4TH EDITION

Pocket Guide to Clinical Microbiology

Christopher D. Doern

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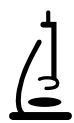

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To Drs. Carey-Ann Burnham, Mike Dunne, and Betz Forbes: invaluable mentors and dear friends.

Contents

Preface

The *Pocket Guide to Clinical Microbiology* is unique among reference texts in that its primary purpose is to present usable information in a concise and accessible manner. Now in its fourth edition, this iconic text has remained popular over the years because it has successfully condensed most of the information needed by the practicing clinical microbiologist into a pocket-sized guide. As Dr. Patrick Murray articulated in the preface of the third edition, with each iteration of this text the format has been honed to enhance its usability. Although the overall format of the fourth edition remains true to the template of previous editions, this edition reflects the tremendous evolution that has occurred in clinical microbiology over the past decade. In particular, three key advances have reshaped the practice of clinical microbiology, and have therefore, reshaped this edition.

First, the widespread adoption of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for organism identification has altered the way many laboratories practice clinical microbiology. Despite the fact that those using MALDI-TOF MS are less dependent on biochemical reactions to identify organisms, understanding biochemical profiles is still required for the troubleshooting of failed or ambiguous MALDI-TOF MS identifications. As such, this text has updated, but maintained, much of the biochemical reactions required to identify organisms the "old school" way. It is my belief that understanding and using these reactions to confirm organism identifications, remains one of the foundations of good clinical microbiology. This pocket guide should provide quick reference to everything the microbiologist needs to confidently identify most organisms encountered in the clinical laboratory. In addition, new information has been added in Section 8 that outlines the specifics of MALDI-TOF MS performance for individual bacteria, mycobacteria, and fungi.

Second, the development of molecular techniques has revolutionized the diagnosis of infectious diseases. Most notably, nucleic acid amplification testing (NAATs), also referred to as polymerase chain reaction (PCR), has drastically changed the practice of clinical

virology to the extent that many laboratories no longer perform viral culture. Information regarding viral culture has been retained in this guide for labs still performing those techniques, but significant additions have been made throughout this edition to reflect the use of molecular diagnostics. Also, because gene sequencing is now a common technique used to identify bacteria and fungi, Section 8 contains new information to help interpret these results.

Third, the continued emergence of antimicrobial resistance poses significant challenges to clinical microbiologists as we struggle to provide treatment options for increasingly difficult-to-treat organisms. To reflect this reality, significant changes have been made to this pocket guide to help the microbiologist better perform and interpret antimicrobial susceptibility testing in the era of multidrug resistance. Acknowledging the international appeal of this text, I added new tables outlining guidance provided by both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The goal of these tables is to help the reader easily understand what methods can be used, and what interpretive criteria exist, for most organism/antibiotic combinations. Also included are tables with important intrinsic resistance profiles for commonly encountered organisms, as well as key mechanisms of resistance.

Sir Isaac Newton once said, "If I have seen further than others, it is by standing on the shoulders of giants." Although I have no illusions that I have "seen further" than anyone else, the humility expressed in this quote resonates with me as I have undertaken the task of updating a text that was conceived and authored by Dr. Patrick Murray, one of the true giants of clinical microbiology. So first and foremost, I thank him for his work making this pocket guide the respected resource that it is. I hope that the fourth edition will do justice to the tradition of this text. In addition, I want to thank the talented, and patient, professionals at ASM Press. Specifically, Christine Charlip and Larry Klein, along with what I'm sure are countless others at ASM, warrant special thanks for all their work. And last, I thank my wife Kelli, who provided support and understanding through the many late nights and weekends it took to complete this project. I could not have finished this project were it not for her support.

The practice of clinical microbiology is a wonderful discipline, requiring judgement, investigation, and critical decision-making to produce quality results. It is my hope that you will find this pocket guide to be a user-friendly reference that enhances your ability to do all of these things, and ultimately provide the best patient care possible.

Christopher D. Doern

About the Author

Christopher Doern, PhD, D(ABMM), is an Assistant Professor of Pathology and the Director of Clinical Microbiology at the Virginia Commonwealth University Health System, Richmond, Virginia. He earned his undergraduate and doctoral degrees from Wake Forest University in Winston-Salem, North Carolina. Doern went on to a fellowship in Medical and Public Health Microbiology at the Washington University School of Medicine, St. Louis, Missouri, and is certified by the American Board of Medical Microbiology (ABMM).

Doern is an active member of the clinical microbiology community and serves on the ABMM, ASM Clinical Laboratory Practices Committee, Clinical Chemistry Trainee Council, and several Clinical and Laboratory Standards Institute document development and revision committees. He is an editor for the *Clinical Microbiology Newsletter* and serves on the *Journal of Clinical Microbiology* and *Pediatric Infectious Diseases Journal* editorial boards.

Doern is involved in educational programs that reach an international audience. Among these is the Medical Microbiology Question of the Day (www.pathquestions.com), for which he has been an editor since 2011. This service provides freely accessible educational material to participants in more than 60 countries

SECTION 1

Taxonomic Classification of Medically Important Microorganisms

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In order to remain true to the tradition set forth by the first three editions of this pocket guide, the first section will be devoted to describing the taxonomy of common (and some uncommon) organisms which are associated with humans and may be isolated by the Clinical Microbiology Laboratory. Unfortunately, in the 13 or so years that have passed since the third edition of the Pocket Guide was published, the rate of taxonomic changes has continued to increase such that publishing a taxonomic list of organisms would be out of date before this book goes to publication. This is a product of continued proliferation of new species of organisms which are being identified by increasingly sophisticated genomic analyses. As such, the revised goal of this section will be to outline some high-level taxonomic groupings and provide the resources and references one would need to identify the most up-to-date taxonomic classifications.

It should be appreciated that despite appearances, changes in nomenclature are regulated by a system of rules with oversight governed by the International Code of Biological Nomenclature (www.biosis.org.uk/zrdocs/codes/codes.htm). The International Code of Nomenclature of Bacteria governs bacterial taxonomy, and all bacteria named after 1980 must be validly published in the *International Journal of Systematic and Evolutionary Microbiology.* A current listing of bacteria can be found at http://www.bacterio.net, http://www.bacterio.cict.fr/, and https://www.dsmz.de/. The International Committee on Taxonomy of Viruses (ICTV) governs viral taxonomy, and all currently recognized viruses can be found at https://talk.ictvonline.org//. The International Code of Botanical Nomenclature governs fungal classification, and additional information can be found at http://www.iapt-taxon.org/nomen/main.php/.

