the bloodstream and resides in the subcutaneous tissue in the human host. The worm may grow up to 300 µm.

Epidemiology

The parasite is found within the rain forests of West and Central Africa. The organism is transmitted through a bite of the tabanid fly or deer fly of the genus *Chrysops*. The female lays her eggs on the leaves of small plants

near the water. The larvae feed on small insects and develop in wet soil. The male fly feeds on pollen and the female feeds on a blood meal.

Pathogenesis and spectrum of disease

The organism is often associated with asymptomatic infection. The larvae develop into adult worms in approximately 6 to 12 months, but can persist in the human host for up to 17 years. The infection is typically identified when the adult worm is seen migrating within the subconjunctiva of the host. Symptoms associated with infection include episodic “calabar swelling,” which are localized areas of transient angioedema in response to the production of parasitic metabolic products. Predominant swelling on the extremities with inflammation of nearby joints and peripheral nerves may occur. Immunemediated encephalopathy, nephropathy, and cardiomyopathy may occur.

Laboratory diagnosis

Infections with *Loa loa* may be asymptomatic for many years before the appearance of microfilariae in the peripheral blood. Therefore, patient diagnosis is often made on the basis of the patient’s clinical symptoms including calabar swelling, eosinophilia, and travel or residency in an endemic area.

Direct Detection

Definitive diagnosis is made by identification of the adult worm from the eye, in tissue or in the peripheral blood. The organism contains a sheath that does not stain with Giemsa. The adult females are larger than the adult males. The nuclei extend to the tail in an irregularly arranged fashion.

Serologic Detection

As with other filarial infections, serologic assays have limited use for diagnosis. A *Loa*-specific recombinant protein has been used in the development of an enzymelinked immunosorbent assay (ELISA) and has demonstrated improved specificity but limited sensitivity.

Molecular Diagnostics

PCR assays are currently available but are limited to research laboratories.

Therapy

DEC is the treatment of choice. In heavy infections, inflammation and allergic reactions may occur, requiring the administration of antiinflammatory medications. Allergic responses can result in central nervous system damage, encephalitis, coma, and death.

Prevention

Prophylactic treatment with DEC has been used to prevent infection.

Onchocerca volvulus

General characteristics

Onchocerca volvulus predominantly resides in tissue nodules within the host. Adult worms measure approximately 300 µm long by 5-9 µm wide.

Epidemiology

O. volvulus is found throughout Africa, Central America, and South America. The parasite is transmitted by the black fly, *Simulium* spp. The black fly lays its eggs in running water where the larvae attach to the rocks. The larvae feed on algae and bacteria. The adults emerge as a flying insect. The females require a blood meal, whereas the males are nectar feeders. The flies feed predominantly during the day.

Pathogenesis and spectrum of disease

Onchocerciasis, commonly referred to as river blindness, is a result of subcutaneous infection with the parasite. The infections are typically localized to the skin, lymph nodes, and eyes. Skin infections result in pruritus, edema, and erythema. Hypo- or hyperpigmentation can occur following a lengthy infection. Nodules, containing the adult worm, vary in size and are firm and tender. Lymphadenopathy may be found in the inguinal or femoral regions. Enlargement of the lymph node may result in a condition referred to as “hanging groin” that may result in a hernia. Onchocercal eye disease may be seen in moderate to heavy infections. Infections of the eye may lead to serious damage and blindness. Mortality increases in adults that experience blindness and systemic infection.

Laboratory diagnosis

Direct Detection

Definitive diagnosis is made from the identification of the adult worm from tissue such as in a nodule or skin snip. Skin samples are placed in physiologic buffered saline for up to 24 hours. Following incubation, the

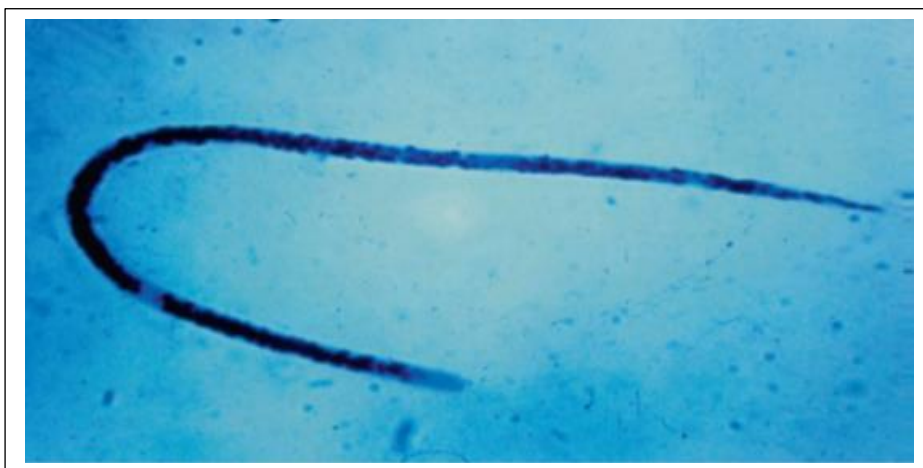


Figure 46 Microfilaria of *Onchocerca volvulus*.

worms will emerge from the tissue and can be visualized microscopically. Occasionally the adult worms may be found in blood or urine following treatment. Microfilariae may also be visible in the cornea of the eye. The microfilariae lack a sheath. The tail is tapered, appears bent or flexed, and does not include nuclei (Figure 46).

Serologic Detection

Although serologic tests generally lack specificity, recombinant ELISAs using multiple antigens have demonstrated increased sensitivity and specificity for the diagnosis of onchocerciasis.

Molecular Diagnostics

PCR amplification assays have been developed and are currently limited to research laboratories.

Therapy

Ivermectin is the recommended treatment. However, in Africa, where *O. volvulus* and *L. loa* are coendemic, ivermectin treatment is often associated with encephalopathy in patients with heavy microfilaria infections. Surgical excision of nodules containing adult worms is recommended when they are located on the head.

Prevention

Mosquito control using insecticides in endemic areas has been used to assist in the control of transmission of *O. volvulus*. In addition, a mass-treatment program with ivermectin is effective in preventing infection.

MANSONELLA SPP. (*M. OZZARDI*, *M. STREPTOCERCA*, *M. PERSTANS*)

General characteristics

Mansonella spp. are generally not associated with serious infections. The adult worms of all species are very similar in size, ranging from approximately 200-225 µm long.

Epidemiology

The parasites are transmitted by biting midges of the genus *Culicoides*. The female requires a blood meal for the maturation of eggs and typically bites in the early evening or morning hours. Transmission of *M. ozzardi* has also been associated with bites from the black fly (*Simulium amazonicum*).

Pathogenesis and spectrum of disease

M. streptocerca may be found in the skin; however, most infected individuals appear asymptomatic. Patients may present with a pruritic rash and pigmentation changes. In addition, lymphadenitis may occur. *M. perstans* resides in the pericardial, pleural, and peritoneal cavities. Symptomatic patients present with swelling of the arms or face similar to infection with *L. loa*. *M. ozzardi* and *M. perstans* are found in the blood.

M. perstans and *M. ozzardi* do not demonstrate periodicity when circulating within the bloodstream. *M. ozzardi* infections are not well characterized.

Laboratory diagnosis

Mansonella spp. microfilariae do not possess sheaths. *M. streptocerca* and *M. perstans* tails contain nuclei that extend to the end of the tail. The tail of *M. streptocerca* is often referred to as a “shepherd’s crook.” *M. ozzardi* have tails with nuclei that do not extend to the tip.

Therapy

Ivermectin is effective in the treatment of *M. streptocerca* and *M. ozzardi* infections. Treatment of *M. perstans* infections has not been effective in most cases.

Prevention

Prevention relies on the use of insect repellents and adequate clothing.

Intestinal Cestodes:

Intestinal Cestodes (Tapeworms):

Diphyllobothrium latum

Dipylidium caninum

Hymenolepis nana

Hymenolepis diminuta

Taenia solium

Taenia saginata

The intestinal cestodes are commonly referred to as tapeworms. Tapeworms have a long, segmented, ribbonlike body with a specialized structure for attachment, or **scolex**, at the anterior end. The adult tapeworm consists of a chain of egg-producing units called **proglottids**, which develop posteriorly from the neck region of the scolex. The crown of the scolex, **rostellum**, may be smooth or armed with hooks. The body of the worm (proglottids) varies in the geometric characteristics or number of segments according to the genus and species of the cestode. The mature cestode is **hermaphroditic**.

In other words, the organism contains both male and female reproductive organs. Food is absorbed from the host through the worm’s **integument**, the outer covering or skin of the organism. Adult worms typically inhabit the small intestine; however, humans may be host to either the adult or the larval forms, depending on the infecting species. Humans infected with a cestode pass the eggs in the feces. The embryo may be visible within the tapeworm egg as an **oncosphere** (larva tapeworm within an embryonic envelope, infective stage) or **hexacanth** embryo. The intermediate host ingests feces containing the adult tapeworm eggs, which further develop into the larva of the cestode. Cestodes generally require one or more intermediate hosts for the completion of their life cycle. Intestinal tapeworm infections are generally asymptomatic. However, if the larval stage develops in human organs outside the intestine, they may cause additional life-threatening complications. Serologic tests are not available for the diagnosis of tapeworm infections, therefore requiring skilled laboratorians for proper morphologic identification of the organism. Fresh or preserved stools are the specimen

of choice for ova and parasites (O&P) examination and cestode identification. Preserved stool containing adult worm segments (**strobila**) or the scolex may also be used for Diagnosis.

Diphyllobothrium latum

General characteristics

Diphyllobothrium latum, the freshwater broad fish tapeworm, is the largest human tapeworm. Adults have been known to reach up to 10 m in length, with more than 3000 to 4000 proglottids, and reside within a host for 30 years or more. The proglottids are characteristically wider than long with a central rosette-shaped uterine structure (Figure 47). The scolex is spatulate and contains two shallow sucking grooves referred to as **bothria** (Figure 47). *D. latum* has unembryonated eggs. The eggs are operculated with a terminal knob, similar to trematode eggs (Figure 47). The intermediate hosts include crustaceans and freshwater fish.

Pathogenesis and spectrum of disease

Diphyllobothrium latum is the only cestode to have an aquatic life cycle (Figure 54-2). Fish serve as the reservoir host, with humans serving as the definitive host. *D. latum* eggs are found in the feces of infected humans and other fish-eating mammals. Once passed into a water source, such as a lake, the life cycle requires two intermediate hosts. After incubation in freshwater for approximately 2 weeks, the mature eggs release the first larval stage (coracidium). The coracidia are ingested by



Figure 47, A ,*Diphyllobothrium latum* scolex. **B.**, *latum* scolex, bothria visible. **C.**, *latum* ovum, *Diphyllobothrium latum* scolex,

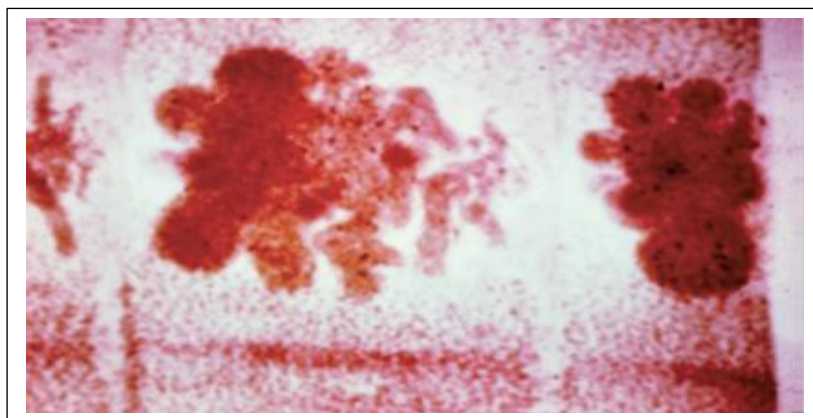


Figure 48: Proglottid demonstrating rosette-shaped uterus in *D. latum*.

copepods. The fish feed upon the small crustaceans ingesting the proceroid larvae. Within the freshwater fish, the larvae develop into the infective larvae. *D. latum* infection occurs through the ingestion of poorly cooked freshwater fish containing the plerocercoid larval form. *D. latum* matures to an adult tapeworm within the human small intestine. Infection is usually asymptomatic, but mild gastrointestinal symptoms may occur such as diarrhea, abdominal pain, fatigue, vomiting, or dizziness. Symptoms vary depending on the worm burden and the host's immune response to the organism. The tapeworm nutritional requirements may decrease the host's vitamin B12 level, resulting in megaloblastic anemia .

Laboratory diagnosis

Both eggs and proglottids may be found in patient's feces. Visualization of the eggs is enhanced using a wet preparation of the patient's stool sample. Diagnosis is made by identification of the ovoid, operculated, nyellowbrown eggs (58 to 75 μm by 40 to 50 μm) passed in abundance in the stool. They are sometimes confused with the eggs of *Paragonimus*. The mature gravid proglottids are wider than long (3 $\times$ 11 mm), often in chains, and contain a rosette-shaped central uterus (Figure 48).

Therapy

Humans infected with *D. latum* develop little or no protective immunity. Reinfection is common. Treatment with praziquantel or niclosamide is effective and nontoxic. Subsequent stool specimens should be reexamined 6 weeks following treatment. The patient may require a vitamin B12 supplement if anemia develops.

Prevention

Prevention simply includes avoiding the consumption of raw fish. The larva stage is destroyed when food is thoroughly cooked or frozen.

Dipylidium caninum

General characteristics

D. caninum, the cat or dog tapeworm (Figure 49), is a double-pored tapeworm consisting of many small proglottids.

As the tapeworm matures, the proglottids separate and pass in the stool. They may be recognized on

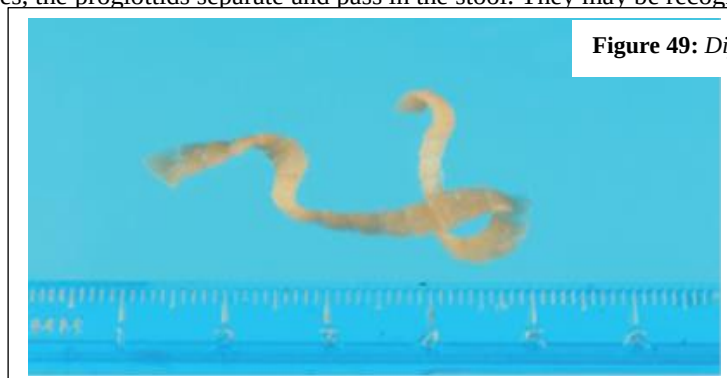


Figure 49: *Dipylidium caninum*

the basis of their characteristic “cucumber seed” appearance when they are wet, as well as their resemblance to a dried grain of rice. Adult tapeworms measure 10 to 70 cm in length. The scolex contains four suckers and an armed rostellum. Egg packets may also be found in the feces of the host.

Pathogenesis and spectrum of disease

Ingestion of an infected flea may result in *D. caninum* infection. The flea is the intermediate host in which infective cysticercoids develop; humans, dogs, and cats are the reservoir hosts. The larval stage of the egg is ingested by a dog or cat and develops into cysticercoid larvae. The adult worm develops and matures within the reservoir host. An infected human host will usually pass proglottids in a bowel movement or they may stick to the skin around the anal area. Humans usually have very mild symptoms such as indigestion, appetite loss, weight loss, perianal itching, persistent diarrhea, and vague abdominal pain. The severity of the disease is dependent on the worm burden. Human infection is usually self-limited.

Laboratory diagnosis

Symptoms of *Dipylidium* infection are similar to those of pinworm infection; however, the treatments are very different. The laboratory should confirm suspected infections. Proglottids (8 to 23 μm) may be seen in the stool. *D. caninum* is also referred to as the “cucumber seed” tapeworm as previously described (see Figure 48). The first sign of infection may be the appearance of seedlike particles in the stool or undergarments of the patient. These particles are the egg-bearing segments of the tapeworm. Groups of egg packets may be found in the stool. The adult worms have a scolex with four suckers and a conical/retractile rostellum armed with four to seven rows of small hooklets. Patients may also develop a moderately elevated eosinophilia.

Therapy

D. caninum infection is usually asymptomatic and is self-limiting. When treated, praziquantel is typically effective.

Prevention

To reduce the risk of infection, flea control of pets in the household will reduce exposure to humans via the intermediate host. To limit the exposure to fleas by household cats, it is recommended to keep cats indoors to prevent infection.

HYMENOLEPIS NANA

General characteristics

Hymenolepis nana, also known as the dwarf tapeworm, is very small in comparison to other tapeworms. The organism may reach up to 4 cm in length. The proglottid contains a scolex with a short-armed rostellum. It is the most common tapeworm with worldwide distribution. An intermediate host is not required, thus making person-to-person spread possible. An adult dwarf tapeworm can live within the host for approximately 4 to 6 weeks.

Pathogenesis and spectrum of disease

H. nana has an unusual life cycle; ingestion of the egg can lead to the development of the adult worm in humans, thus bypassing the need for an intermediate host. Humans can serve as both intermediate and definitive hosts. Infection occurs by accidentally ingesting dwarf tapeworm eggs. This happens most commonly through direct fecal-oral transmission or accidental ingestion of an infected arthropod. The worm resides within the upper ileum of the intestinal tract. Once infected, the dwarf tapeworm may reproduce inside the body, thus causing **autoinfection**. Autoinfection is essentially a reinfection or constant reproduction of the parasite within the host. Massive infection with several thousand worms may follow autoinfection, resulting in **hyperinfection**. Hyperinfection refers to a large parasitic burden within the host. Autoinfection appears to initiate a cellular and humoral immune response. The immune response will provide the host with some protective immunity. Most patients are asymptomatic. Symptomatic patients may experience weight loss, nausea, weakness, loss of appetite, diarrhea, and abdominal discomfort. Young children, especially those with a heavy infection, may develop headache, itchy bottom, or difficulty sleeping. Dwarf tapeworm infection may be misdiagnosed as infection.

Laboratory diagnosis:

Adult worms and proglottids are rarely seen in stool specimens. (see Figure 50). Some patients may demonstrate a low-grade eosinophilia.

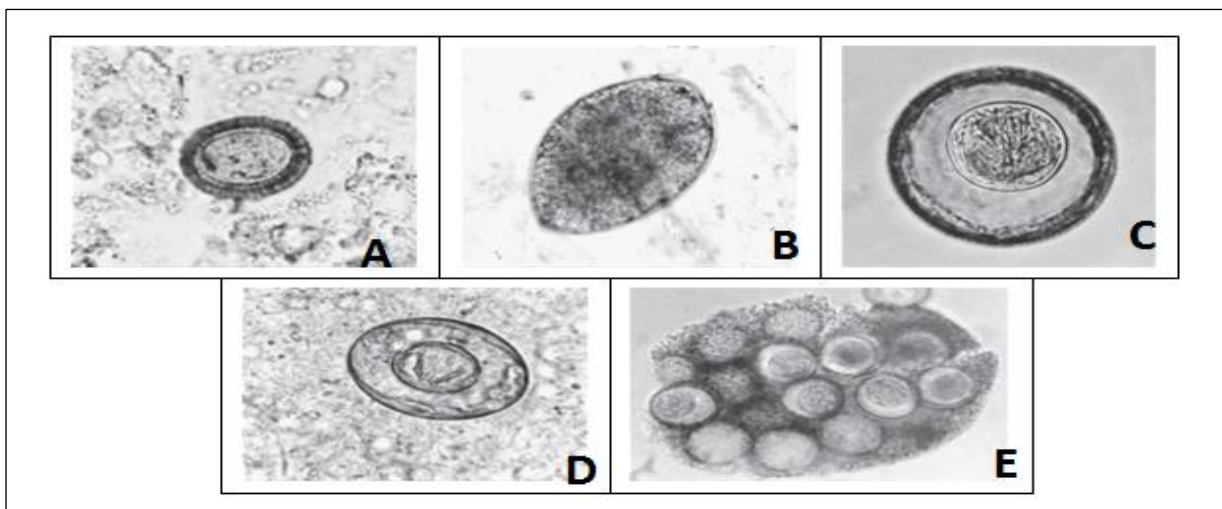


Figure 50, *Taenia* spp. egg. **B,** *Diphylllobothrium latum* egg. **C,** *Hymenolepis diminuta* egg. **D,** *Hymenolepis nana* egg. **E,** *Dipylidium*

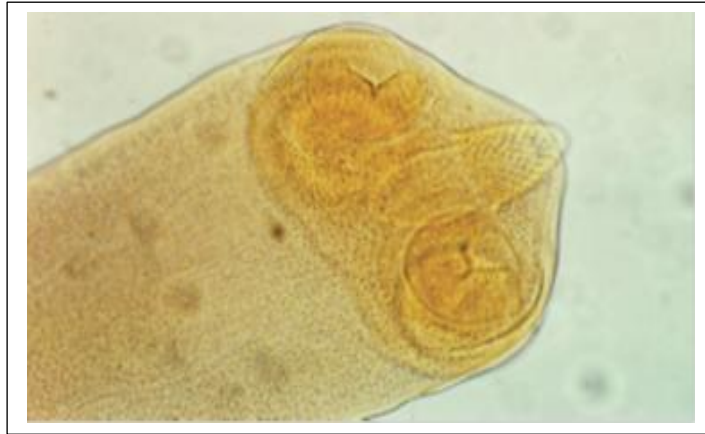


Figure 51 *D. caninum* scolex demonstrating the armed rostellum.

Therapy

Praziquantel remains the therapy of choice. Niclosamide is also effective and can be repeated with reinfection.

Prevention

Good hygiene is the best method for control and prevention.

HYMENOLEPIS DIMINUTA

General characteristics

Hymenolepis diminuta, the rat tapeworm, is larger than *H. nana* and can measure 20 to 60 cm in length.

Outbreaks of human infection are rarely seen.

Pathogenesis and spectrum of disease

The life cycle of *H. diminuta* involves insects, similar to the life cycle of *H. nana*. *H. diminuta* rarely infects humans, but may do so if a human accidentally ingests an arthropod infected with cysticercoids. Multiple adult worms may mature in the human intestine. Infections are usually tolerated well by the host because of the small size of the organism. Symptoms may include diarrhea, anorexia, nausea, headache, and dizziness. The infection is more common in children, causing mild diarrhea, remittent fever, and abdominal pain.

Laboratory diagnosis

Proglottids are rarely seen in the stool; diagnosis is made by the identification of eggs. (see Figure 50). The eggs are clearly differentiated from *H. nana* because of the absence of polar filaments.

Therapy

H. diminuta is readily treated with praziquantel, although the disease is self-limiting and treatment is often not necessary.

Prevention

Prevention is attained primarily by controlling the mice and rat populations along with good hygiene and sanitation.

Taenia solium

General characteristics

T. solium, the pork tapeworm, is the intestinal cestode capable of causing serious pathologic damage to the human host. Humans serve as the definitive host, whereas pigs serve as the intermediate host. Humans can also serve as the intermediate host. *T. solium* may result in an intestinal infection in which the larvae mature and reside in the small intestine for up to 25 years. The organisms can grow to be 1.5 to 8 m long and produce more than 1000 proglottids, each containing about 50,000 eggs. Cysticercosis is the extraintestinal form of the disease and can be much more severe. The disease is life threatening if the organism invades the central nervous system.

Pathogenesis and spectrum of disease

T. solium infection can result in the presence of both adult and larval stages in the human host. Infection begins when the intermediate host ingests embryonated eggs in feces. Once the egg is ingested, the hexacanth embryo is released into the intestine where the embryo penetrates the mucosa. The embryo then matures into a cyst (cysticercus) in the tissue. Humans may become infected when they eat raw or undercooked pork containing embedded cysts. Pork tapeworm infection is usually caused through the ingestion of multiple worms. During ingestion and subsequent digestion of the infected meat, the cysticercus is released and attaches to the mucosa within the small intestine of the human host. The cysticercus matures into an adult worm within approximately 5 to 12 weeks. The eggs are then released in the host's feces. Accidental ingestion of the eggs by the human host may also result in migration of the embryo through the intestine to other areas of the body, including the eyes, brain, muscle, or bone. In addition, the proglottids are motile and may migrate out of the anus. Infection of the adult tapeworm causes few clinical symptoms, although abdominal pain, diarrhea, indigestion, and loss of appetite may be present as a result of irritation to the mucosa of the intestinal wall. The major complication with *T. solium* is cysticercosis (larval forms throughout the body), in which the human host becomes the intermediate host and harbors the larvae in tissues as previously described.

Laboratory diagnosis

Serologic diagnosis is unreliable for infections with *T. solium*. Diagnosis of *Taenia* tapeworm infection is through the examination of stool samples. Individuals suspected of infection with *T. solium* should be asked if they have passed any notable tapeworm segments in their stool.

Additional laboratory findings may include a low-grade eosinophilia, increased serum IgE level, and the presence of atypical lymphocytes in the cerebrospinal fluid.

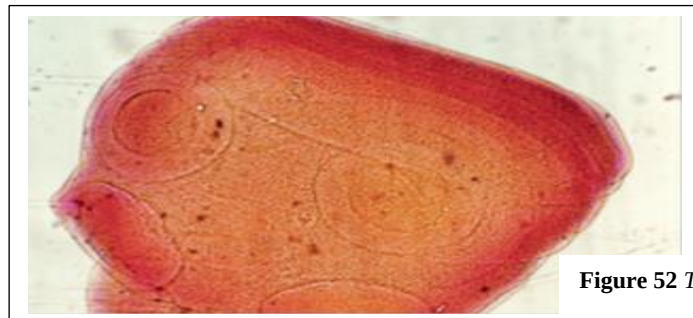


Figure 52 *T. saginata* scolex with suckers.

Therapy

Adult worms can be eradicated with praziquantel or niclosamide. Expulsion of the scolex must be assured to assume satisfactory treatment.

Prevention

Good hygiene and immediate treatment are essential for the prevention of autoinfection.

Taenia saginata

General characteristics:

T. saginata or beef tapeworm has a worldwide distribution and is more common than *T. solium*. The worm can grow 4 to 12 m and contain 1000 to 2000 segments. *T. saginata* may produce 100,000 eggs and live up to 25 years in the human intestine.

Epidemiology

T. saginata has a similar life cycle to that of *T. solium*. Cattle are the intermediate hosts and humans are infected through the ingestion of cysticerci (larval form) in raw or undercooked beef.

Pathogenesis and spectrum of disease

The life cycle of *T. saginata* begins with human ingestion of undercooked or raw meat infected with larvae. The larvae are ingested in the meat and, following digestion, released into the small intestine where the worm attaches to the mucosa and matures. In about 3 months, the worm may grow up to 4 to 5 m in length and gravid segments begin to break off and pass in stool. Following deposition of gravid segments in the soil, an intermediate bovine host may ingest the segments. The segments are digested and the eggs hatch, releasing an oncosphere that penetrates the muscle tissue. Following penetration of the mucosa the organisms are carried via the lymphatic vessels and bloodstream throughout the intermediate host. Humans then ingest the infected meat of the intermediate host, as previously indicated. Humans typically are asymptomatic, or have very mild indigestion, loss of appetite, vomiting, and abdominal discomfort. A rare case of severe infection may result in intestinal obstruction and appendicitis. Patients are often unaware of their infection until gravid motile segments are passed in the feces and cause psychological distress.

Laboratory diagnosis

The stool should be examined for proglottids and eggs; eggs may also be present on anal swabs. The eggs of *T. saginata* are indistinguishable from those of *T. solium*. The uterus of *T. saginata* is longer than wide and typically contains 15 to 18 lateral branches on each side. The scolex has four suckers and is unarmed or does not contain any hooklets (Figure 52). Stool specimens should be handled with care since the eggs cannot be distinguished from those of *T. solium*. Slight eosinophilia may develop.

Therapy

Recommended treatment includes praziquantel or niclosamide. Treatment of *T. saginata* can be considered

successful when no proglottids are passed for 4 consecutive months.

Prevention

Beef should be inspected for cysticerci and thoroughly cooked before ingesting

Tissue Cestodes:

Cestodes (Tapeworms) Tissue (Larval Forms):

Taenia solium

Echinococcus granulosus

Echinococcus multilocularis

Taenia multiceps

Spirometra mansonioides

Tissue cestodes do not reach the adult stage in the human host. The organism infect the human in their intermediate or cyst stage. The infections are much more serious than those caused by the adult tapeworm. The parasites can cause serious disease, or even death. Larval cestodes cause infection by accidental ingestion of eggs excreted from the intermediate host and they lodge in various organs and tissues in the human body. Diagnosis of larval infections can be problematic.

TAENIA SOLIUM

General characteristics

Taenia solium, also known as the pork tapeworm, causes an intestinal infection from eating contaminated pork. The adult worm usually causes no clinical disease. Humans may accidentally become the intermediate host and ingest eggs from human feces. This typically occurs when an individual is already infected with adult *T. solium*. Autoinfection occurs when the individual swallows eggs from improper hand washing. Humans may develop the larval infection, which could result in cysticercosis. Cysticercosis is usually asymptomatic unless larvae invade the central nervous system (CNS), the globe of the eye, or other muscle and tissues.

Pathogenesis and spectrum of disease

Clinical signs and symptoms depend on the location, viability, and number of the cysticerci present. Cysticerci can develop in any organ or tissue of the body. The severity of the symptoms depends on the body site involved and may not appear for years after the initial infection. The most severe cases are found in the central nervous system and the eye. Once cysticerci localize in the brain, the organism causes a condition referred to as **neurocysticercosis**. Infection can cause epileptic-type seizures, headaches, mental disturbances, meningitis, or sudden death. Cysticerci can also be found in the eye and must be removed to avoid permanent eye damage, including blindness. Much of the damage from cysticercosis is caused by the severe inflammatory host response that occurs after the cysticerci have died. Antibodies are produced and offer the patient secondary immunity.

Laboratory diagnosis

Cysticercosis can be difficult to diagnose. *T. solium* eggs are found in stools in fewer than half the patients with cysticercosis. Demonstration of eggs or proglottids in the feces is an indication of *Taenia* infection but does not provide a diagnosis for cysticercosis. Definitive diagnosis usually requires the identification of cysticercus in the tissue. The organism is surgically removed and microscopically examined for the presence of suckers and hooks on the scolex. The cysticercus is round to oval, translucent, and about 5 mm or more in diameter. The organism has a scolex with four suckers and a rostellum with a circle of hooks. Fine needle aspiration cytology may be helpful in the diagnosis and eliminates the need for surgical biopsy. Diagnosis may also be made using computed tomography (CT) scans and magnetic resonance imaging (MRI). Radiographs may also be useful in detecting calcifying cysticerci within tissue. Ocular cysticercosis may be diagnosed by visual identification of the larval worm. Serologic procedures (such as enzyme-linked immunosorbent assay [ELISA]) may also be used as a useful tool to aid in diagnosis, but may not be sensitive enough in light infections. The Centers for Disease Nucleic acid-based methods and species-specific polymerase chain reaction (PCR) have been described to differentiate *Taenia* species.

Therapy

Cysticercosis should be treated with corticosteroids, anticonvulsants, and surgery if deemed appropriate. Surgery may be required for ocular, spinal, or brain involvement.

Prevention

Education, meat inspection, and improvement of sanitation measures are the key preventive measures.

ECHINOCOCCUS GRANULOSUS

General characteristics

Echinococcus is the smallest of all tapeworms (3 to 9 mm long) with three to five proglottids. It contains a scolex with four suckers and a rostellum with hooks to attach to the intestinal wall. *E. granulosus* is a tapeworm found in the small intestine of the definitive host, the canine. Eggs are ingested by the intermediate hosts and include a variety of mammals including sheep, cattle, moose, and humans. There are several strains of *Echinococcus granulosus* that have been identified, with the dog-sheep strain being the most common. Humans are typically accidental hosts and are considered a dead-end since the life cycle of the organism is unable to continue in a human host. Oncospheres hatch in the intestine of the intermediate host and invade the circulatory system, where they develop into hydatid cysts. Disease symptoms vary with the site and size of the cyst. Echinococcosis (**hydatid disease**) results from the presence of one or more cysts (**hydatids**), which can develop in any tissue.