Taxonomic Classification of Bacteria

Classification and taxonomy of prokaryotes (bacteria) is complicated and is governed by the International Code of Nomenclature of Bacteria (last revised in 1990). By definition, each prokaryotic species must include a genus-level name that is included within a hierarchy or ranks, which includes (from highest to lowest rank) subtribe, tribe, subfamily, family, suborder, order, subclass, class, division (or phylum), and domain (or empire). To further complicate matters, the tribe and subtribe do not actually include names and are therefore not used.

Most importantly, there is no such thing as an official classification of prokaryotes. This is because in contrast to eukaryotes, the prokaryotic designations are a matter of scientific judgment. Therefore, the closest things that we have to "official" taxonomic designations are those names that are generally accepted by the microbiology community. Despite this fact, microbiologists have

achieved some amount of consensus by relying on resources such as the International Journal of Systematic and Evolutionary Microbiology and Bergey's Manual of Systematic Bacteriology.

As of the writing of this fourth edition Pocket Guide, the prokaryotes were divided into 2 domains, 35 phyla, 80 classes, 1 subclass, 178 orders, 20 suborders, 402 families, and 2,552 genera. The following is a consolidated taxonomic outline, which will focus on the taxonomic organization or those organisms that are most likely to be encountered in the clinical microbiology laboratory. This is not meant to be an exhaustive list of all bacteria. Rather, it is intended to provide some context to the relationships between some of the most commonly encountered organisms in human clinical specimens.

The taxonomy of bacterial classification is arranged in the following way. . .

Domain
Phyla
Class
Subclass
Order
Suborder
Family
Genera

Domain: Bacteria

Class. Actinobacteria
- Family. *Actinomycetaceae*
  - Genus. *Actinobaculum*
  - Genus. *Actinomyces*
  - Genus. *Arcanobacterium*
  - Genus. *Mobiluncus*
  - Genus. *Trueperella*
- Family. *Corynebacteriaceae*
  - Genus. *Corynebacterium*
  - Genus. *Turicella*
- Family. *Dietziaceae*
  - Genus. *Dietzia*
- Family. *Mycobacteriaceae*
  - Genus. *Mycobacterium*
- Family. *Nocardiaceae*
  - Genus. *Gordonia*
  - Genus. *Nocardia*
  - Genus. *Rhodococcus*
- Family. *Tsukamurellaceae*
  - Genus. *Tsukamurella*
- Family. *Propionibacteriaceae*
  - Genus. *Propionibacterium*

Family. *Streptocmycetaceae*
Genus. *Streptomyces*
Family. *Nocardiopsaceae*
Genus. *Nocardiopsis*
Family. *Bifidobacteriaceae*
Genus. *Alloscardovia*
Genus. *Bifidobacterium*
Genus. *Gardnerella*
Family. *Brevibacteriaceae*
Genus. *Brevibacterium*
Family. *Cellulomonadaceae*
Genus. *Cellulomonas*
Genus. *Oerskovia*
Genus. *Tropheryma*
Family. *Dermabacteraceae*
Genus. *Dermabacter*
Genus. *Helcobacillus*
Family. *Dermacoccaceae*
Genus. *Dermacocccus*
Genus. *Kytococcus*
Family. *Microbacteriaceae*
Genus. *Leifsonia*
Genus. *Microbacterium*
Family. *Micrococcaceae*
Genus. *Arthrobacter*
Genus. *Kocuria*
Genus. *Micrococcus*
Genus. *Rothia*
Genus. *Stomatococcus*
Class. Coriobacteriia
Family. *Atopobiaceae*
Genus. *Atopobium*
Family. *Eggerthellaceae*
Genus. *Eggerthella*
Genus. *Slackia*
Class. Bacteroidia
Family. *Bacteroidaceae*
Genus. *Bacteroides*
Family. *Porphyromonadaceae*
Genus. *Dysgonomonas*
Genus. *Microbacter*
Genus. *Parabacteroides*
Genus. *Porphyromonas*
Genus. *Tannerella*
Family. *Prevotellaceae*
Genus. *Prevotella*

Class. Flavobacteriia
- Family. *Flavobacteriaceae*
  - Genus. *Bergeyella*
  - Genus. *Capnocytophaga*
  - Genus. *Chryseobacterium*
  - Genus. *Elizabethkingia*
  - Genus. *Empedobacter*
  - Genus. *Flavobacterium*
  - Genus. *Weeksella*

Class. Sphingobacteriia
- Family. *Sphingobacteriaceae*
  - Genus. *Sphingobacterium*

Class. Chlamydiae
- Family. *Chlamydiaceae*
  - Genus. *Chlamydia*
  - Genus. *Chlamydophila*

Class. Bacilli or Fibribacteria
- Family. *Bacillaceae*
  - Genus. *Bacillus*
- Family. *Listeriaceae*
  - Genus. *Listeria*
- Family. *Paenibacillaceae*
  - Genus. *Paenibacillus*
- Family. *Staphylococcaceae*
  - Genus. *Staphylococcus*
- Family. Unassigned
  - Genus. *Gemella*
- Family. *Aerococcaceae*
  - Genus. *Abiotrophia*
  - Genus. *Aerococcus*
  - Genus. *Dolosicoccus*
  - Genus. *Facklamia*
  - Genus. *Globicatella*
- Family. *Carnobactericeae*
  - Genus. *Alloiococcus*
  - Genus. *Dolosigranulum*
  - Genus. *Granulicatella*
- Family. *Enterococcaceae*
  - Genus. *Enterococcus*
  - Genus. *Vagococcus*
- Family. *Lactobacillaceae*
  - Genus. *Lactobacillus*
  - Genus. *Pediococcus*
- Family. *Leuconostocaceae*
  - Genus. *Leuconostoc*
  - Genus. *Weissella*

- Family. *Streptococcaceae*
  - Genus. *Lactococcus*
  - Genus. *Streptococcus*
- Class. Clostridia
  - Family. *Clostridiaceae*
    - Genus. *Clostridium*
    - Genus. *Sarcinia*
  - Family. *Peptococcaceae*
    - Genus. *Peptococcus*
  - Family. Peptostreptococcaceae
    - Genus. *Peptostreptococcus*
  - Family. Unassigned
    - Genus. *Anaerococcus*
    - Genus. *Finegoldia*
    - Genus. *Helcococcus*
    - Genus. *Peptoniphilus*
- Class. Erysipelotrichia
  - Family. *Erysipelotrichaceae*
    - Genus. *Erysipelothrix*
- Class. Negativicutes
  - Family. *Veillonellaceae*
    - Genus. *Veillonella*
- Class. Fusobacteriia
  - Family. *Fusobacteriaceae*
    - Genus. *Fusobacterium*
  - Family. *Leptotrichiaceae*
    - Genus. *Leptotrichia*
    - Genus. *Sneathia*
    - Genus. *Streptobacillus*
- Class. Alphaproteobcteria
  - Family. *Caulobacteraceae*
    - Genus. *Brevundimonas*
  - Family. *Bartonellaceae*
    - Genus. *Bartonella*
  - Family. *Brucellaceae*
    - Genus. *Brucella*
    - Genus. *Ochrabactrum*
  - Family. *Rhizobiaceae*
    - Genus. *Agrobacterium*
    - Genus. *Rhizobium*
  - Family. *Rhodobacteraceae*
    - Genus. *Paracoccus*
  - Family. *Acetobacteraceae*
    - Genus. *Roseomonas*
  - Family. *Rhodospirllaceae*
    - Genus. *Inquilinus*
  - Family. *Anaplasmataceae*