Pathogenesis and spectrum of disease

Hydatid disease in humans is potentially dangerous depending on the size and location of the cyst. Some cysts may remain undetected for many years until they grow large enough to affect other organs. Many humans live

day-to-day without ever knowing they are infected. The cyst is very slow growing in humans. It is usually fluid-filled and has a germinal layer from which many thousands of scolices are budded. These are known as daughter cysts (**brood capsules**), which attach to the germinal layer or free-float in the cyst. The scolices in the hydatid fluid resemble grains of sand and are called **hydatid sand** (Figures 53). The result is a unilocular cyst containing future adult worms. The cyst may resemble a slow-growing tumor. Infections in the liver or lungs may be asymptomatic for many years, but the pressure eventually causes noticeable symptoms. The majority of the hydatid cysts occur within the liver. Cysts within the liver cause chronic abdominal pain and allergic reactions and may result in cholangitis (infection of the common bile duct) and cholestasis (interference with flow of bile from the liver). Cysts that develop in the lungs may cause infections and abscesses and result in chronic cough, shortness of breath, and chest pain. During the life cycle of the cyst, there may be occasional seepage of fluid into the host tissue and circulation causing sensitization or activation of the immune response from the presence of the parasite. The rupture and release of the fluid of a hydatid cyst may cause anaphylactic shock as a result of the primary sensitization in a previously asymptomatic individual. If a cyst bursts within the human body, many new cysts may be released that are typically eliminated via the host's cellular immune response. Leaking fluid from a cyst may cause notable eosinophilia.

Laboratory diagnosis

Clinical symptoms of a slow-growing abdominal tumor with or without eosinophilia are suggestive of infection. Human infection ranges from asymptomatic to severe, including death. Diagnosis is made through the identification of cysts in the infected organ, accompanied with positive serologic tests. A variety of serologic tests are available including ELISA, indirect hemagglutination, and latex agglutination. Both false positives and false negatives may occur; therefore clinical history is extremely important for diagnosis. Ultrasound, magnetic resonance imaging (MRI), and computed tomography (CT) have

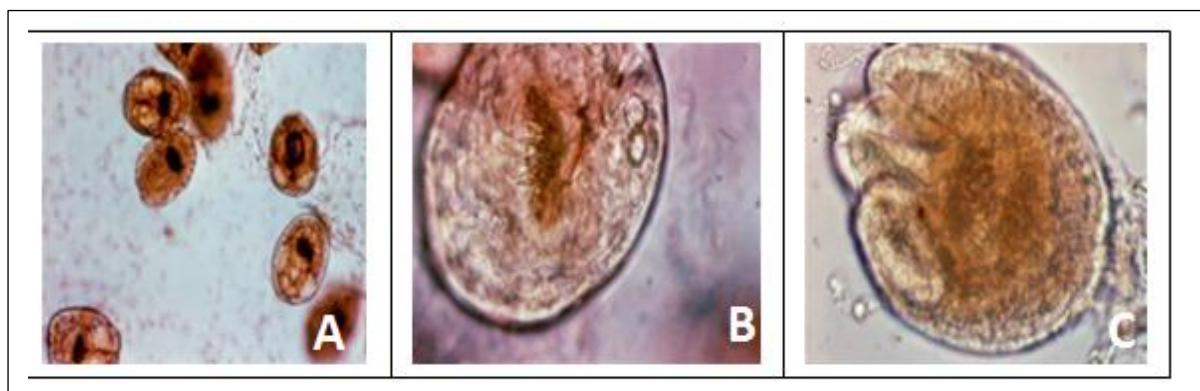


Figure 53: *Echinococcus* granules. **A** B, Ovum. **C**, Scolex.

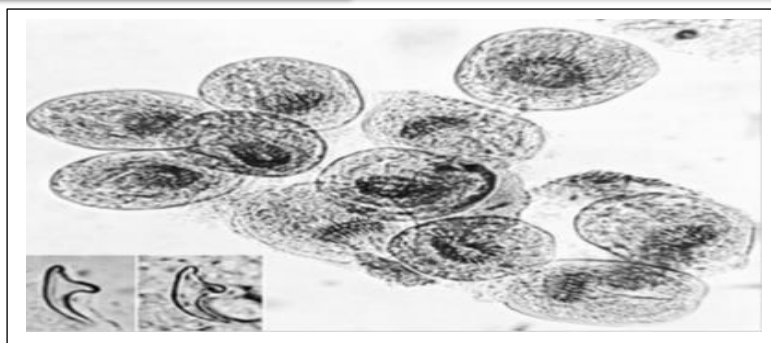


Figure 54: *Echinococcus granulosus*, hydatid sand (300×). (Inset)

Two individual hooklets (1000×)

improved the diagnosis and may provide visualization of the fluid-filled cysts. Calcified cysts can be visualized using conventional x-ray. Microscopic examination of the cyst fluid for the identification of the scolices can be useful in diagnosis. A 1% eosin stain may be added to the fluid to assist in the visualization and determination as to whether or not the cyst is viable. Nonviable scolices will stain with the eosin whereas viable scolices will not.

Therapy

Surgery is the most common form of treatment. The procedure involves surgical removal of cysts or inactivation of hydatid sand by injecting the cyst with 10% formalin and then removing it. Extreme care must be taken to avoid spillage. Albendazole is the drug of choice to kill the scolices within the cyst, reduce the size of the cyst, and prevent recurrence. Mebendazole and praziquantel have also been shown to be effective.

Prevention

Preventive measures include avoiding contact with infected dogs and deworming animals regularly. Effective control includes educating the population concerning the danger and means of transmission of hydatid disease as well as maintaining good hygiene and practicing safe disposal of dog feces. Slaughtered animals must be disposed of properly, to prevent dogs from exposure to contaminated materials and interrupt the *Echinococcus* life cycle.

ECHINOCOCCUS MULTILOULARIS

General characteristics

Although rarely found in the brain of humans, *E. multilocularis* causes alveolar hydatid disease, which is a fatal form of echinococcosis. It is the most lethal of all helminthic diseases. The cyst is extremely dangerous because it lacks a laminated membrane and develops a series of connected chambers. The chambers contain little or no fluid and rarely contain a scolex. The morphology of the cyst is very similar to that of *E. granulosus*, but the adult organisms are much smaller (1.2 to 3.7 mm). The cysts are very resistant to cold temperatures.

Pathogenesis and spectrum of disease

Alveolar hydatid disease is a highly lethal, destructive disease. The cyst of *E. multilocularis* grows slowly and may take years to produce clinical symptoms. Many cysts are asymptomatic during the life of the infected individual and are sometimes found during autopsy, surgery, or imaging scans related to other clinical conditions. The severity of symptoms depends on the location of the cyst and the size, as seen with *E. granulosus*. Cysts form primarily in the liver and metastasize to the lung or brain. Cysts in the liver are not restricted with a laminated cyst wall and are capable of expansion into a multicystic structure. This **multilocular** (many chambers) hydatid cyst is often mistaken for a hepatic sarcoma, making diagnosis difficult. This disease is often fatal.

Laboratory diagnosis

Ultrasound, CT scans, and MRI are used to visualize the cyst and can be supported with serologic testing. Serologic tests, such as ELISA, are sensitive and highly specific.

Therapy

The most common treatment is to remove the parasite Surgically. Treatment with mebendazole and albendazole has been used successfully

Prevention

Controlling rodents is an important means of prevention along with educating the public at risk to avoid exposure to infective feces. Practicing good hygiene and periodically deworming household pets are also helpful.

Taenia multiceps

General characteristics

T. multiceps is a tapeworm that causes coenurosis in humans. The **coenurus** (larval form) may cause destructive damage or death, but is an extremely rare disease in humans. The coenurus is a unilocular cyst similar to cysticercus, although the worm has multiple scolices. Daughter cysts may also be seen. The body of *T. multiceps* is 5 to 6 cm long and consists of 200 to 250 segments. The scolex has 4 suckers and a proboscis (tubular appendage) with 22 to 32 hooks arranged in 2 rows.

Pathogenesis and spectrum of disease

The oncosphere hatches and penetrates the intestinal wall of the intermediate host. The embryo is carried via the bloodstream to various parts of the body including the brain, eyes, and central nervous system, where the organism lodges and the coenurus develops. The coenurus develops into multiple daughter cysts. Symptoms include headache, vomiting, paralysis, and blindness. The coenurus causes a serious disease called coenurosis in sheep and in dogs that have eaten the brains of infected sheep. This clinical condition is known as gid, sturdy, or staggers.

Laboratory diagnosis

Diagnosis is similar to that for *Echinococcus* infection. CT and MRI may be useful for detecting the cysts. Microscopic identification can be used if the cyst has been removed surgically. Currently, there are no serologic tests available.

Therapy

Treatment is similar to that for *Echinococcus*. The most common treatment is surgery if possible, although the drugs used for cysticercosis may also be effective against coenurus infection.

Prevention

dogs associated with sheep and other livestock should not be fed the brain or spinal cord from infected animals and should be dewormed regularly. good hygiene should be practiced and care taken not to eat or drink anything contaminated with dog feces.

Spirometra mansonoides

General characteristics

Sparganosis is an infection caused by the plerocercoid larvae of *Spirometra*. The larvae (spargana) are white, wrinkled, and ribbon-shaped. They may be 3 mm wide and up to 30 cm long. The sparganum has **bothria** (longitudinal grooves) instead of suckers. No scolex is present, which can help differentiate *Spirometra* from *Taenia solium*.

Pathogenesis and spectrum of disease

Spargana migrate and lodge anywhere in the human body. Clinical symptoms depend on which organs or tissues are involved. Spargana can live for several years before symptoms develop. Sparganosis is usually asymptomatic until the larvae grow and cause an inflammatory reaction. Painful nodules can develop in the tissues. A variety of symptoms may occur, including seizure, weakness, headache, and eye pain that can lead to blindness if left untreated.

Laboratory diagnosis

Definitive diagnosis is usually made by removal and identification of the sparganum from infected tissue. Clinical history, ELISA, MRI, and CT can all be used together to presumptively diagnose sparganosis. Eosinophilia may also be present.

Therapy

Praziquantel has been used with limited success. Surgical removal of the complete sparganum is the treatment of choice.

Prevention

Prevention strategies should include safe drinking water practices, and awareness of the dangers of consuming raw fish and amphibians. Water in contaminated areas should be boiled before consumption.

Intestinal Trematodes:

Helminths Trematodes (Flukes) Intestinal:

Fasciolopsis buski

Heterophyes heterophyes

Metagonimus yokogawai

The intestinal trematodes (flukes) are members of the phylum Platyhelminthe (flatworms), are dorsoventrally flattened, and require at least one intermediate host (a freshwater snail).

Human infection occurs by ingestion of metacercariae encysted on freshwater vegetation or fish. Most trematodes are hermaphroditic (both ovaries and testes are contained within each adult worm).

The parasites are typically identified from eggs shed in the feces. The adult worms are located in the small intestine, where they lay eggs that may be embryonated or remain unembryonated until they are shed from the body via feces. The egg continues developing after reaching the water, and a ciliated, free-swimming miracidium larva is released. The miracidium enters a snail host and develops into a redia (cylindrical larvae), followed by development into tailed cercariae.

The cercariae emerge from the snail and encyst as a metacercariae (encrusted larvae) on water plants or fish. A human host ingests raw or undercooked plants (*Fasciolopsis buski*) or fish (*Heterophyes heterophyes*, *Metagonimus yokogawai*) containing the metacercariae, which excyst in the intestinal tract, attach, and mature into adults.

Fasciolopsis buski

General characteristics

The adults of *F. buski* have an elongated shape and range from 20 to 75 mm long to approximately 8 to 20 mm wide. They have an oral sucker at the anterior end and a ventral sucker located about midway to the posterior end. The eggs, which are indistinguishable from those of *Fasciola hepatica* (Figure 55), are oval and elongated, transparent, and yellow-brown with an operculum (lid) at one end, and they range in size from 130 to 140 µm long to 80 to 85 µm wide and may be unembryonated.

Pathogenesis and spectrum of disease

The intestinal attachment site of the adult worms often becomes locally inflamed and ulcerated, and may hemorrhage. Moderate to heavy infections may cause abdominal pain, diarrhea, intestinal obstruction, and edema of the abdomen and lower extremities, and may result in inadequate absorption of vitamin B12. Eosinophilia is commonly observed.

Prevention

Infection can be prevented by making sure that water plants and fish are properly cooked before eating. In addition, changes are needed in agricultural practices and health education in the endemic areas.

Heterophyes heterophyes: and *metagonimus yokogawai*

General characteristics

Adult *H. heterophyes* worms range in size from 1.0 to 1.7 mm in length by 0.3 to 0.4 mm in width, and have a broadly rounded posterior. *M. yokogawai* adults range in size from 1.0 to 2.5 mm long to approximately 0.4 to 0.8 mm wide.

The adult *H. heterophyes* also has an additional sucker, the genital sucker, which surrounds the genital pore. The eggs are small, yellow-brown, embryonated, and operculated and may have minimal opercular shoulders. Eggs range in size from 26 to 30 long μm to 15 to 17 μm wide, and may be indistinguishable between the two species.

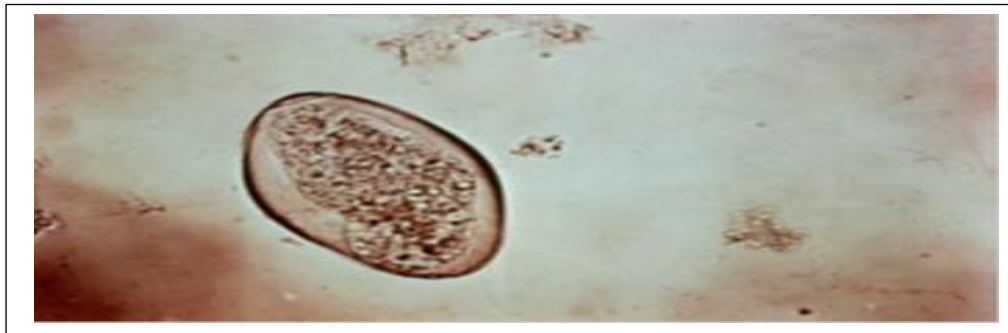


Figure 55 *Fasciola* egg. The eggs of *F. buski* and *F. hepatica* are indistinguishable morphologically.

Pathogenicity and spectrum of disease

Infections with a small number of worms may be asymptomatic. Symptoms in heavy infections may include abdominal pain, diarrhea with a large amount of mucus, and ulceration of the intestinal wall. Eggs may gain entry into intestinal capillaries and lymphatics, where they can be carried to the heart, brain, spinal cord, or other tissues, causing emboli or granuloma formation.

Prevention

Avoid ingestion of raw, inadequately cooked, and pickled or salted fish. The risk of infection could be greatly reduced by improved sanitary conditions and health education programs.

Laboratory diagnosis

Identification of the intestinal trematodes is made by recovery of eggs, or in rare cases adults, from stool specimens using a sedimentation method such as formalinethyl acetate. The sediment may be examined in a wet mount with or without iodine. Because the eggs of *Fasciolopsis buski* are identical to those of *Fasciola*

hepatica, and those of *Heterophyes heterophyes* and *Metagonimus yokogawai* are very similar, diagnosis may also require assessment of symptoms, obtaining a travel history, and/or recovery of adult worms.

Treatment

The drug of choice for treatment of intestinal trematode infection is praziquantel (Biltricide), an isoquinoline derivative administered orally in three doses for 1 day.

Liver and Lung Trematodes:

Trematodes (Flukes) Liver/Lung:

Clonorchis (Opisthorchis) sinensis

Opisthorchis viverrini

Fasciola hepatica

Paragonimus westermani

Paragonimus mexicanus

Clonorchis sp., *Opisthorchis* sp. and *Fasciola* sp. Live in the biliary ducts of humans. *Paragonimus* spp. are found in the lungs and in other body sites.