Genus. *Anaplasma*
Genus. *Ehrlichia*
Genus. *Wolbachia*
Family. *Rickettsiaceae*
Genus. *Orientia*
Genus. *Rickettsia*
Family. *Sphingomonadaceae*
Genus. *Sphingomonas*
Class. Betaproteobacteria
Family. *Alcaligenaceae*
Genus. *Achoromobacter*
Genus. *Alcaligenes*
Genus. *Bordetella*
Genus. *Oligella*
Family. *Burkholderiaceae*
Genus. *Burkholderia*
Genus. *Cupriavidis*
Genus. *Pandoraea*
Genus. *Ralstonia*
Family. *Comamonadaceae*
Genus. *Acidovorax*
Genus. *Comamonas*
Genus. *Defltia*
Family. *Oxalobacteraceae*
Genus. *Herbaspirillum*
Family. *Neisseriaceae*
Genus. *Eikenella*
Genus. *Kingella*
Genus. *Neisseria*
Family. *Sprillaceae*
Genus. Genera
Genus. *Sprillium*
Class. Epsilonproteobacteria
Family. *Campylobacteraceae*
Genus. *Arcobacter*
Genus. *Campylobacter*
Family. *Helicobacteraceae*
Genus. *Helicobacter*
Class. Gammaproteobacteria
Family. *Enterobacteriaceae*
Genus. *Cedecea*
Genus. *Citrobacter*
Genus. *Cronobacter*
Genus. *Edwardsiella*
Genus. *Enterobacter*
Genus. *Erwinia*
Genus. *Escherichia*

Genus. *Hafnia*
Genus. *Klebsiella*
Genus. *Kluybera*
Genus. *Leclercia*
Genus. *Morganella*
Genus. *Pantoea*
Genus. *Plesiomonas*
Genus. *Proteus*
Genus. *Providencia*
Genus. *Raoltella*
Genus. *Salmonella*
Genus. *Serratia*
Genus. *Shigella*
Genus. *Yersinia*
Genus. *Yokenella*
Family. *Vibrionaceae*
Genus. *Vibrio*
Family. *Aeromonadaceae*
Genus. *Aeromonas*
Family. *Shewanellaceae*
Genus. *Shewanella*
Family. *Cardiobacteriaceae*
Genus. *Cardiobacterium*
Genus. *Suttonella*
Family. *Coxiellaceae*
Genus. *Coxiella*
Family. *Legionellaceae*
Genus. *Legionella*
Family. *Pasteurellaceae*
Genus. *Actinobacillus*
Genus. *Aggregatibacter*
Genus. *Haemophilus*
Genus. *Pasteurella*
Family. *Moraxelaceae*
Genus. *Acinetobacter*
Genus. *Branhamella*
Genus. *Moraxella*
Family. *Pseudomonadaceae*
Genus. *Chryseomonas*
Genus. *Flavimonas*
Genus. *Pseudomonas*
Family. *Francisellaceae*
Genus. *Francisella*
Class. Spirochaetes
Family. *Brachyspiraceae*
Genus. *Brachyspira*

Family. *Leptospiraceae*
Genus. *Leptospira*
Family. *Borreliaceae*
Genus. *Borrelia*
Family. *Spirochaetaceae*
Genus. *Treponema*
Class. Mollicutes
Family. *Mycoplasmataceae*
Genus. *Mycoplasma*
Genus. *Ureaplasma*

Taxonomic Classification of Human Viruses

The taxonomy of viral classification is arranged in the following way. . .

Order
Family
Subfamily
Genus
Species

Practically speaking, most clinical microbiologists organize viruses in terms of genome structure, family, and genus, and only rarely are the subfamily or species designations utilized. As such, the following taxonomic structure is presented in terms of what would be most useful to the practicing clinical microbiologist.

Single-stranded, nonenveloped DNA viruses
Family. *Parvoviridae*
Genus. *Erythrovirus*
Species. Human parvovirus B19 virus

Double-stranded, nonenveloped DNA viruses
Family. *Polyomaviridae*
Genus. *Polyomavirus*
Species. JC polyomavirus, BK polyomavirus
Family. *Papillomaviridae*
Genus. *Papillomavirus*
Species. Human papillomavirus
Family. *Adenoviridae*
Genus. *Mastadenovirus*
Species. Human adenoviruses (species A to G)

Double-stranded, enveloped DNA viruses
Family. *Poxviridae*
Genus. *Orthopoxvirus*

Species. Vaccinia virus, variola virus smallpox virus, cowpox virus, monkeypox virus
Genus. *Molluscipoxvirus*
Species. Molluscum contagiosum virus
Genus. *Parapoxvirus*
Species. Orf virus
Family. *Hepadnaviridae*
Genus. *Orthohepadnavirus*
Species. Hepatitis B virus
Family. *Herpesviridae*
Genus. *Simplexvirus*
Species. Human herpesvirus 1 (herpes simplex virus type 1; HHV-1), human herpesvirus 2 (herpes simplex virus type 2; HHV-2)
Genus. *Varicellovirus*
Species. Human herpesvirus 3 (varicella-zoster virus [VZV]; HHV-3)
Genus. *Lymphocryptovirus*
Species. Human herpesvirus 4 (Epstein-Barr virus [EBV]; HHV-4)
Genus. *Cytomegalovirus*
Species. Human herpesvirus 5 (CMV; HHV-5)
Genus. *Roseolovirus*
Species. Human herpesvirus 6 (roseola virus; HHV-6), human herpesvirus 7 (HHV-7)
Genus. *Rhadinovirus*
Species. Human herpesvirus 8 (HHV-8)

Single-stranded, positive-sense, nonenveloped RNA viruses
Family. *Picornaviridae*
Genus. *Enterovirus*
Species. *Enterovirus A* (human coxsackievirus A2, human enterovirus 71) *Enterovirus B* (human coxsackievirus B1, human echovirus), *Enterovirus C* (human poliovirus 1 to 3, human coxsackievirus A1), *Enterovirus D* (human enterovirus 68, 70, and 94), *Rhinovirus A*, *B*, and C.
Genus. *Aphthovirus*
Species. Foot-and-mouth disease virus
Genus. *Hepatovirus*
Species. Human hepatitis A virus (HHAV)
Family. *Caliciviridae*
Genus. *Norovirus*
Species. Norwalk virus
Genus. *Sapovirus*
Species. Sapporo virus
Family. *Astroviridae*

Genus. *Astrovirus*
Species. Human astrovirus

Single-stranded, positive-sense, enveloped RNA viruses
Family. *Coronaviridae*
Genus. *Coronavirus*
Species. Human coronavirus, Severe acute respiratory syndrome (SARS) virus, Middle eastern respiratory syndrome (MERS) virus
Genus. *Torovirus*
Species. Human torovirus
Family. *Togaviridae*
Genus. *Alphavirus*
Species. Sindbis virus, Eastern equine encephalitis (EEE) virus, Western equine encephalitis (WEE) virus, Venezuelan equine encephalitis (VEE) virus, Chickungunya virus, many other viruses
Genus. *Rubivirus*
Species. Rubella virus
Family. *Flaviviridae*
Genus. *Flavivirus*
Species. Yellow fever virus, West Nile virus, St. Louis encephalitis (SLE) virus, Japanese encephalitis (JE) virus, Dengue virus (types 1 through 4), Zika virus, many other viruses
Genus. *Hepacivirus*
Species. Hepatitis C virus (HCV)

Single-stranded, negative-sense, enveloped RNA viruses
Family. *Rhabdoviridae*
Genus. *Lyssavirus*
Species. Rabies virus
Family. *Filoviridae*
Genus. "Marburg-like viruses"
Species. Marburg virus
Genus. "Ebola-like viruses"
Species. *Ebola* virus
Family. *Orthomyxoviridae*
Genus. *Influenzavirus* A
Species. Influenza A virus
Genus. *Influenzavirus* B
Species. Influenza B virus
Genus. *Influenzavirus* C
Species. Influenza C virus
Family. *Paramyxoviridae*
Genus. *Respirovirus*

Species. Sendai virus, Human parainfluenza virus (types 1 and 3)
Genus. *Rubulavirus*
Species. Mumps virus, Human parainfluenza virus (types 2 and 4)
Genus. *Morbillivirus*
Species. Measles virus
Genus. *Henipavirus*
Species. Hendra virus, Nipah virus
Genus. *Pneumovirus*
Species. Human respiratory syncytial virus (RSV)
Genus. *Metapneumovirus*
Species. human metapneumovirus
Family. *Bunyaviridae*
Genus. *Orthobunyavirus*
Species. Bunyamwera virus, California encephalitis virus, La Crosse virus, many other viruses
Genus. *Hantavirus*
Species. Hantaan virus, Sin Nombre virus, other viruses
Genus. *Nairovirus*
Species. Crimean-Congo hemorrhagic fever virus (CCFV), other viruses
Genus. *Phlebovirus*
Species. Rift Valley fever virus, other viruses
Family. *Arenaviridae*
Genus. *Arenavirus*
Species. Lymphocytic choriomeningitis (LCM) virus, Lassa virus, Junin virus, Machupo virus, Sabia virus, other viruses

Double-stranded, enveloped RNA viruses
Family. *Retroviridae*
Genus. *Deltaretrovirus*
Species. Human T-lymphotropic virus type 1 (HTLV-1), human T-lymphotropic virus type 2 (HTLV-2)
Genus. *Lentivirus*
Species. Human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2)
Family. *Reoviridae*
Genus. *Rotavirus*
Species. Rotavirus (types A, B, and C)
Genus. *Coltivirus*
Species. Colorado tick fever virus

Taxonomic Classification of Fungi

The taxonomic classification of fungal organisms is complex because fungi can be classified by different methods. The phylogentic taxonomy for fungi is represented in this chapter and is subject to the International Code of Nomenclature (ICN) for algae, fungi, and plants (http://www.iapt-taxon.org). This organization was formally known as the International Code of Botanical Nomenclature (ICBN).

Fungi are divided into four divisions (phylum or subphylum): Mucormycotina, Entomophthoromycotina, Ascomycota, and Basidiomycota. The Protozoa and Chromista kingdoms include some members that possess a fungus-like appearance and are clinically relevant, such as *Rhinosporidium* and *Pythium*.

The taxonomy of fungal classification is arranged in the following way. . .

Phylum
Subphylum
Class
Order
Family
Genus
Species

Since the last writing of this Pocket Guide, a significant change has occurred in the world of fungal taxonomy. As of January 1, 2013, only one name will be used to identify fungi, and the "correct" name will be that which was first identified. Why has this occurred? Most scientists agree that the convention of having multiple names for fungi to represent the differing states of a fungus is no longer necessary with the use of DNA sequence analyses. The multi-name convention is confusing, and is especially so for the clinical microbiologist who is trying to communicate understandable and actionable information to those caring for patients.

Because fungal taxonomic naming conventions are changing more quickly than for bacteria, parasites, and viruses, publishing an extensive list of fungal taxonomy would be rendered inaccurate as soon as this Pocket Guide was published. In fact, many of the names that would be included as of the writing of this book will likely cease to exist.

Taxonomic Classification of Parasites[a]

The term "parasite" refers to a group of eukaryotic organisms, about 200 of which are medically relevant helminths, and 80 of which are medically relevant protozoan species. Within this subset of nearly

300 parasites, about 100 species are commonly found in humans, and an even smaller number within that cause a disproportionate number of important diseases. Presented in the following table are the taxonomic classifications of some of the most important human parasites.

Since the last edition of this Pocket Guide was published, some significant taxonomic changes have occurred. The two most clinically relevant changes relate to the Microsporidia and *Blastocystis hominis.* Due to recent genome-wide analyses, Microsporidia now belong to the kingdom Fungi. The taxonomy of *Blastocystis hominis* has been controversial, and it has previously been considered a fungus and also as protozoa. Recent genome analyses suggest it is most closely related to *Proteromonas,* though this organism is a flagellate and *B. hominis* does possess a flagellum and is not motile. It is now part of the kingdom Chromista rather than the Protozoa.