The liver flukes:

General characteristics

The adults of these trematodes live in the biliary ducts and in heavy infections may be also found in the gallbladder. Two of these, *Clonorchis sinensis* (the Chinese liver fluke) and *Opisthorchis viverrini* (the Southeast Asian liver fluke), are elongated and narrow and much smaller than *Fasciola* (the sheep liver fluke). These flukes also all require a freshwater snail as an intermediate host.

Life cycle

The life cycle of the liver flukes is very similar to that of the intestinal flukes. The adult worms produce eggs in the biliary ducts that are then excreted from the body in the feces. The free-swimming miracidium is released from the egg in freshwater and enters the snail host where it develops into a redia and then a cercariae, which leaves the snail and enters the water (Figure 56).

The cercariae of *Clonorchis* and *Opisthorchis* are ingested by a second intermediate host, a freshwater fish. The cercariae then encyst and develop into the metacercariae within the intermediate host. The metacercaria is the infective stage for humans. When infected freshwater fish are eaten raw or undercooked, the metacercariae will excyst in the duodenum and then travel to the bile duct where they mature. The cercariae of *Fasciola* encyst on freshwater vegetation, such as watercress and water chestnuts, and develop into metacercariae. When the infected vegetation is eaten raw, the metacercariae will excyst in the duodenum and then travel to the bile duct and mature.

Pathogenesis and spectrum of disease:

Light infections with *C. sinensis* or *O. viverrini* are most common, and may be asymptomatic. Heavier infections with these flukes may present with fever, abdominal pain, and jaundice. Eosinophilia and increased serum levels of immunoglobulin E (IgE) may be observed. Severe infections may cause obstruction of the biliary ducts, resulting in enlargement and tenderness of the liver, cirrhosis, cholecystitis (inflammation of the gallbladder), and cholangiocarcinoma (cancerous growth in bile duct epithelium).

Even light infections with *Fasciola* may cause fever, abdominal pain, nausea, diarrhea, enlargement and tenderness of the liver, jaundice, nonproductive cough, eosinophilia, and elevated serum IgE levels. More severe infections may result in obstruction of the biliary ducts, cirrhosis, cholecystitis, and cholangiocarcinoma. During migration in the human body, the larvae may penetrate the peritoneal cavity, and adult flukes may then be found in the intestinal walls, lungs, heart, or brain.

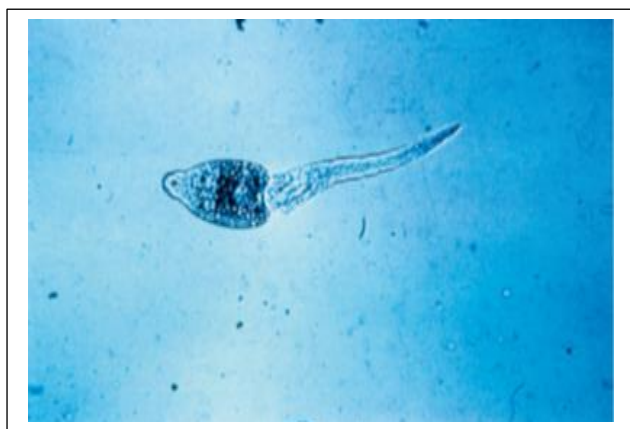


Figure 56 Cercaria of a liver fluke.

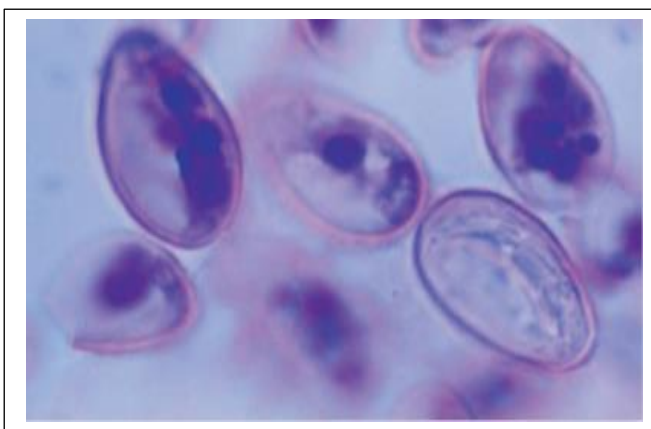


Figure 57 *Clonorchis sinensis* egg.

Laboratory diagnosis

Identification of the liver flukes is primarily made by recovery of the eggs in feces using a sedimentation method and a wet mount with or without iodine staining. There is also serologic testing available in the United States for diagnosis of *Fasciola*. Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) serum IgG antibody testing is performed at private references laboratories.

Therapy :

The drug of choice for treatment of infections with *Clonorchis* and *Opisthorchis* is praziquantel given orally three times for 1 day. An alternative drug is albendazole, a benzimidazole group drug, given once daily for 7 days. The drug of choice for *Fasciola* is bithionol (praziquantel is not effective) given orally every other day for 10 to 15 doses.

Prevention :

Human infection can be prevented by ensuring that fish and aquatic vegetation are properly cooked before consumption, as well as by the improvement of sanitary conditions along with the education of good personal hygiene.

THE LUNG FLUKES:

General characteristics

The genus *Paragonimus* contains several species known to infect humans. *Paragonimus westermani* is the most common and widely distributed lung fluke. The adult worms live in the lungs and produce eggs that may be present in sputum, or if expectorated and swallowed, may be present in feces. Like other trematodes, a freshwater snail is required as an intermediate host.

Life cycle:

Reservoir hosts for *P. westermani* include dogs and cats, and those for *P. mexicanus* include domestic and wild pigs, and dogs. Species of *Paragonimus* may also be found in other freshwater crab- or crayfish-eating mammals. The adult worms, encapsulated in the lungs, produce eggs that leave the lung via the bronchioles, stimulating a cough response. The eggs are then swallowed and eventually excreted in the feces. The free-swimming miracidium is released from the egg in freshwater and enters the snail host where it develops into a redia and then a cercariae, which leaves the snail and enters the water. The cercariae then enter a second intermediate host, a crab or crayfish, where they encyst and develop into metacercariae. The metacercaria is the infective stage for humans. When infected freshwater crabs and crayfish are eaten raw or undercooked, the metacercariae will excyst in the duodenum and then migrate through the intestinal wall, and eventually through the diaphragm and into the lungs where they encapsulate (usually in pairs) and mature.

Pathogenesis and spectrum of disease

Light infections may be asymptomatic. The migration of the metacercariae through muscle and tissue may cause local pain and immune response to tissue damage.

In the lungs, the immune response causes infiltration of eosinophils and neutrophils. Serum IgE levels are usually elevated. Eventually the adult worms are encapsulated in a granuloma. Presence of the worms in the lungs usually results in a chronic cough, with possible production of blood-tinged sputum. The cough provides a mechanism to transport eggs up into the throat where they are swallowed and then may be excreted in the feces. The larvae of *P. mexicanus* may migrate to other areas of the body, frequently causing the formation of subcutaneous or lower abdominal nodules. The larvae of *Paragonimus* may even enter the brain (rarely), where they can cause severe damage.

Laboratory diagnosis

The eggs may be recovered from sputum, and occasionally in feces using a sedimentation concentration method. The eggs may be observed in a wet mount (with/without iodine stain) (Figure 58).

Charcot-Leyden crystals may also be observed in sputum or lung tissue specimens. Charcot-Leyden crystals are slender and pointed at both ends. The crystals normally appear colorless and stain purplish to red with trichrome. Elevated levels of eosinophils in whole blood and elevated IgE levels in serum may be present. Lesions in the lungs may be observed in x-ray. Serum IgG EIA and immunoblot testing, and EIA serum and

cerebrospinal fluid (CSF) IgG antibody testing is performed at private reference laboratories; cross reactivity with other species and trematodes may occur.

Therapy

The drug of choice for treatment of *Paragonimus* infections is praziquantel given three times a day for 2 days.

Prevention

Human infection can be prevented by not eating pickled, raw, or undercooked crabs and crayfish. Care should also be taken to properly clean utensils used in the preparation of these foods. Improvement of sanitary conditions and practices may also help to reduce the prevalence of these infections.

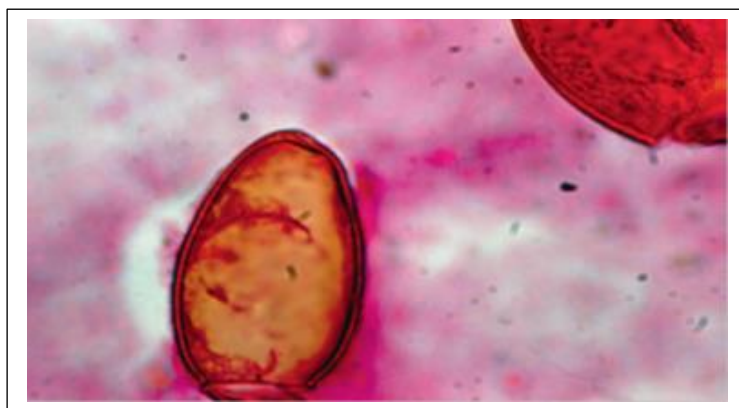


Figure 58: *Paragonimus westermani* egg.

Blood Trematodes:

Schistosoma mansoni

Schistosoma haematobium

Schistosoma japonicum

Schistosoma intercalatum

Schistosoma Mekongi

There are four species of blood flukes that are primarily associated with disease in humans (known as schistosomiasis, bilharziasis, or snail fever), all belonging to the genus *Schistosoma*. These four species are *Schistosoma haematobium*, *S. japonicum* (Oriental blood fluke), *S. mekongi*, and *S. mansoni*. A fifth species, *S. intercalatum*, is a pathogen primarily in animals but has been associated with human disease. The blood flukes differ

in morphology and life cycle characteristics from the other trematodes, but because they all belong to the same genus, they are very similar, and may be difficult to distinguish from each other. They do, however, require a freshwater snail as the only intermediate host.

General characteristics

Unlike the other trematodes, adult schistosomes are not flattened, but are rather long, thin, and rounded in shape. There is an oral sucker surrounding the mouth and a ventral sucker located just slightly below the oral sucker. The adult male averages 1.5 cm in length and is wider than the female, having a ventral fold that wraps around the female when they mate (Figure 59). The adult female averages 2 cm in length and is very thin. The eggs of each species are distinct, and can be distinguished by size, spine morphology, and sometimes specimen type (Figure 60). The size range for eggs of *S. haematobium* is 110 to 170 μm long by 40 to 70 μm wide and they have a sharply pointed terminal spine. They are fully embryonated without an operculum. The size range for the eggs of *S. japonicum* is 70 to 100 μm long by 50 to 65 μm wide, and they have a small lateral spine that is sometimes difficult to detect.

S. mekongi eggs are smaller than those of *S. japonicum*, ranging in size from 50 to 65 μm long by 30 to 55 μm wide. They are fully embryonated without an operculum and have a small lateral spine. The size range for eggs of *S. mansoni* is 115 to 180 μm long by 40 to 75 μm wide, and they have a large lateral spine. *S. mansoni* eggs are unoperculate, immature when released, and take up to 8 to 10 days to develop a miracidium. *S. intercalatum* eggs are fully embryonated without an operculum, have a terminal spine, and range in size from 140 to 240 μm long by 50 to 85 μm wide. *S. intercalatum* eggs resemble those of *S. haematobium* and can be differentiated by Ziehl-Neelsen acid-fast positivity. In addition, *S. intercalatum* eggs are only found in feces, not in urine specimens. One of the main differences in the schistosomes from other trematodes is that instead of being hermaphroditic, there are separate male and female adult worms. In human infection, the adult worms live in either the veins that supply the intestine (*S. japonicum* and *S. mansoni*) or the veins that supply the urinary bladder (*S. haematobium*).

The eggs are passed from the body in either the feces or the urine. To reach the inside of the intestine or bladder, the eggs must penetrate the tissue from the veins. This is accomplished via a spine that is distinctive among the major species. The embryonated egg will release the miracidium (Figure 62) once it reaches freshwater, and will enter the snail host, where it will develop into the infectious cercaria. The freeswimming cercariae are capable of penetrating through the human skin directly and do not encyst on aquatic vegetation or other aquatic wildlife (Figure 63). The cercariae penetrate the host tissue until they reach a vein; then they travel to capillaries near the lungs and then to the portal vein of the liver, where they mature. When they are mature, the adult males will pair with the females and then travel to the veins of either the intestine or the bladder, where the eggs are produced.

Pathology and spectrum of disease

Infection with only a small number of worms may be asymptomatic. Quite often, penetration of the skin by the cercariae causes localized swelling and itching. The migration of the larvae through the body may cause transient symptoms of fever, malaise, cough (when they migrate in the lungs), or hepatitis (when in the liver). The adults are able to acquire some host antigens on their outer surface, and so may not elicit an immune response, although the eosinophil count may be high.

Severe tissue damage, with associated pain, fever, and chills, may occur when the eggs travel through the tissue to reach the intestine or bladder. There may also be bloody diarrhea or blood in the urine (hematuria). Necrosis, lesions, and granulomas may develop, as well as obstruction of the bowel or ureters. Penetration of



Figure 59: Mating of *Schistosoma mansoni* male and female Worms.

human skin by the cercariae of blood flukes that commonly infect other mammals or aquatic birds may cause a schistosomal dermatitis known as “swimmer’s itch.” Erythema, edema, and intense itching may develop that usually disappear within 1 week. The cercariae of these species are not able to complete the life cycle by entering the human bloodstream, and are destroyed by the host immune system.

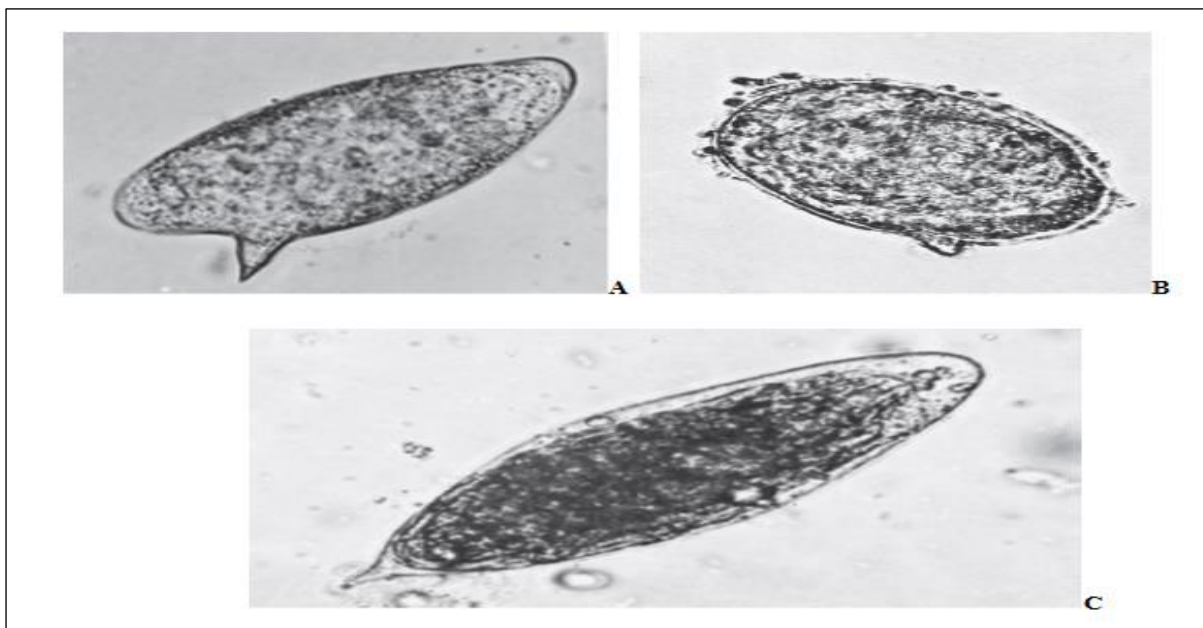


Figure 60, *Schistosoma mansoni* egg. **B,** *Schistosoma japonicum* egg. **C,** *Schistosoma haematobium* egg.