Kingdom. Protozoa

- Phylum. Metamonada (flagellates)
  - Class. Trepomonadea (intestinal flagellates)
    - Order. Diplomonadida
      - Genus. *Giardia duodenalis*
  - Class. Retortamonadea (intestinal flagellates)
    - Order. Retortamonadida
      - Genus. *Chilomastix mesnili,*
      - Genus. *Retortamonas intestinalis*
  - Class. Trichomonadea (intestinal and related flagellates)
    - Order. Trichomonadida
      - Genus. *Dientamoeba fragilis*
      - Genus. *Trichomonas vaginalis*
      - Genus. *Trichomonas tenax*
      - Genus. *Pentatrichomonas hominis*
- Phylum. Percolozoa
  - Class. Heterolobosea (flagellated amoebae)
    - Order. Schizopyrenida
      - Genus. *Naegleria fowleri*
- Phylum. Euglenozoa
  - Class. Kinetoplastea (blood and tissue flagellates)
    - Order. Trypanosomatida
      - Genus. *Leishmania Donovan*
      - Genus. *Leishmania infantum* (= *L. chagasi*)
      - Genus. *Leishmania major*
      - Genus. *Leishmania tropica*
      - Genus. *Leishmania braziliensis*
      - Genus. *Leishmania mexicana*
      - Genus. *Trypanosoma cruzi*
      - Genus. *Trypanosoma brucei gambiense*

Genus. *Trypanosoma brucei rhodesiense*
Genus. *Trypanosoma rangeli*
Phylum. Amoebozoa
Class. Amoebaea
Order. Acanthopodida
Genus. *Acanthamoeba* spp.
Genus. *Balamuthia mandrillaris*
Class. Archamoebea (intestinal amoebae)
Order. Euamoebida
Genus. *Entamoeba histolytica*
Genus. *Entamoeba coli*
Genus. *Entamoeba dispar*
Genus. *Entamoeba hartmanni*
Genus. *Entamoeba gingivalis*
Genus. *Entamoeba polecki*
Genus. *Endolimax nana*
Genus. *Iodamoeba buetschlii*
Phylum. Sporozoa (sporozoans)
Class. Coccidea
Order. Eimeriida
Genus. *Cryptosporidium parvum*
Genus. *Toxoplasma gondii*
Genus. *Cyclospora cayetanensis*
Genus. *Cystoisospora* (*Isospora*) *belli*
Genus. *Sarcocystis hominis*
Order. Piroplasmida
Genus. *Babesia microti*
Genus. *Babesia divergens*
Genus. *Babesia gibsoni*
Order. Haemosporida
Genus. *Plasmodium falciparum*
Genus. *Plasmodium malariae*
Genus. *Plasmodium ovale*
Genus. *Plasmodium vivax*
Genus. *Plasmodium knowlesi*
Phylum. Ciliophora (ciliates)
Class. Litostomatea
Order. Trichostomatia
Genus. *Balantidium coli*

Kingdom. Chromista
Phylum. Bigyra
Class. Blastocystea
Genus. *Blastocystis hominis*

Kingdom. Animalia
Phylum. Nemathelminthes (Nematodes, Roundworms)

Class. Adenophorea (Asphasmidea)
Family. Trichinellidae (Trichuridae)
Genus. *Trichinella spiralis*
Genus. *Trichuris trichiura*
Genus. *Capillaria* spp.
Class. Secernentea (Phasmidea)
Family. Ancylostomatidae
Genus. *Ancylostoma duodenale*
Genus. *Necator americanus*
Family. Angiostrongylidae
Genus. *Parastrongylus* (*Angiostrongylus*) *cantonensis*
Genus. *Parastrongylus* (*Angiostrongylus*) *costaricensis*
Family. Ascarididae
Genus. *Ascaris lumbrioides*
Genus. *Toxocara canis*
Genus. *Toxocara cati*
Genus. *Bayliascaris procyonis*
Family. Dracunculidae
Genus. *Dracunculus medinensis*
Family. Onchocercidae
Genus. *Brugia malayi*
Genus. *Loa loa*
Genus. *Wuchereria bancrofii*
Genus. *Onchocerca volvulus*
Genus. *Brugia timori*
Genus. *Dirofilaria immitis*
Genus. *Mansonella ozzardi*
Genus. *Mansonella perstans*
Family. Oxyuridae
Genus. *Enterobius vermicularis*
Family. Strongyloididae
Genus. *Strongyloides stercoralis*
Genus. *Strongyloides fuelleborni*
Family. Gnathostomatidae
Genus. *Gnathostoma spinigerum*

Phylum. Platyhelminthes
Class. Trematoda (flukes)
Order. Diplostomida
Family. Schistosomatidae
Genus. *Schistosoma haematobium*
Genus. *Schistosoma japonicum*
Genus. *Schistosoma mansoni*
Genus. *Schistosoma mekongi*
Genus. *Schistosoma intercalatum*

Order. Plagiorchiida
Family. Fasciolidae
Genus. *Fasciola hepatica*
Genus. *Fasciola gigantica*
Genus. *Fasciolopsis buski*
Family. Heterophyidae
Genus. *Heterophyes heterophyes*
Family. Opisthorchidae
Genus. *Clonorchis sinensis*
Genus. *Opisthorchis felineus*
Genus. *Opisthorchis viverrini*
Family. Paragonimidae
Genus. *Paragonimus westermani*
Genus. *Paragonimus kellicotti*
Family. Paragonimidae
Genus. *Dicrocoelium dentriticum*
Class. Cestoda (tapeworms)
Order. Pseudophyllidea
Family. Diphyllobothriidae
Genus. *Diphyllobothrium latum*
Order. Cyclophyllidea
Family. Dipylidiidae
Genus. *Dipylidium caninum*
Family. Hymenolepididae
Genus. *Hymenolepis nana*
Genus. *Hymenolepis diminuta*
Family. Taeniidae
Genus. *Taenia saginata*
Genus. *Taenia solium*
Genus. *Echinococcus granulosus*
Genus. *Echinococcus multilocularis*

[a]Adapted from J. H. Jorgensen, M. A. Pfaller, K. C. Carroll, G. Funke, M. L. Landry, S. S. Richter, D. W. Warnock (ed.), *Manual of Clinical Microbiology*, 11th ed., ASM Press, Washington, D.C., 2015.

SECTION 2

Indigenous and Pathogenic Microbes of Humans

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The relationship between humans and microbes can be defined in one of three ways: 1) transient colonization, 2) persistent colonization, 3) or pathogenic infection. The majority of organisms are unable to establish permanent colonization/infection on the skin or mucosal surfaces and are considered an insignificant finding when recovered in clinical specimens. Examples include the molds and many of the nonfermentative Gram-negative bacilli that can be isolated in soil, vegetation, water, and food products. These organisms are unable to compete with the normal microbial population of the body or cannot survive on the skin surface.

Other organisms are able to establish long-term residency on or in the human body. The successes of these interactions are influenced by complex microbial and host factors (e.g., favorable environment [pH, atmosphere, moisture, available nutrients], ability to adhere to surfaces, resistance to bacteriocins, antibiotics, and phagocytic cells). These microbes generally exist in a symbiotic relationship with their human host and produce disease only when they invade normally sterile body sites such as tissues and body fluids. Table 2.1 is a listing of the organisms most commonly recovered from the body surfaces of healthy individuals. This table is intended to serve as an interpretive guideline for cultured specimens. It should be remembered that many organisms cannot be detected when present in a mixed population (typical of many body sites). With the emergence of next generation sequencing microbiome experimentation, our understanding of the human microbiome has expanded significantly and demonstrated a greater diversity of commensal organisms than had been previously appreciated.

Additionally, recent advances in diagnostic technology have changed the practice of clinical microbiology forever. In particular, the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has allowed microbiologists to identify organisms rapidly and with more accuracy than ever before. Consequently, the microbiologist must understand a greater depth of organisms than ever before, and it remains critical that only those organisms which are clinically significant be reported. MALDI-TOF MS is a powerful tool that has greatly improved our ability to diagnose infectious diseases. However, if used incorrectly, MALDI-TOF MS can lead to misdiagnoses, provider confusion, and unnecessary antimicrobial therapy.

The quantitative and qualitative presence of specific microbes will also vary with the individual host, including dramatic changes in the indigenous flora in hospitalized patients. Thus, only qualitative data (presence or absence of the organisms) are presented. Data for viruses are not listed because replication of viruses generally is associated with host tissue destruction or an immunologic response (although this can range from a clinically asymptomatic infection to host death).

Most diseases in humans are caused by infections with endogenous bacteria and yeasts or exposure to opportunistic molds, parasites, and viruses. However, some interactions between microbes and humans commonly lead to disease. The most common microbes responsible for human disease are summarized in this section.

Selected pathogens are monitored routinely, with all clinical laboratories required to report specific organisms or diseases to their state public health department. This group of organisms and the diseases associated with them are reported weekly in *Morbidity and Mortality Weekly Report*. Data for 2015 are summarized in this section. In addition, the Department of Health and Human Services (HHS) and the U.S. Department of Agriculture (USDA) have published a list of select agents and toxins. This list is presented in this section and can be found at this website (https://www.selectagents.gov/selectagentsandtoxinslist.html).

Arthropods, parasites in their own right, can also serve as vectors for human disease. A listing of the most common arthropod vectors and their associated diseases is included in Table 2.2. Tables 2.3 and 2.4 are listings of fungi and parasites isolated from humans and their geographic distribution. For additional information about indigenous and pathogenic microbes, please consult the reference texts listed in the Bibliography.

Table 2.1 Human indigenous flora[a]

Organism	Prevalence of carriage in[b]: Resp tract	GI tract	GU tract	Skin, ear, and eye
Abiotrophia defectiva	+	0	0	0
Acholeplasma laidlawii	+	0	0	0
Acidaminococcus fermentans	+	+	0	0
Acinetobacter baumannii	0	0	0	+
Acinetobacter junii	+	0	0	+
Acinetobacter lwoffii	+	0	0	+
Acinetobacter radioresistens	0	0	0	+
Actinomyces spp.	+	+	+	+
Aggregatibacter actinomycetemcomitans	+	0	0	0
Aerococcus christensenii	0	0	+	0
Aerococcus viridans	0	0	0	+
Aerococcus urinae	0	0	+	0
Aeromonas spp.	0	+	0	0
Alistipes spp.	+	+	+	+
Alloiococcus otitis	0	0	0	+
Anaerococcus hydrogenalis	0	+	+	+
Anaerococcus lactolyticus	0	+	+	0
Anaerococcus prevotii	0	+	+	0
Anaerorhabdus forcosus	0	+	0	0
Arcanobacterium spp.	+	0	0	+
Atopobium spp.	+	0	0	0
Bacillus spp.	0	+	0	+
Bacteroides caccae	+	+	+	+

Bacteroides distasonis	0	+	0	0
Bacteroides eggerthii	0	+	0	0
Bacteroides fragilis	+	+	+	0
Bacteroides merdae	0	+	0	0
Bacteroides ovatus	0	+	0	0
Bacteroides splanchnicus	0	+	0	0
Bacteroides thetaiotaomicron	+	+	0	+
Bacteroides vulgatus	+	+	+	+
Bifidobacterium adolescentis	0	+	0	0
Bifidobacterium bifidum	0	+	+	0
Bifidobacterium breve	0	+	+	0
Bifidobacterium catenulatum	0	+	+	0
Bifidobacterium dentium	+	+	+	0
Bifidobacterium longum	0	+	+	0
Bilophila wadsworthia	+	+	+	0
Blastocystis hominis	0	+	0	0
Blastoschizomyces capitatus	0	0	0	+
Brevibacterium casei	0	0	0	+
Brevibacterium epidermidis	0	0	0	+
Burkholderia cepacia complex	+	0	+	+
Butyrivibrio fibrisolvens	0	+	0	0
Campylobacter concisus	+	+	0	+
Campylobacter curvus	+	+	0	0
Campylobacter gracilis	+	+	0	0
Campylobacter jejuni	0	+	0	0

(continued)

Table 2.1 Human indigenous flora[a] *(continued)*

	Prevalence of carriage in[b]:			
Organism	**Resp tract**	**GI tract**	**GU tract**	**Skin, ear, and eye**
Campylobacter rectus	+	+	0	+
Campylobacter showae	+	+	0	+
Candida albicans	+	+	+	+
Candida glabrata	+	+	+	+
Candida guilliermondii	+	+	+	+
Candida kefyr	+	+	+	+
Candida krusei	+	+	+	+
Candida lusitaniae	+	+	+	+
Candida parapsilosis	+	+	+	+
Candida tropicalis	+	+	+	+
Capnocytophaga gingivalis	+	0	+	+
Capnocytophage granulosum	+	0	0	0
Capnocytophaga haemolytica	+	0	0	0
Capnocytophaga ochracea	+	+	+	+
Capnocytophaga sputigena	+	0	0	+
Cardiobacterium hominis	+	0	0	0
Cantipeda periodontii	+	0	0	0
Chilomastix mesnili	0	+	0	0
Citrobacter freundii	0	+	0	0
Citrobacter koseri	0	+	0	0
Clostridium spp.	0	+	0	0
Corynebacterium accolens	+	0	0	+
Corynebacterium afermentans	+	0	0	+