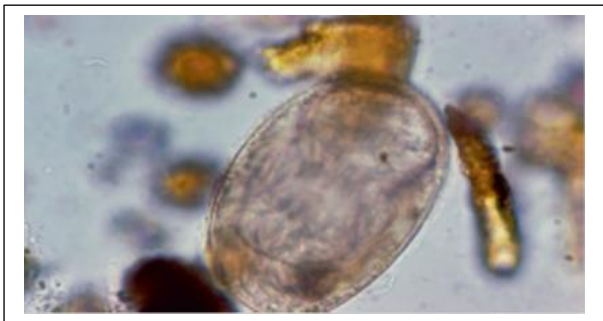


Figure 61 : *S. japonicum* egg.

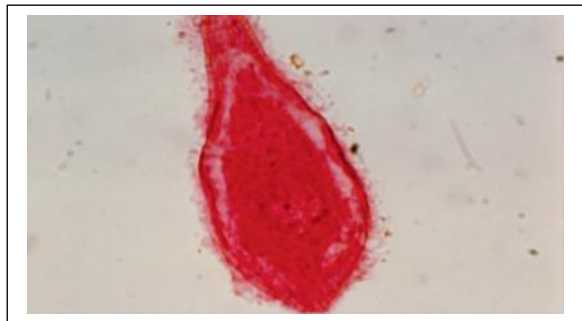


Figure 62 : *S. mansoni* miracidium.



Figure 63: *S. mansoni* cercaria.

Laboratory diagnosis

The standard method of diagnosis is by the detection of characteristic eggs in feces or rectal biopsy, for *S. japonicum*, *S. mekongi*, *S. mansoni*, and *S. intercalatum* (and perhaps *S. haematobium* if these worms have migrated to a bladder vein that is close to the intestine); and in urine (usually concentrated before examination) or bladder tissue biopsy for *S. haematobium*.

A wet mount with/without iodine from a sedimentation or concentration method can be examined for eggs.

Figure provides images of three different schistosome eggs. To optimize recovery of *S. haematobium* in urine, the specimen should be collected between noon and 2 pm. There are some antibody-based assays that are available for diagnosis of schistosomal IgG antibody (enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], and immunoblot), but these methods cannot distinguish between current and previous infections. This type of assay may, however, be useful for travelers who have returned from endemic areas.

Several nucleic acid-based testing methods have been developed that demonstrate high sensitivity and specificity using genomic or mitochondrial sequences. In addition, schistosome DNA has been identified in patients' plasma using real-time polymerase chain reaction (PCR).

Therapy

The drug of choice for treatment of schistosome infections is praziquantel, given in two or three doses in a day. Infection with *S. mansoni* may require a larger dose than that for the other species.

Prevention

Because human infection is by direct penetration of the cercariae, prevention of schistosome infection is more difficult to achieve. Educational programs are required to help people in endemic areas understand how to help prevent infection.

Sanitary conditions need to be improved with proper disposal not only of human wastes but also that of domestic animals (in areas with *S. japonicum* and *S. mekongi*). A safe water supply for bathing and washing

clothes is also necessary. Various snail control methods have been tried, but these methods are very costly and would need to be repeated on a regular basis to have the desired effect.

Table Body Sites and Parasite Recovery (Trophozoites, Cysts, Oocysts, Spores, Adults, Larvae, Eggs, Amastigotes, Trypomastigotes)

Site	Parasites
Blood	Plasmodium spp.
Red cells	Babesia spp.
White cells	Leishmania spp. Toxoplasma gondii
Whole blood/plasma	Trypanosoma spp. Microfilariae
Bone marrow	Leishmania spp. Trypanosoma cruzi Plasmodium spp.
Central Nervous System Cutaneous ulcers	Taenia solium (cysticerci) Echinococcus spp. Naegleria fowleri Acanthamoeba spp. Balamuthia mandrillaris Sappinia diploidea Toxoplasma gondii Microsporidia Trypanosoma spp
Intestinal tract	Leishmania spp. Acanthamoeba spp. Entamoeba histolytica Entamoeba dispar Entamoeba coli Entamoeba hartmanni Endolimax nana Iodamoeba bütschlii Blastocystis hominis Giardia lamblia Chilomastix mesnili Dientamoeba fragilis Pentatrichomonas hominis Balantidium coli Cryptosporidium spp. Cyclospora cayetanensis Isospora belli Microsporidia

	<i>Ascaris lumbricoides</i> <i>Enterobius vermicularis</i> Hookworm <i>Strongyloides stercoralis</i> <i>Trichuris trichiura</i> <i>Hymenolepis nana</i> <i>Hymenolepis diminuta</i> <i>Taenia saginata</i> <i>Taenia solium</i> <i>Diphyllobothrium latum</i> <i>Clonorchis sinensis</i> (Opisthorchis) <i>Paragonimus</i> spp. <i>Schistosoma</i> spp. <i>Fasciolopsis buski</i> <i>Fasciola hepatica</i> <i>Metagonimus yokogawai</i> <i>Heterophyes heterophyes</i>
Liver, spleen	<i>Echinococcus</i> spp. <i>Entamoeba histolytica</i> <i>Leishmania donovani</i> Microsporidia
Lung	<i>Cryptosporidium</i> spp.* <i>Echinococcus</i> spp. <i>Paragonimus</i> spp. Microsporidia
Muscle	<i>Taenia solium</i> (cysticerci) <i>Trichinella</i> spp. <i>Onchocerca volvulus</i> (nodules) <i>Trypanosoma cruzi</i> Microsporidia
Skin	<i>Leishmania</i> spp. <i>Onchocerca volvulus</i> Microfilariae
Urogenital system	<i>Trichomonas vaginalis</i> <i>Schistosoma</i> spp. Microsporidia Microfilariae
Eye	<i>Acanthamoeba</i> spp. <i>Toxoplasma gondii</i> <i>Loa loa</i> Microsporidia

Note: This table does not include every possible parasite that can be found in a particular body site; the most likely organisms have been listed.

*Disseminated in severely immunosuppressed individuals

Table Epidemiology of the More Common Groups of Human Parasites

Parasite Group	Habitat (Reservoir)	Mode of Transmission	Prevention
Protozoa, Intestinal Amebae	Single-celled organisms generally found in humans. Although certain animals harbor some of these organisms, they are not considered important reservoir hosts.	Humans acquire infections by ingesting food and water contaminated with fecal material containing the resistant, infective cyst stage of the protozoa. Various sexual practices have also been documented in transmission.	Preventive measures include increased attention to personal hygiene and sanitation measures; elimination of sexual activities that may involve fecal-oral contact.
Flagellates	The flagellates are generally found in humans. Although certain animals harbor some of these organisms, they are not considered important reservoir hosts; one exception may be animals, such as the beaver, that harbor <i>Giardia lamblia</i> . Contaminated water supplies are also a source.	Humans acquire infections by ingesting food and water contaminated with fecal material containing the resistant, infective cyst stage of the protozoa; in some cases (<i>Dientamoeba fragilis</i>), no cyst stage has been identified; the trophozoite forms may be transmitted from person to person in certain helminth eggs.	Preventive measures include increased attention to personal hygiene and sanitation measures; elimination of sexual activities that may involve fecal-oral contact; adequate water treatment (including filtration) is required; also awareness of environmental sources of infection.
Ciliates	<i>Balantidium coli</i> is generally found in humans, but it is also found in pigs. In some areas of the world,	Humans acquire infections by ingesting food and water contaminated with fecal material containing the	Preventive measures include increased attention to personal hygiene and sanitation

	pigs are considered important reservoir hosts.	resistant, infective cyst stage of the protozoa	measures, as well as elimination of sexual activities that may involve fecal-oral contact.
Coccidia	Coccidia are found in humans. In some cases (e.g., cryptosporidiosis) animal reservoirs (cattle) can serve as important hosts. The muscle of various animals may contain sarcocysts that are infective for humans through the consumption of raw or poorly cooked meat. Numerous waterborne outbreaks with <i>Cryptosporidium</i> spp. have been reported throughout the world. Coccidian oocysts are extremely resistant to environmental conditions, particularly if they are kept moist	These protozoa are acquired through ingestion of various meats or by fecal-oral transmission through contaminated food and/or water. The infective forms are called <i>oocysts</i> (<i>Cryptosporidium</i> spp., <i>Isospora</i> (<i>Cystoisospora</i>) <i>belli</i> , <i>Cyclospora</i> <i>cayetanensis</i>) or sarcocysts (<i>Sarcocystis</i> spp.), which are contained in infected meat. <i>Cryptosporidia</i> have also been implicated in nosocomial infections.	Preventive measures include increased attention to personal hygiene and sanitation measures; elimination of sexual activities that may involve fecal-oral contact. Adequate water treatment (including filtration) is mandatory; awareness of environmental sources of infection also is important.
Microsporidia	Microsporidia can infect every living animal, some of which probably serve as reservoir hosts for human infection. However, host specificity	Infection with microsporidial spores usually occurs through ingestion; however, inhalation of spores and direct inoculation from the environment almost certainly occur.	Preventive measures include increased attention to personal hygiene and sanitation measures; increased awareness of environmental

	has not been well defined to date. The spores are environmentally resistant and can survive years if kept moist.		exposure possibilities; and adequate water treatment.
Protozoa, Other Sites Amebae	Free-living amebae are associated with warm, freshwater environments; they are also found in soil. Although humans can harbor these organisms, person-to-person transfer is thought to be rare. Environmental sources are the primary link to human infection. Contaminated eye care solutions have been linked to organisms that cause keratitis.	Infection occurs through contact with contaminated water; organisms enter through the nasal mucosa and may travel via the olfactory nerve to the brain. Disease can be very severe and life-threatening; keratitis is also caused by these organisms, and infection can be linked to blindness or severe corneal damage. Eye infections can be linked to contaminated lens solutions or direct, accidental inoculation of the eye from environmental water and/or soil sources.	Avoidance of contaminated environmental water and soil sources; adequate care of contact lens systems
Flagellates	<i>Trichomonas vaginalis</i> infection is found in a large percentage of humans; humans may present as symptomatic or asymptomatic.	<i>T. vaginalis</i> is found in the genitourinary system and is usually acquired by sexual transmission.	Awareness of sexual transmission; treatment of all partners when infection is diagnosed in an individual

	Person-to-person transfer is very common; reinfection is also common, particularly if sexual partners are not treated.		patient.
Protozoa, Blood and Tissue Malaria, Babesiosis	Humans harbor the five species of malaria (<i>Plasmodium vivax</i> , <i>P. ovale</i> , <i>P. malariae</i> , <i>P. knowlesi</i> , and <i>P. falciparum</i>). Other animals can carry <i>Babesia</i> spp., and animal reservoir hosts play a large role in human transmission.	These organisms are arthropod-borne, <i>Plasmodium</i> spp. by the female anopheline mosquito and <i>Babesia</i> spp. by one or more genera of ticks. These infections can also be transmitted transplacentally, via shared needles, through blood transfusions, and from organ transplants.	Vector control; awareness of transmission through blood transfusions, shared drug needles, congenital infections, and organ transplants. Careful monitoring of the blood supply. Malaria prophylaxis if traveling to endemic areas.
Flagellates (leishmaniae)	Some strains of leishmaniae have reservoir hosts (e.g., dogs for the Mediterranean strain of <i>Leishmania donovani</i> and wild rodents for the African strains of <i>L. donovani</i> .) <i>L. tropica</i> also has been linked to the same two animal reservoirs	Transmission is through the bite of infected sandflies. Infection can also occur from person to person (cutaneous lesions), from blood transfusion, shared needles, and organ transplants.	Vector control; avoiding environmental sources (e.g., dogs, wild rodents); careful handling of all clinical specimens from infected patients.
Flagellates (trypanosomes)	Humans are the only known hosts for <i>Trypanosoma brucei gambiense</i> (West African	Transmission is through the bite of the infected tsetse fly and through blood transfusion, shared needles,	Vector control; awareness of potential exposure/infection from blood sources

	trypanosomiasis); <i>Trypanosoma brucei</i> <i>rhodesiense</i> (East African trypanosomiasis) infections are found in a number of antelope and other ungulates that act as reservoir hosts. Rodents and some mammals are reservoir hosts for <i>Trypanosoma cruzi</i>	and organ transplants. Transmission of <i>T. cruzi</i> is through the infected feces of the triatomid bug; the bug takes a blood meal, immediately defecates, and the human host scratches the infected feces into the bite site; bug saliva contains an irritant that stimulates scratching	(transfusions, shared needles, organ transplants). Laboratory accidents while handling infected blood have been reported.
Nematodes, intestinal	These roundworms generally do not have animal reservoirs relevant to human infection. One exception is the pig ascarid; human infections have been reported. These worms are found worldwide; <i>Ascaris lumbricoides</i> is probably the most common parasite of humans, although some would argue that <i>Enterobius vermicularis</i> is number one. <i>Strongyloides stercoralis</i> is particularly important as the causative agent of severe disease in the compromised host.	<i>A. lumbricoides</i> and <i>Trichuris trichiura</i> eggs must undergo development in the soil before they are infective; thus children who play in the dirt are a particularly high-risk group. Ingestion of food and water contaminated with infective eggs is the primary route of infection. Hookworm and <i>S. stercoralis</i> infections are initiated by larval penetration of the skin from contaminated soil. Pinworm infection (<i>E. vermicularis</i>) is acquired through ingestion of infective eggs from the environment	Avoiding ingestion of contaminated soil and/or avoiding frequenting soil contaminated with hookworm eggs (pets, soil, water, warmth, warm weather); treatment for pinworm is recommended, but reinfection is common

		(hand-to-mouth).	
Nematodes, Tissue	<i>Trichinella</i> spp. have a number of animal reservoir hosts, including bears, walruses, pigs, rodents, and other animals. Dog and cat hookworms cause cutaneous larva migrans (CLM), and the dog and cat ascarid, <i>Toxocara</i> spp., causes visceral and ocular larva migrans (VLM, OLM). These infections can be serious and cause severe disease if not treated.	<i>Trichinella</i> organisms are acquired by ingestion of raw or poorly cooked infected meat. CLM is caused by skin penetration of infective larvae from the soil; children should avoid sandboxes where dogs and cats are known to defecate. Larval migration is limited to the skin. VLM and OLM are caused by accidental ingestion of <i>Toxocara</i> spp. eggs from contaminated soil; larval migration occurs throughout the body, including the eyes.	Adequate cooking of infected meat; awareness of possibility of contaminated soils for dog and cat hookworms and/or ascarids; covering of all sandboxes where pets have access to defecation and children play.
Nematodes, filarial	<i>Wuchereria bancrofti</i> , <i>Loa loa</i> , and <i>Onchocerca volvulus</i> have no animal reservoirs and are found only in humans, whereas <i>Brugia</i> spp. can also be found in cats and monkeys. <i>Dracunculus medinensis</i> can infect dogs, cats, and monkeys and	Filarial nematodes are transmitted through the bite of a blood-sucking arthropod (midges, mosquitoes, flies). <i>Dracunculus</i> infections are acquired through ingestion of water contaminated with small crustaceans, <i>Cyclops</i> spp., which contain infective larvae.	Vector control; protection of well-water sources

	also humans.		
Cestodes, Intestinal	The human serves as the definitive host for beef (<i>Taenia saginata</i>) and pork (<i>Taenia solium</i>) tapeworms; cows/ camels and pigs serve as intermediate hosts, respectively. Humans also serve as the intermediate host for <i>T. solium</i> (cysticercosis). <i>Diphyllobothrium latum</i> adult tapeworms can be found in a number of wild animals, the most important being dogs, bears, seals, and walruses, which serve as reservoir hosts; humans are the definitive host. <i>Hymenolepis nana</i> (dwarf tapeworm) can occur in rodents; humans can serve as both intermediate and definitive hosts, with development from the egg to adult worm occurring in the human intestine.	Human infection with the adult worm occurs through ingestion of raw or poorly cooked meat (beef, camel, pork) containing the intermediate forms, the cysticerci. Humans become the accidental intermediate host when eggs from an adult <i>T. solium</i> tapeworm are ingested. The cysticerci develop in the muscle and tissues of the human rather than the pig. Infection with the adult <i>D. latum</i> tapeworm occurs through ingestion of poorly cooked freshwater fish containing the sparganum or plerocercoid larval form. Infection with <i>H. nana</i> is primarily acquired through accidental ingestion of eggs from an adult tapeworm.	Adequate cooking of infected meat; treatment of patients harboring adult tapeworms (accidental ingestion of eggs can lead to infection
Cestodes, Tissue	adult worms are found in a variety of animals; the human becomes the accidental intermediate host after ingestion of eggs from the adult worms. Reservoir hosts include dogs, cats, and rodents	Ingestion of certain tapeworm eggs or accidental contact with certain larval forms can lead to tissue infection with <i>Taenia solium</i> , <i>Echinococcus</i> spp., and several others.	Preventive measures involve increased attention to personal hygiene and sanitation measures.
Trematodes, intestinal	Fish-eating wild and domestic animals	Ingestion of water chestnut and caltrop	Avoiding eating raw water plants