Corynebacterium amycolatum	0	0	0	+
Corynebacterium auris	0	0	0	+
Corynebacterium diphtheriae	+	0	0	+
Corynebacterium duram	+	0	0	0
Corynebacterium glucuronolyticum	0	0	+	0
Corynebacterium jeikeium	0	0	0	+
Corynebacterium kroppenstedtii	0	0	0	+
Corynebacterium macginleyi	0	0	0	+
Corynebacterium matruchotii	+	0	0	0
Corynebacterium minutissimum	0	0	0	+
Corynebacterium propinquum	+	0	0	0
Corynebacterium pseudodiphtheriticum	+	0	0	0
Corynebacterium riegelii	0	0	+	0
Corynebacterium simulans	0	0	0	+
Corynebacterium striatum	+	0	0	+
Corynebacterium ulcerans	+	0	0	0
Corynebacterium urealyticum	0	0	+	+
Cryptococcus albidus	+	0	0	0
Dermabacter hominis	0	0	0	+
Dermacoccus nishinomiyaensis	0	0	0	+
Desulfomonas pigra	0	+	0	0
Dysgonomonas spp.	0	+	0	0
Eggerthella lenta	0	+	0	+
Eikenella corrodens	+	+	0	+
Endolimax nana	0	+	0	0

(continued)

Table 2.1 Human indigenous flora[a] *(continued)*

	Prevalence of carriage in[b]:			
Organism	**Resp tract**	**GI tract**	**GU tract**	**Skin, ear, and eye**
Entamoeba coli	0	+	0	0
Entamoeba gingivalis	+	0	0	0
Entamoeba hartmanni	0	+	0	0
Enterobacter spp.	0	+	0	0
Enterococcus faecalis	+	+	+	+
Enterococcus faecium	0	+	0	**0**
Enterococcus gallinarum	0	+	0	**0**
Epidermophyton floccosum	0	0	0	+
Escherichia coli	+	+	+	+
Escherichia fergusonii	0	+	0	0
Escherichia hermanii	0	+	0	0
Escherichia vulneris	0	+	0	0
Eubacterium spp.	+	+	0	0
Ewingella americana	+	0	0	0
Finegoldia magnus	0	+	+	+
Fusobacterium alocis	+	0	0	0
Fusobacterium gonidiaformans	0	+	+	0
Fusobacterium mortiferum	0	+	0	0
Fusobacterium naviforme	0	+	+	0
Fusobacterium necrophorum	+	+	0	0
Fusobacterium nucleatum	+	0	+	+
Fusobacterium periodonticum	+	0	0	0
Fusobacterium russii	0	+	0	0

Fusobacterium sulci	+	0	0	0
Fusobacterium varium	0	+	0	0
Gardnerella vaginalis	+	+	+	+
Gemella haemolysans	+	+	0	+
Gemella morbillum	+	+	0	+
Geotrichum spp.	+	+	+	+
Granulicatella spp.	+	0	0	0
Haemophilus spp.	+	+	0	+
Hafnia alvei	0	+	0	0
Helcococcus kunzii	0	0	0	+
Helicobacter spp.	0	+	+	0
Kingella spp.	+	0	0	0
Klebsiella spp.	+	+	0	+
Kocuria spp.	0	0	0	+
Kytococcus sedantarius	0	0	0	+
Lactobacillus acidophilus	+	+	+	0
Lactobacillus breve	+	0	0	0
Lactobacillus casei	+	0	+	0
Lactobacillus cellobiosus	0	0	+	0
Lactobacillus fermentum	+	+	+	0
Lactobacillus reuteri	0	+	0	0
Lactobacillus salivarius	+	+	0	0
Lactococcus spp.	0	0	+	0
Leclercia adeocarboxylata	0	+	0	0
Leminorella spp.	0	0	+	0

(continued)

Table 2.1 Human indigenous flora[a] *(continued)*

	Prevalence of carriage in[b]:			
Organism	**Resp tract**	**GI tract**	**GU tract**	**Skin, ear, and eye**
Leptotrichia bucalis	+	0	0	+
Leuconostoc spp.	0	0	+	0
Listeria monocytogenes	+	+	0	+
Malassezia spp.	0	0	0	+
Megasphaera elsdenii	0	+	0	0
Micrococcus luteus	+	0	0	+
Micrococcus lylae	+	0	0	+
Micromonas micros	+	0	0	0
Microsporum spp.	0	0	0	+
Mitsuokella multiacidus	0	+	0	0
Mobiluncus curtisii	0	+	+	0
Mobiluncus mulieris	0	+	+	0
Moellerella wisconsensis	0	+	0	0
Moraxella catarrhalis	+	0	0	0
Morganella morganii	0	+	0	0
Mycoplasma buccale	+	0	0	0
Mycoplasma faucium	+	0	0	0
Mycoplasma fermentans	+	0	+	0
Mycoplasma genitalium	+	0	+	0
Mycoplasma hominis	+	0	+	0
Mycoplasma lipophilum	+	0	0	0
Mycoplasma orale	+	0	0	0
Mycoplasma penetrans	0	0	+	0

Mycoplasma pneumoniae	+	0	0	0
Mycoplasma primatum	0	0	+	
Mycoplasma salivarium	+	0	0	0
Mycoplasma spermatophilum	0	0	+	0
Neisseria cinerea	+	0	0	0
Neisseria flavescens	+	0	0	0
Neisseria lactamica	+	0	0	0
Neisseria meningitidis	+	0	0	+
Neisseria mucosa	+	0	0	0
Neisseria polysaccharea	+	0	0	0
Neisseria sicca	+	0	+	+
Neisseria subflava	+	0	0	+
Oligella ureolytica	0	0	+	0
Oligella urethralis	0	0	+	0
Pantoea spp.	+	+	0	+
Pasteurella bettyae	0	0	+	0
Pasteurella multocida	+	0	0	0
Parabacteroides distasonis	+	+	+	0
Pentatrichomonas hominis	0	+	0	0
Peptococcus niger	0	0	+	+
Peptoniphilus asaccharolyticus	0	+	+	+
Peptoniphilus lacrimalis	+	0	0	0
Peptostreptococcus anaerobius	+	+	0	0
Peptostreptococcus productus	0	+	0	0
Peptostreptococcus vaginalis	0	0	+	+

(continued)