	serve as reservoir hosts. The definitive host of <i>Fasciolopsis buski</i> is the pig.	(raw, peeled with the teeth) is the source of infection; metacercariae are encysted on the plant material. Pig feces are used to fertilize various water plant crops.	that may contain encysted larval forms of the flukes; adequate waste disposal of farm animal feces (pigs).
Trematodes, liver, lung	Cats, dogs, and wild fish-eating mammals can serve as reservoir hosts for <i>Opisthorchis</i> spp., <i>Clonorchis sinensis</i> , and <i>Paragonimus</i> spp. <i>Fasciola hepatica</i> is normally a parasite of sheep, and <i>F. gigantica</i> is a parasite of cattle; humans are accidental hosts.	Infection occurs through ingestion of raw or poorly cooked fish, crabs, crayfish, and certain plants in or on which metacercariae are encysted. Infection with <i>Fasciola</i> spp. is not easily acquired (the parasite is not that well adapted to the human host).	Thorough cooking of potentially infected fish, crabs, crayfish; avoiding eating raw water plants that may contain encysted metacercariae.
Trematodes, Blood	<i>Schistosoma mansoni</i> and <i>S. haematobium</i> appear to be restricted to the human host; <i>S. japonicum</i> can be found in cattle, deer, dogs, and rodents. The worms mature in the blood vessels, and eggs make their way outside the body in stool and/or urine. The freshwater snail is a mandatory part of the life cycle (contains developmental forms of schistosome).	Infection occurs through skin penetration by infected cercariae released from a freshwater snail containing the intermediate stages of the schistosome life cycle. Cercariae can be released from the snail intermediate host singly or in groups	Protection from potentially contaminated water sources; awareness of mode of transmission; proper handling of human waste containing eggs (continued infection of snail intermediate hosts).

Introduction

Mycology is the study of fungi. The name “fungi” is derived from “*mykos*” meaning mushroom. The fungi are eukaryotic organisms and they differ from the bacteria, which are prokaryotic organisms, in many ways. The fungi possess rigid cell walls, which possess two characteristic cell structures: chitin and ergosterol.

Chitin: The fungi consist primarily of chitin, unlike peptidoglycan present in cell wall of bacteria. Hence, fungi are not sensitive to action of penicillin and other antibiotics that inhibit peptidoglycan synthesis. Chitin is a polysaccharide consisting of long chains of N-acetylglucosamine. In addition to chitin, the fungal cell wall also contains mannan and other polysaccharides. Of these, beta-glucan is most important, because it is the target of antifungal drug caspofungin.

Ergosterol: The cell membrane of fungus contains ergosterol, unlike human cell membrane which contains cholesterol. The antifungal agents, such as amphotericin B, fluconazole, and ketoconazole have selective action on the fungi due to this basic difference in membrane sterols.

Fungal infections are **mycoses**. Most pathogenic fungi are exogenous, their natural habitats being water, soil, and organic debris. The mycoses with the highest incidence— candidiasis and dermatophytosis—are caused by fungi that are part of the normal human microbiota and highly adapted

to survival on the human host. For convenience, mycoses may be classified as superficial, cutaneous, subcutaneous, or systemic, invading the internal organs (Table 4-1). The systemic mycoses may be caused by endemic fungi, which are usually primary pathogens, or by ubiquitous, often secondary opportunistic pathogens.

Category	Mycosis	Causative Fungal Agents
Superficial	Pityriasis versicolor Tinea nigra White piedra Black piedra	<i>Malassezia</i> species <i>Hortaea werneckii</i> <i>Trichosporon</i> species <i>Piedraia hortae</i>
Cutaneous	Dermatophytosis Candidiasis of skin, mucosa, or nails	<i>Microsporum</i> species, <i>Trichophyton</i> species, and <i>Epidermophyton floccosum</i> <i>Candida albicans</i> and other <i>Candida</i> species
Subcutaneous	Sporotrichosis Chromoblastomycosis Mycetoma Phaeohyphomycosis	<i>Sporothrix schenckii</i> <i>Phialophora verrucosa</i> , <i>Fonsecaea pedrosoi</i> , and others <i>Pseudallescheria boydii</i> , <i>Madurella mycetomatis</i> , and others <i>Exophiala</i> , <i>Bipolaris</i> , <i>Exserohilum</i> , and other dematiaceous molds
Endemic (primary, systemic)	Coccidioidomycosis Histoplasmosis Blastomycosis Paracoccidioidomycosis	<i>Coccidioides posadasii</i> and <i>Coccidioides immitis</i> <i>Histoplasma capsulatum</i> <i>Blastomyces dermatitidis</i> <i>Paracoccidioides brasiliensis</i>
Opportunistic	Systemic candidiasis Cryptococcosis Aspergillosis Hyalohyphomycosis Phaeohyphomycosis Mucormycosis (zygomycosis) <i>Pneumocystis pneumonia</i> Penicilliosis	<i>Candida albicans</i> and many other <i>Candida</i> species <i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i> <i>Aspergillus fumigatus</i> and other <i>Aspergillus</i> species Species of <i>Fusarium</i> , <i>Paecilomyces</i> , <i>Trichosporon</i> , and other hyaline molds <i>Cladophialophora bantiana</i> ; species of <i>Alternaria</i> , <i>Cladosporium</i> , <i>Bipolaris</i> , <i>Exserohilum</i> and numerous other dematiaceous molds Species of <i>Rhizopus</i> , <i>Lichtheimia</i> , <i>Cunninghamella</i> , and other zygomycetes <i>Pneumocystis jiroveci</i> <i>Penicillium marneffei</i>

Table(4-1)The Major Mycoses and Causative Fungi

Taxonomical Classification

The fungi are placed in the phylum Thallophyta. There are four classes of fungi: Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes or Fungi Imperfecti .

Pathogenesis of Fungal Infection

Most fungi are obligate aerobes or facultative anaerobes, but none are obligate anaerobes. The natural habitat of most fungi is environment, because all these fungi require a preformed organic source of carbon, hence their constant association with decaying matter. *C. albicans* is exception and is an important fungus, which is a part of the normal human flora. The effects of fungi on humans can be grouped in three major ways as follows: (a) colonization and disease, (b) hypersensitive diseases, and (c) diseases caused by mycotoxins or fungal toxins.

Colonization and Disease

Most fungal infections are mild and self-limited. Intact skin is an effective host defense against certain fungi. But if the skin is broken, organisms, the fungi enter through that broken skin and initiate the infection. Fatty acid content, pH, epithelial turnover, and normal bacterial flora of the skin

contribute to host resistance against fungi. For example, the mucous membrane of the nasopharynx traps inhaled fungal spores. Cell-mediated immunity is much important in conferring protection against fungi. Suppression of cell-mediated immunity can lead to reactivation and dissemination of asymptomatic

fungal infection and to diseases caused by opportunistic fungi. The humoral immunity is mediated by production of IgG and IgM antibody. But their role in protection from fungal disease is uncertain. Fungal infection that occurs in the immunocompromised hosts is called as opportunistic mycosis.

Hypersensitivity Diseases

Humans are continually exposed to air-borne fungal spores and other fungal elements present in the environment. These spores can be antigenic stimulants and depending on individual's immunological status may induce a state of hypersensitivity by production of immunoglobulins or sensitized lymphocytes. Rhinitis, bronchial asthma, alveolitis, and various forms of atopy are the clinical manifestations of hypersensitive pneumonitis. The clinical manifestations of the hypersensitivity disease are seen only in sensitized person, after repeated exposure to the fungus, fungal metabolites, or other cross-reactive materials. Allergies to the fungal spores are manifested primarily by an asthmatic reaction including rapid bronchial constriction mediated by IgE, eosinophilia, and positive hypersensitivity skin test reaction. These are caused due to immediate hypersensitivity reactions of the host to fungal spores.

Diseases Caused by Fungal Toxins

Mycotoxicosis is caused by ingested fungal toxins. Mycotoxicosis caused by eating amanita mushroom is the best example of mycotoxicosis. This group of fungi produces five toxins. Of these, amanitine and phalloidin are the two most potent hepatotoxins. The toxicity of amanitine is due to its ability to inhibit cellular RNA polymerase, which prevents mRNA synthesis. Aflatoxin is another fungal toxin produced by *Aspergillus flavus* that causes disease in humans. Aflatoxin-B causes a mutation in the P53 tumor-suppressor gene, resulting in a loss of P53 protein, thereby in a resultant loss of growth control in the hepatocytes. Hence, it causes damage to liver, and it induces tumor in liver in animals and is associated with hepatic carcinomas in humans.

Laboratory Diagnosis

Laboratory diagnosis of fungal infections depends on: (a) direct microscopy, (b) culture, (c) serological tests, (d) nonculture methods, and (e) molecular methods.

Direct Microscopy

Direct microscopic examination depends on demonstration of characteristic asexual spores, hyphae, or yeast in various clinical specimens by light microscopy. The commonly used clinical specimens are sputum, lung biopsy material, and skin scrapings.

The specimen is either treated with 10% KOH or stained with special fungal stains. Use of 10% KOH dissolves tissue material, leaving the alkali-resistant fungi intact. Calcofluor dye is a fluorescent dye that combines with fungal cell wall and is useful in identification of fungi in tissue specimens. Methenamine silver stain is useful for demonstration of fungi in tissues. India ink preparation of cerebrospinal fluid (CSF) is a useful method for demonstration of white capsule of *C. neoformans* in CSF. Gram staining is also useful to demonstrate Gram-positive *Candida* species in the specimen.

The disadvantages of microscopy are that it shows low sensitivity and requires an experienced microscopist for specific identification.

Culture

Fungal culture is a frequently used method for confirming the diagnosis of fungal infection. SDA is the most commonly used medium for fungal culture. Other media include CHROM agar, blood agar, etc. The low pH of the medium and addition of chloramphenicol and cycloheximide to the medium inhibit the growth of bacteria in the specimen and thereby facilitate the appearance of slow-growing fungi. Fungal colony is identified by rapidity of growth, color, and morphology of the colony at the obverse and pigmentation at the reverse.

Microscopy of the fungal colony is carried out in lactophenol cotton blue (LPCB) mount to study the morphology of hyphae, spores, and other structures. The appearance of the mycelium and the nature of the asexual spores are very much helpful to identify the fungus.

Serological Tests

Demonstration of the antibodies in patient's serum or CSF is useful for diagnosis of fungal infections, especially in systemic fungal infections. A significant rise of antibody titer in a paired sera sample confirms the diagnosis. The complement fixation test was the earliest test used in fungal serology and is still used in the diagnosis of suspected cases of histoplasmosis, blastomycosis, or coccidiomycosis. Recently, newer tests like ELISA (enzyme-linked immunosorbent assay), Western blot, and radioimmunoassays are increasingly used for serodiagnosis of fungal infections.

Nonculture Methods

These methods include (a) detection of fungal antigen, (b) detection of fungal cell wall markers, and (c) detection of fungal metabolites.

Antigen detection: It is useful in immunocompromised hosts where antibody detection is not as sensitive. Detection of fungal antigen in serum, CSF, and urine is increasingly used for diagnosis of many fungal infections. Demonstration of antigen indicates recent or active infection. Latex agglutination test is a frequently used test to demonstrate polysaccharide capsular antigen of *C. neoformans* in CSF for diagnosis of cryptococcal meningitis. False-positive reactions due to *Trichosporon beigelli* and *Capnocytophaga canimorsus* are known.

Detection of fungal cell wall markers: Mannan is a highly immunogenic component of the candidal cell wall. Mannan antigen detection, therefore, is most widely used method in the diagnosis of candidiasis. Galactomannan is a heat-stable heteropolysaccharide found in the cell walls of all *Aspergillus* species. Production of the

galactomannan antigen is proportional to fungal load in tissue,

hence is being used as the prognostic marker for diagnosis

of invasive aspergillosis. A sandwich ELISA using rat monoclonal

antibody EB-A2 against galactomannan antigen is being

currently used in Europe for diagnosis of invasive aspergillosis.

Most pathogenic fungi have 1, 3-beta-D-glucan in their cell walls and minute quantities are secreted into the circulation during the life cycle. Detection of this antigen can also be used as an indicator of invasive fungal infections. Detection of 1, 3-beta-D-glucan is based on its ability to activate a coagulation cascade within amebocytes derived from the hemolymph of horseshoe crabs. This uses a different cascade than endotoxin to cause coagulation, hence is specific for fungi. The test does not detect certain species, such as *C. neoformans* and *Zygomycetes*.

Detection of fungal metabolites: Detection of distinctive fungal metabolites is another approach for the diagnosis of fungal infections. Gas liquid chromatography is being used to quantify arabinitol for diagnosis of *C. albicans* infections.

Antifungal Drugs

A few drugs are available for therapy of systemic fungal infection, unlike a large number of antibiotics available to treat bacterial infections. The drugs used to treat bacterial disease have no effect on fungal diseases. Amphotericin B and various azoles are the most effective antifungal drugs. They act on the ergosterol of fungal cell membrane that is not found in bacterial or human cell membrane. Similarly, caspofungin inhibits synthesis of beta-glucan, which is found only in fungal membrane but not in bacterial or human cell membrane. Table 4-2 summarizes the common antifungal agents and their primary sites of activity.

Table (4-2)Antifungal agents and primary sites of activity

Group of compounds	Antifungal agent	Mechanism of action
Polyenes	Amphotericin B	Bind to ergosterol
	Nystatin	
Azole derivatives	Miconazole	Inhibit cytochrome P-450 dependent enzymes
	Ketoconazole	
	Fluconazole	
	Itraconazole	
Nucleoside analogs	5-fluoro-cytosine	Inhibits DNA and RNA synthesis
Grisans	Griseofulvin	Inhibits microtubular function
Allylamines	Naftifine	Squalene epoxidase inhibitors
	Terbinafine	
Thiocarbamates	Tolnaftate	Squalene epoxidase inhibitors
	Tolciclate	
Morpholines	Amorolfine	Inhibits ergosterol biosynthesis
Echinocandins	Caspofungin, Anidulafungin	B-1, 3 glucan synthetase inhibitors

Superficial ,Cutaneous, and Subcutaneous Mycoses

Fungal infections, depending on the tissues that are initially colonized, can be classified into three major groups as follows:

Superficial mycoses: These are surface infections of the skin, affecting the outermost layers of skin, hair, and mucosa.

Cutaneous mycoses: These are infections of the skin involving the epidermis and its integuments, the hair, and nails.

Subcutaneous mycoses: These are infections of the dermis, subcutaneous tissue, muscle, and fascia

Superficial Mycoses

Superficial mycosis caused by different fungi is restricted to the outer most layers of the skin and hair. The condition usually causes cosmetic problem, which can be easily diagnosed and treated. It includes four important conditions:

(a) pityriasis versicolor, (b) tinea nigra, (c) black piedra, and (d) white piedra

Pityriasis Versicolor

Pityriasis versicolor or tinea versicolor is a superficial infection of the skin caused by *Malassezia furfur* (*Pityrosporum orbiculare*). *M. furfur* requires fatty acids for growth, hence is cultured on the Sabouraud's dextrose agar (SDA) overlaid with a layer of olive oil. On incubation at 37°C, the fungus produces creamy colonies within 5–7 days. The fungus is found in parts of the body rich in sebaceous glands. The lesions of pityriasis versicolor are found most commonly on the upper torso, arms, and abdomen.

Laboratory diagnosis of the condition is usually made by demonstration of both budding yeast cell and hyphae in KOH preparation of skin scrapings. Characteristic "spaghetti and meatballs" appearance of fungus is demonstrated in the microscopy of KOH preparation of the skin. Culture is not carried out routinely for diagnosis. Topical miconazole is treatment of choice.

Tinea Nigra

Tinea nigra is an infection of keratinized layer of skin caused by *Exophiala werneckii* or *Cladosporium werneckii*. *C. werneckii* is a dimorphic fungus that produces melanin. The fungus on the SDA grows as yeast with many cells in various stages of cell division producing typical two-celled oval structure, on primary isolation from clinical specimen. On prolonged incubation, elongated hyphae develop and in older cultures, mycelia and conidia are predominantly found. A well-demarcated brown-black macular lesion, which appears as brownish spot of the skin, is typical manifestation of the condition. These brownish to black lesions are most commonly seen on palms and soles.

Laboratory diagnosis of tinea nigra is made by microscopy of the KOH preparation of skin scrapings collected from the affected part. Typical darkly pigmented yeast-like cells and hyper fragmented hyphae are demonstrated. Culture of the skin scraping on the SDA confirms the diagnosis.

Black Piedra

Black piedra is a superficial infection of the hair caused by *Piedraia hortae*, a dematiaceous fungus. . Culture of specimens on SDA shows slow-growing brown to reddish black mycelium, which is considered asexual or anamorphic stage of the fungus.

Infection of shaft of hairs of beard and scalp is the major clinical feature of black piedra. Laboratory diagnosis of the condition is made by demonstration of nodules containing asci with spindle-shaped ascospores in 10% KOH mount of the hair.

White Piedra

White piedra is an infection of the hair caused by yeast-like organism *Trichosporon beigelli*. The fungus can be grown on SDA and other media containing cycloheximide. On SDA, it forms green-colored colonies, which subsequently become yellowish gray and wrinkled. Microscopic examination of the colony shows septate hyphae that break rapidly to form arthroconidia. The latter subsequently

become round and develop to Blastoconidia The development of a soft, pasty, cream-colored growth along infected hair shaft characterizes the condition. The initial growth of *T. beigelli* occurs beneath the epidermis of hair. The infected hair shaft consists of mycelium that rapidly fragments to arthroconidia.

Laboratory diagnosis of the condition is made by demonstration of fragmented hyphae that develop into arthroconidia or produce blastoconidia in 10% KOH mount of hair. Culture of the fungus from clinical specimen confirms the diagnosis.

Features of the organisms causing superficial mycoses are summarized in (Table 4-4).

Table (4-3) Features of the organisms causing superficial mycoses

Disease	Organism	Tissue	Diagnosis
Pityriasis versicolor	<i>Malassezia furfur</i>	Skin	Microscopy of skin scraping with 10% KOH shows "spaghetti and meatballs" appearance
Tinea nigra	<i>Exophiala werneckii</i>	Skin	Culture on SDA yields black yeast and hyphae
Black piedra	<i>Piedraia hortae</i>	Hair	Direct microscopy; culture yields asexual phase
White piedra	<i>Trichosporon beigelli</i>	Hair	Direct microscopy; culture on SDA; hyphae, arthroconidia, and blastoconidia

Cutaneous Mycoses

Dermatophytoses or cutaneous mycoses are diseases of the skin, hair, and nail. These infections are caused by a homogeneous group of closely related fungi known as dermatophytes. These dermatophytes infect only superficial keratinized structures, such as skin, hair, and nail, but not deeper tissues.

Dermatophytes

The most important dermatophytes that cause infection in humans are classified into the following three genera:

- (i) *Trichophyton* — causes infection of hair, skin, and nail.
- (ii) *Microsporum* — causes infection of hair and skin.
- (iii) *Epidermophyton* — causes infection of skin and nails, but not hair.

The dermatophytes on the basis of their natural habitat and host preferences can be classified into following groups:

- (i) **Anthropophilic species:** These dermatophytes are typically adapted to live on human host. They are transmitted from human to human through fallen hairs, desquamated epithelium, combs, hair brushes, towels, etc. Examples are *Trichophyton rubrum*, *Microsporum audouinii*, and *Epidermophyton floccosum*.

(ii) **Zoophilic species:** These are the dermatophytes that live on animals and often cause infection in their animal host. These zoophilic species are transmitted from infected animals to humans by direct and indirect contacts with domestic animals (e.g., cat and dog) and occasionally wild animals. Examples are *Trichophyton violaceum* and *Microsporum canis*.

(iii) **Geophilic species:** These are saprophytic fungi found in soil or in dead organic substances. They occasionally cause infection in humans and animals. Examples are *Microsporum gypseum* and *Trichophyton ajelloi*. Dermatophytes usually grow only on keratinized skin and do not penetrate the living tissues. In some infected persons, hypersensitivity to fungus antigen may cause secondary eruptions, such as vesicles on the finger. This reaction is known as dermatophytid (id) reaction. This reaction occurs as a result of hypersensitivity response to circulating fungal antigen, and these lesions do not contain any fungal hyphae.

Laboratory diagnosis

Laboratory diagnosis is based on demonstration of fungal element in clinical specimen by microscopy and confirmation by culture. The specimens include skin scrapings and nail clippings or hair taken from the areas suspected to be infected by dermatophytes. These entire specimens are treated with alkali solution to clear epithelial cells and other debris. Direct microscopy is useful only for diagnosis, while culture is always carried out to identify the specific causative fungal agent.

Direct microscopy

Examination of 10% direct KOH mount may show fungal hyphae. Three types of hair infections can be demonstrated in microscopy of 10% KOH wet mount as follows (Fig. 4-1):

Ectothrix: Ectothrix infection is characterized by presence of a layer of arthrospores on the surface of hair shaft. It is caused by *M. audouinii*, *M. canis*, and *Trichophyton mentagrophytes*.

Endothrix: The clusters of arthrospores are found entirely within the hair shaft in endothrix infection. It is caused by *Trichophyton tonsurans*, *T. violaceum*, and *Trichophyton schoenleinii*.

Culture

The clinical specimens are cultured by inoculation on SDA containing antibiotics like cycloheximide. The media after inoculation are incubated at 25–30°C for 3 weeks. At 25°C most of the pathogenic fungi grow well, while saprophytic fungi and bacteria are inhibited.

The cultures are examined at regular intervals, and dermatophytes are identified based on (a) colony morphology, (b) pigment production, and (c) presence of microconidia and macroconidia.

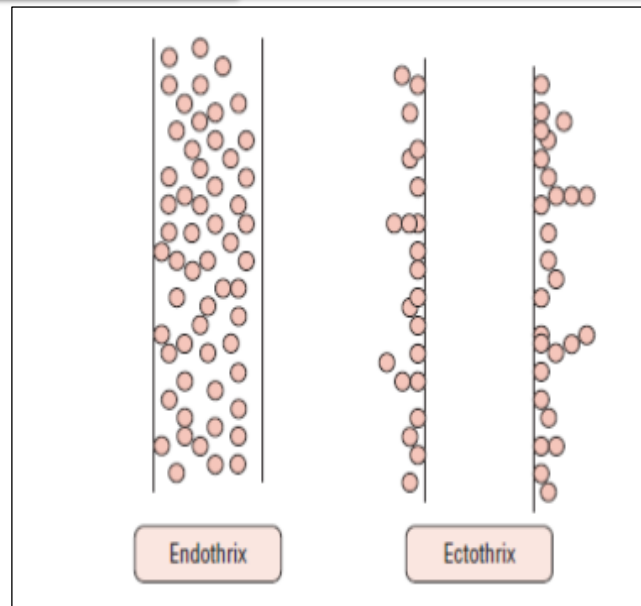


Figure (4-1) Endothrix and Ectothrix infection

Subcutaneous Mycosis

Subcutaneous mycosis is defined as fungal infection associated with development of characteristic lesion in subcutaneous tissue and overlying skin with or without extension to bone and muscle. This is caused by a heterogeneous group of fungal infection of low pathogenic potential introduced in the body percutaneously from a trivial trauma. shows the classification of subcutaneous mycoses.

Mycetoma

Mycetoma is a slowly progressive, chronic granulomatous infection of skin and subcutaneous tissues with occasional involvement of underlying fascia and bone usually affecting extremities.

extremities are most commonly involved. Microabscesses are formed in subcutaneous tissues surrounded by polymorphonuclear inflammatory reaction. The center of the lesion consists of tangled filaments of these organisms.

During the course of infection, microabscesses burst open with the formation of chronic multiple sinuses discharging copious, seropurulent fluid containing granules. The color and consistency of these granules vary depending on the fungi that cause the disease. The condition is characterized by formation of painless, localized, swollen lesions on the affected limbs. Multiple discharging sinuses are present.

Systemic mycoses

Systemic mycoses are caused by fungi of soil, which are inherently virulent and cause disease in healthy humans. The systemic mycoses include coccidioidomycosis, paracoccidioidomycosis, histoplasmosis, blastomycosis, and cryptococcosis.

Histoplasmosis

Histoplasmosis is primarily a disease of reticuloendothelial system caused by an intracellular fungus *Histoplasma capsulatum*. *H. capsulatum* is a dimorphic fungus, which occurs in two stages: as a mold in soil and as yeast at body temperature in mammals. On SDA medium at 37°C, this fungus produces cottony mycelial growth. The colony is characterized by thin, branching, septate hyphae that produce tuberculate macroconidia and microconidia.

Blastomycosis

Blastomycosis is a granulomatous fungal infection caused by *B. dermatitidis*. *B. dermatitidis* is a dimorphic fungus, which occurs in two stages: as mold in soil and as yeast in tissue.

On culture at 37°C and in tissue, the yeast is a round structure with a double refractile wall and a single broad-based bud. This appearance helps to differentiate it from the *Cryptococcus neoformans* yeast, which has a narrow-based bud. On culture at 25°C, the fungus produces a mycelial growth showing typical pyriform microconidia, which measure 2–4 μ m in diameter.

Cryptococcosis

Cryptococcosis, also called European blastomycosis, is an acute to chronic disease caused by an encapsulated yeast, *C. neoformans*.

Cryptococcosis is the most common life-threatening fungal disease in patients with AIDS. Of the 19 species that comprise the genus *Cryptococcus*, human disease is associated with only *C. neoformans*.

Morphology

- *C. neoformans* is a true yeast.
- It is an oval and budding yeast and measures 3–6 μ m in diameter. The yeast may be single or may have a single budding daughter cell.
- Within the host and in certain culture media, the yeast is surrounded by a wide polysaccharide capsule. The polysaccharide capsule is composed of mannose, xylose, and glucuronic acid.
- *C. neoformans* on SDA medium forms smooth, convex, cream-colored colonies at 20–37°C. Lactophenol cotton blue (LPCB) wet mount of the colony shows budding yeast cells.

C. neoformans has two varieties: *C. neoformans* var *neoformans* and *C. neoformans* var *gattii*. Based on antigenic specificity of the capsular polysaccharide, the species has been classified into four serotypes. These are serotypes A and D (*C. neoformans* var *neoformans*) and serotypes B and C (*C. neoformans* var *gattii*).

Pathogenesis and Immunity

The immune status of the host is the crucial factor in pathogenesis

of cryptococcosis. *C. neoformans* usually causes most serious infections in patients with impaired CMI. These include:

- patients with AIDS,
- patients undergoing corticosteroid treatment,
- patients undergoing organ transplantation,
- patients with reticuloendothelial malignancy, and
- patients with sarcoidosis.

C. neoformans is primarily transmitted by inhalation. Following inhalation, the yeasts are deposited into the pulmonary alveoli, in which they survive before they are phagocytosed by alveolar macrophages. Glucosylceramide synthase has been identified as an essential factor in the survival of *C. neoformans* in pulmonary alveoli.

Cryptococcal polysaccharide capsule has antiphagocytic properties. Hence, unencapsulated yeast are readily phagocytosed and destroyed than the encapsulated organisms, which are more resistant to phagocytosis. The antiphagocytic properties of the capsule prevent recognition of the yeast by phagocytes and inhibit leukocyte migration into the area of fungal replication.

Host immunity

The host immunity in cryptococcal infection is mediated by both cellular and humoral responses. CMI is mediated by natural killer cells and T lymphocytes can inhibit or kill cryptococci. An increase in helper T-cell activity, skin test conversion, and a reduction in the number of viable organisms in the tissues indicates a successful host response against the fungus. Humoral immunity is mediated by anticryptococcal antibodies and soluble anticryptococcal factors. Both anticryptococcal antibodies and the complement play a crucial role in facilitating the macrophage- and lymphocyte-mediated immune response to the organism.

Clinical Syndromes

C. neoformans causes (a) pulmonary cryptococcosis in immunocompetent hosts and in immunocompromised hosts, (b) CNS cryptococcosis, and (c) disseminated nonpulmonary non-CNS cryptococcosis.

○ Pulmonary cryptococcosis

The clinical manifestations of pulmonary cryptococcosis are widely variable. Pulmonary disease varies from asymptomatic colonization of the respiratory tract to acute respiratory distress syndrome affecting immunocompromised hosts. It depends on the immune status of the host

○ CNS cryptococcosis

Both the brain and meninges are involved in cryptococcal infection of the CNS infections. Meningitis and meningoencephalitis are the most common manifestations. These are usually subacute or chronic in nature. Without specific therapy, the infection is invariably fatal. The patient dies due to the disease 2 weeks to several years after the symptom onset.

○ Disseminated nonpulmonary non-CNS cryptococcosis

Disseminated cryptococcosis includes the skin, prostate, and medullary cavity of the bones, next only to the lungs and CNS, and it occurs most commonly in patients with AIDS and other immunosuppressed conditions.

Epidemiology

C. neoformans is distributed worldwide. The incidence of cryptococcosis is increasing and now it represents a major lifethreatening fungal infection in patients with AIDS. Most cases of cryptococcosis are caused by serotypes A and D. *C. neoformans* var *gattii* is the most common variety that causes disease in immunocompetent patients. *C. neoformans* var *neoformans* is the most common variety that causes disease in immunocompromised patients, e.g., AIDS. *C. neoformans* is primarily transmitted by inhalation (Figure 4-2). Human-to-human transmission does not occur.

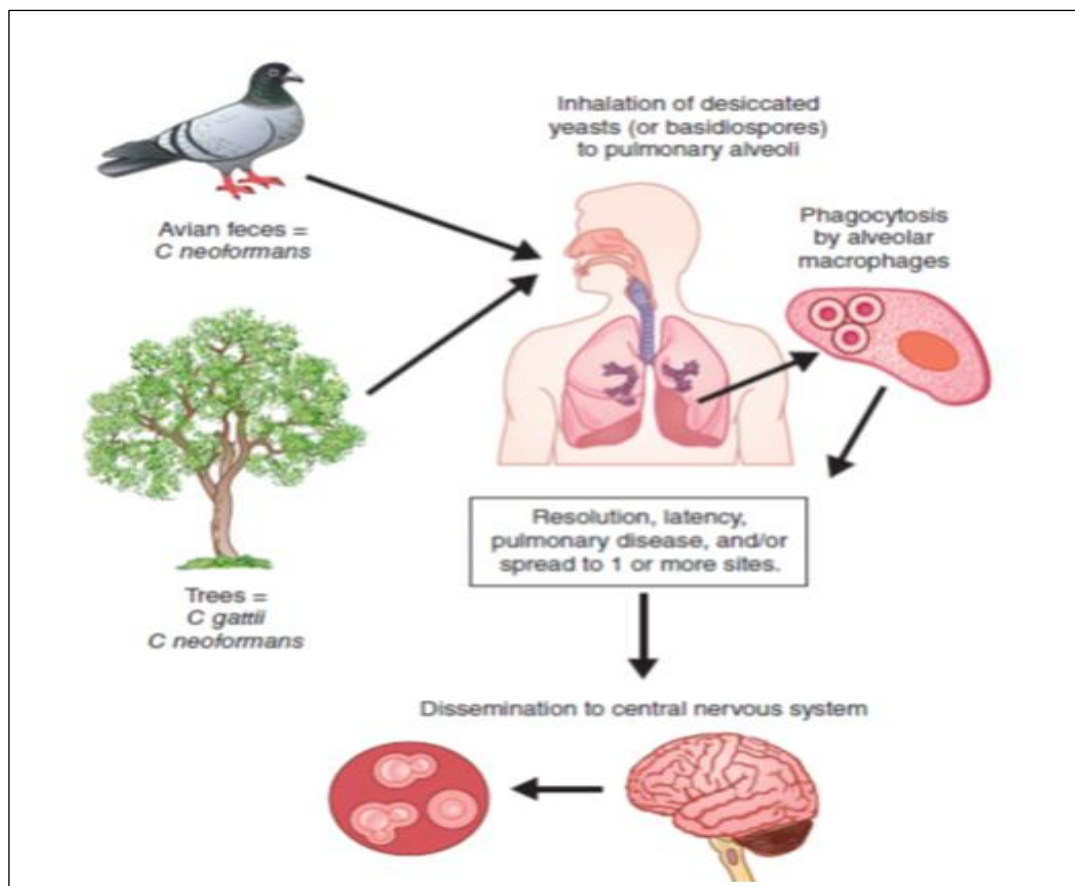
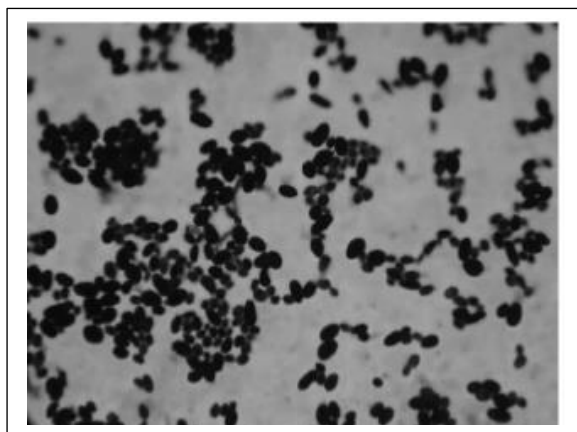


Figure (4-2) Transmission of *Cryptococcus neoformans*

Laboratory Diagnosis

Laboratory diagnosis of cryptococcal infection is made by demonstration of the yeast in CSF, sputum, pus, and brain biopsy tissue by smear and culture. Methenamine silver or periodic acid-Schiff stains are used to stain the tissue specimens for demonstration of the capsule of *C. neoformans*. Fixed tissue may also be stained with mucicarmine, which preferentially stains *C. neoformans*.

India ink preparation is commonly used to detect budding yeast cells in the CSF (Figure 4-3). The capsule appears as a clear halo around the yeast cells. By this method, cryptococci can be demonstrated in 25–50% of patients with cryptococcal meningitis. Gram-stained smear of the CSF shows Gram-positive yeast cells. The culture of centrifuged CSF specimens confirms diagnosis of the condition. This fungus is identified based on the macroscopically appearance, biochemical test result and ability to grow at 37 C°.



Figure(4-3) India ink preparation showing capsule of *Cryptococcus neoformans* (_400).

Opportunistic Fungal infections

The opportunistic fungi usually cause infections in persons with impaired host defense, but do not cause disease in most of the immunocompetent hosts. Since these fungi become pathogens in individuals with impaired immunity by taking

advantage of the host's debilitated conditions, they are called opportunistic fungi.

The most opportunistic infections are caused by *Candida albicans*, *Aspergillus spp.*, *Penicillium marneffeii*, and various Zygomycetes (Table 4-4).

Candidiasis

Candida species are the most common fungal pathogens that affect humans. These species are true opportunistic pathogens that take advantage of the host's debilitated condition and gain access to the

circulation and deep tissues. The genus *Candida* includes more than 100 species, of which only few cause disease in humans. *C. albicans* and occasionally other species cause candidiasis, a major infection in immunocompromised.

able (4-4) Common opportunistic infections

Disease	Causative fungus
Candidiasis	<i>Candida albicans</i> , <i>Candida tropicalis</i> , and other species
Aspergillosis	<i>Aspergillus fumigates</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , and other species
Zygomycosis	<i>Mucor</i> , <i>Rhizopus</i> , and <i>Absidia</i> species
<i>Pneumocystis carinii</i> pneumonia (PCP)	<i>Pneumocystis jiroveci</i>
Penicilliosis	<i>Penicillium marneffei</i>
<i>Pseudoallescheria boydii</i> infection	<i>Pseudoallescheria boydii</i>
<i>Fusarium solani</i> infection	<i>Fusarium solani</i>
Meningitis	<i>Cryptococcus neoformans</i>

Candida albicans

C. albicans is the most common *Candida* species, which causes opportunistic infections in immunocompromised hosts. It forms the part of the normal flora of the mucous membrane of the gastrointestinal, genitourinary, and respiratory tract. Properties

- *C. albicans* is ovoid or spherical yeast with a single bud.
- It forms the part of the normal flora of the mucous membrane

of the gastrointestinal, genitourinary, and respiratory tract. It produces pseudohyphae in the cultures and in tissues. Pseudohyphae are elongated yeast that may resemble hyphae morphologically, but are really not true hyphae.

Candida grows readily on Sabouraud's dextrose agar and on bacteriological culture media. *C. albicans* produces creamy white, smooth colonies with a yeasty odor.

- It can be differentiated from other *Candida* species by carbohydrate fermentation reaction and by characteristic growth properties.
- Only *C. albicans* produces chlamydospores on cornmeal agar culture at 25°C.

Pathogenesis and Immunity

Candida spp. are usually present as part of normal flora on healthy mucosal surface of the oral cavity, gastrointestinal tract, and vagina. *Candida* shows colonization at these sites in more than 80% of healthy people. The organism, however, is rarely present on the surface of normal human skin, except occasionally from certain intertriginous area, such as the groin.

✓ Pathogenesis of *Candida* infection

Under certain conditions, *Candida* gains access to systemic circulation from the oropharynx of the gastrointestinal tract. Colonization of the mucocutaneous surface is the first stage in the pathogenesis of Candidal infection. The fungus causes invasion in human tissue through different routes. Disruption of the skin or mucosa allows the organism access to the blood stream. Massive colonization with large numbers of *Candida*

also permits the organism to pass directly into the blood stream, causing the infection. In immunocompromised hosts, *Candida* may disseminate to many organs, such as lung, spleen, liver, heart, and brain. *Candida*

may induce inflammation of the eye, causing endophthalmitis and also may involve skin in 10–30% of patients with disseminated infection. Deficiency in host defence mechanisms plays a significant role in development of *Candida* infection.

✓ Host immunity

Both cell-mediated and humoral antibodies confer protection against *Candida* in healthy adults. Cell-mediated immunity (CMI) is, however, most important. Alteration in CMI may cause extensive superficial candidiasis, despite having normal or elevated humoral antibodies. The humoral antibodies appear to play minimal role in protection against the disease. Humoral antibodies confer protection against *Candida* in healthy adults.

✓ Clinical Syndromes

Candida causes a wide spectrum of clinical illnesses as follows:

Cutaneous candidiasis: *Candida* species in immunocompetent

host can cause infection of any warm and moist part of the body exposed to environment. It causes infection of the nail, rectum, and other skin folds.

Mucocutaneous candidiasis: Mucocutaneous candidiasis (thrush, perianal disease, etc.) is the most common manifestation of candidiasis, but usually does not cause any mortality.

In patients with advanced immunodeficiency due to HIV infection, *Candida* species can cause severe oropharyngeal and esophageal candidiasis that result in poor intake of food, leading to malnutrition, wasting, and early death. These patients are also usually resistant to treatment with antifungal therapy.

Systemic candidiasis: These include endocarditis, gastrointestinal tract candidiasis, respiratory tract candidiasis, genitourinary candidiasis, and hepatosplenic candidiasis. Systemic candidiasis may be candidemia and disseminated candidiasis. In patients with AIDS, oral thrush and *Candida* esophagitis are more common but not candidemia and disseminated candidiasis. *Candida* endophthalmitis and central nervous infection (CNS) infection due to *Candida* species are other complications of *Candida* infection.

Disseminated candidiasis: This is increasingly becoming a problem in patients with serious hematologic malignancies that are treated with immunosuppressive drugs for over a long period of time. Severe neutropenia in these patients is the most important predisposing condition for life-threatening infection caused by *Candida*. In this condition, *Candida* usually spreads through the circulation and involves many organs, such as lungs, spleen, kidney, liver, heart, and brain. However, disseminated candidiasis is not a major problem in patients with AIDS. In such patients, serious infection of the oropharynx and the upper gastrointestinal tract is the

Laboratory Diagnosis

○ **Specimens**

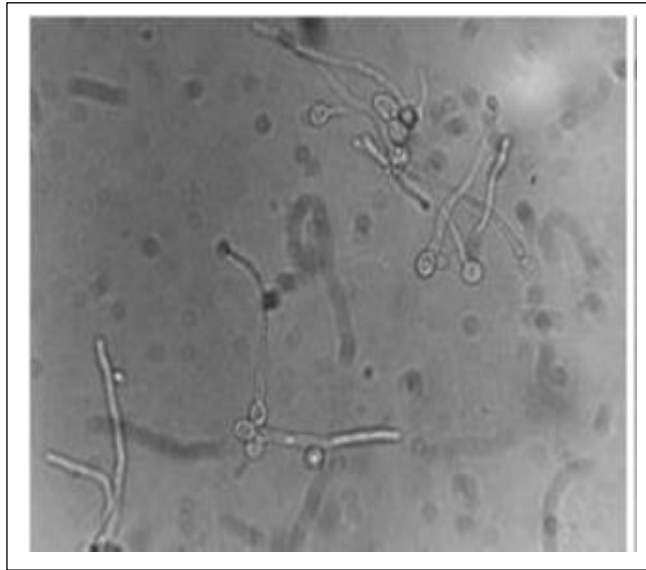
These include exudates or tissues for microscopy obtained from skin or nails examined by microscope for demonstration of pseudohyphae or budding yeast cells of *Candida*.

○ **Microscopy**

Gram-stained smear of the exudates or tissue shows Gram positive, oval, budding yeast and pseudohyphae. Since *Candida* is found as a part of normal flora on normal skin or mucosa, only the presence of large numbers of *Candida* is of significance. Demonstration of pseudohyphae indicates infection, and tissue invasion is of more diagnostic value.

○ **Culture**

Culture on Sabouraud's dextrose agar (SDA) produces typical creamy white, smooth colonies. Different *Candida* species are identified by their growth characteristics, sugar fermentation, and assimilation tests. Germ tube is a rapid method for identification of *C. albicans* and *Candida dubliniensis*. This test depends on the ability of *C. albicans* to produce germ tube within 2 hours when incubated in human serum at 37°C. This phenomenon is called Reynold-Braude phenomenon (Figure 4-4).



Figure(74-2) *Candida albicans* showing formation of the germ tube (_400).

- **Nonculture Candida detection tests**

These include (a) *Candida* mannan assay, (b) *Candida* heat-labile-antigen assay, (c) D-arabinitol assay, (d) D-inositol assay, and (e) 1,3-beta-D-glucan assay. Beta-D-glucan assay is a broad-spectrum test that detects *Candida* as well as *Aspergillus*, *Fusarium*, *Acremonium*, and *Saccharomyces* species. This test depends on the principle that beta-D-glucan is a component of the cell wall of these fungi, which can be detected by its ability to activate factor G of the horseshoe crab coagulation cascade. This test is a highly specific and sensitive test.

Treatment

Antifungal therapy forms the mainstay of treatment of the infections caused by *Candida*. These agents include azoles (fluconazole, triazole, ketoconazole), nystatin, and amphotericin is becoming increasingly important worldwide .

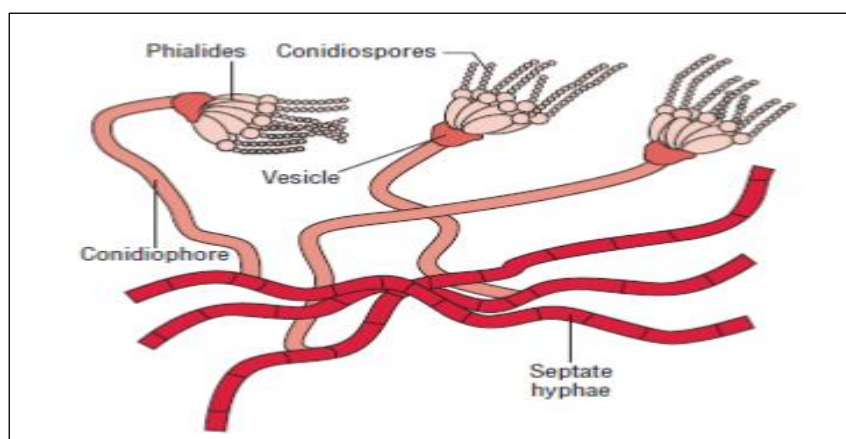
Aspergillosis

A broad spectrum of diseases in humans ranging from direct invasion to hypersensitive reactions are caused by *Aspergillus* species. Although more than 100 species have been described, the majority of human diseases are caused by *Aspergillus fumigates* and *Aspergillus niger*, and less frequently by *Aspergillus flavus* and *Aspergillus clavatus*.

Aspergillus Species

Properties

- Aspergillus species are molds.
- They have septate hyphae that form V-shaped dichotomous branches (Figure 4-5). The *Aspergillus* species are identified by (a) their morphological features, (b) the pattern of conidiophores development, and (c) the color of the conidia.
- The presence of septate hyphae that branch at 45° angles is the typical feature of *Aspergillus* species hyphae. The hyphae in tissues are best demonstrated with silver stains. The walls of the hyphae are more or less parallel, unlike those of *Mucor* and *Rhizopus*, which are more or less irregular.



Figure(4-5) *Aspergillus* species with septate hyphae that form V-shaped dichotomous branches.

Pathogenesis and Immunity

Aspergillus species rarely cause infections in immunocompetent individuals. They cause invasive infections mostly in the patients who are immunocompromised either due to (a) use of immunosuppressive drugs, (b) underlying lung diseases, or (c) immunodeficiency diseases, such as HIV. In immunocompromised host, *Aspergillus* species cause invasion of the blood, thereby causing infarction, hemorrhage, and necrosis of lung tissues. *Aspergillus* spp. also produces toxic metabolites that inhibit macrophage and neutrophil phagocytosis, facilitating dissemination of the infection. *Aspergillus* species unlike *Candida* species do not form the part of normal flora of humans. They are ubiquitous in the environment; hence transmission of infection is mostly exogenous.

Clinical Syndromes

In immunocompetent hosts, *Aspergillus* species may primarily affect the lungs, causing four main syndromes including (a) allergic bronchopulmonary aspergillosis, (b) chronic necrotizing aspergillus pneumonia, (c) aspergilloma, and (d) invasive aspergillosis.

Allergic bronchopulmonary aspergillosis: It is a hypersensitivity reaction to *A. fumigatus* organisms, which have colonized in tracheobronchial tree. This condition occurs often in association with asthma and cystic fibrosis.

Chronic necrotizing pulmonary aspergillosis: It is a subacute infection seen in patients with some degree of immunosuppression. The condition occurs in conjunction with alcoholism, underlying lung disease, or chronic corticosteroid therapy.

Aspergilloma: It is a condition that occurs in a preexisting cavity in the lung parenchyma. This cavity may have been caused earlier by tuberculosis, sarcoidosis, cystic fibrosis, and emphysematous bullae. The condition is characterized by the presence of a ball of fungus within the cavity. The fungus, however, does not invade the cavity. It may cause hemoptysis.

Invasive aspergillosis: It is a rapidly progressive infection in patients who are severely immunocompromised. The condition is mostly fatal. In immunocompromised host, *Aspergillus* organisms cause a disseminated disease, leading to endophthalmitis, endocarditis, and abscesses in the viscera, such as liver, spleen, kidney, soft tissues, and bone.

Laboratory Diagnosis

Laboratory diagnosis of invasive aspergillosis or chronic necrotizing aspergillus pneumonia depends on demonstration of *Aspergillus* in tissue by direct microscopy and culture.

يَمْعُونَ اللَّهَ