Table 2.1 Human indigenous flora[a] *(continued)*

	Prevalence of carriage in[b]:			
Organism	**Resp tract**	**GI tract**	**GU tract**	**Skin, ear, and eye**
Porphyromonas asaccharolytica	0	+	+	0
Porphyromonas catoniae	+	0	0	0
Porphyromonas endodontalis	+	0	0	0
Porphyromonas gingivalis	+	0	0	+
Prevotella bivia	0	0	+	0
Prevotella buccae	+	0	0	0
Prevotella buccalis	+	0	+	0
Prevotella corporis	+	0	0	0
Prevotella dentalis	+	0	0	0
Prevotella denticola	+	0	0	0
Prevotella disiens	0	0	+	0
Prevotella enoeca	+	0	0	0
Prevotella heparinolytica	+	0	0	0
Prevotella intennedia	+	0	0	0
Prevotella loescheii	+	0	+	0
Prevote/la melaninogenica	+	0	+	0
Prevotella nigrescens	+	0	0	0
Prevotella oralis	+	0	+	0
Prevotella oris	+	0	0	0
Prevotella oulorum	+	0	0	0
Prevotella tannerae	+	0	0	0
Prevotella veroralis	+	0	+	0
Prevotella zoogleoformans	+	0	0	0

Propionibacterium acnes	+	+	+	+
Propionibacterium avidum	0	0	0	+
Propionibacterium granulosum	0	0	0	+
Propionibacterium propionicum	+	0	0	0
Propionferax innocuum	0	0	0	+
Proteus mirabilis	0	+	+	+
Proteus penneri	0	+	+	0
Proteus vulgaris	0	+	+	0
Providencia rettgeri	0	+	0	0
Providencia stuartii	0	+	0	0
Pseudomonas aeruginosa	+	+	0	0
Retortamonas intestinalis	0	+	0	0
Rothia dentocariosa	+	0	0	0
Rothia mucilaginosa	+	+	+	+
Ruminococcus productus	0	+	0	0
Saccharomyces spp.	+	+	+	0
Selenomonas spp	+	0	0	0
Serratia liquefaciens	0	+	0	0
Serratia marcescens	0	+	0	0
Serratia odorifera	0	+	0	0
Staphylococcus aureus	+	+	+	+
Staphylococcus auricularis	0	0	0	+
Staphylococcus capitis	0	0	0	+
Staphylcoccus caprae	0	0	0	+
Staphylococcus epidermidis	+	+	+	+
Staphylococcus haemolyticus	0	0	+	+

(continued)

Table 2.1 Human indigenous flora[a] *(continued)*

	Prevalence of carriage in[b]:			
Organism	**Resp tract**	**GI tract**	**GU tract**	**Skin, ear, and eye**
Staphylococcus hominis	0	0	0	+
Staphylococcus lugdunensis	0	0	0	+
Staphylococcus pasteuri	0	0	0	+
Staphylococcus saccharolyticus	0	0	0	+
Staphylococcus saprophyticus	0	+	+	+
Staphylococcus simulans	0	0	0	+
Staphylococcus xylosus	0	0	0	+
Staphylococcus warneri	0	0	0	+
Streptobacillus moniliformis	+	0	0	0
Streptococcus agalactiae	0	+	+	+
Streptococcus anginosus	+	+	+	0
Streptococcus bovis	0	+	0	0
Streptococcus constellatus	+	+	+	0
Streptococcus cricetus	+	0	0	0
Streptococcus crista	+	0	0	0
Streptococcus dysdalactiae	+	+	0	0
Streptococcus equisimilis	+	0	0	0
Streptococcus gordonii	+	+	0	+
Streptococcus intermedius	+	+	+	0
Streptococcus mitis	+	+	+	+
Streptococcus mutans	+	+	0	0
Streptococcus oralis	+	0	0	0
Streptococcus parasanguis	+	0	0	0

Streptococcus pneumoniae	+	+	0	+
Streptococcus pyogenes	+	0	0	+
Streptococcus salivarius	+	0	0	0
Streptococcus sanguinis	+	+	0	+
Streptococcus sobrinus	+	0	0	0
Streptococcus vestibularis	+	0	0	0
Succinivibrio dextrinosolvens	0	+	0	0
Tissierella praeacuta	0	+	0	0
Treponema denticola	+	0	0	0
Treponema maltophilum	+	0	0	0
Treponema minutum	0	0	+	0
Treponema phagedenis	0	0	+	0
Treponema refringens	0	0	+	0
Treponema socranskii	+	0	0	0
Treponema vincentii	+	0	0	0
Trichomonas tenax	+	0	0	0
Trichophyton spp.	0	0	0	+
Trichosporon spp.	0	0	0	+
Turicella otitidis	0	0	0	+
Ureaplasma parvum	0	0	+	0
Ureaplasma urealyticum	+	0	+	0
Veillonella spp.	+	+	0	0
Weeksella virosa	0	0	+	0

[a]Adapted from P. R. Murray, Human microbiota, p. 295–306, *in* L. Collier, A. Balows, and M. Sussman (ed.), *Topley & Wilsons Microbiology and Microbial Infections,* 9th ed., Arnold, London, 1998, and Human Microbiome Project Consortium, *Nature*. 2012. Jun 13; 486:207–14.

[b]Resp, respiratory tract including nasopharynx and oropharynx; GI, gastrointestinal tract; GU, genitourinary tract; **+**, commonly present; 0, not typically isolated in healthy individuals.

BONE AND JOINT INFECTIONS

Arthritis

- Bacteria
  - *Neisseria gonorrhoeae*
  - *Staphylococcus aureus*
  - *Borrelia burgdorferi*
  - *Brucella* spp.
  - *Pseudomonas aeruginosa*
  - *Pasteurella multocida*
  - *Eikenella corrodens*
  - *Streptobacillus moniliformis*
  - *Mycoplasma hominis*
  - *Ureaplasma urealyticum*
  - *Mycobacterium marinum* (and other *Mycobacterium* spp.)
  - *Kingella kingae* (children)
- Viruses
  - Rubella virus
  - Hepatitis B virus
  - Mumps virus
  - Lymphocytic choriomeningitis virus
  - Parvovirus B19
  - Human immunodeficiency virus
  - Chickungunya
  - Dengue
- Fungi
  - *Sporothrix schenckii*
  - *Candida* spp.
  - *Coccidioides immitis*

Osteomyelitis

- Bacteria
  - *Staphylococcus aureus* (and other *Staphylococcus* spp.)
  - *Streptococcus*, beta-hemolytic groups
  - *Streptococcus pneumoniae*
  - *Escherichia coli*
  - *Salmonella* spp. (and other *Enterobacteriaceae*)
  - *Pseudomonas aeruginosa*
  - *Mycobacterium tuberculosis* (and other *Mycobacterium* spp.)
- Fungi
  - *Candida* spp.
  - *Aspergillus* spp.

Cryptococcus neoformans
Blastomyces dermatitidis
Coccidioides immitis

CARDIOVASCULAR INFECTIONS

Endocarditis

Bacteria
- *Staphylococcus aureus* (and other *Staphylococcus* spp.)
- *Streptococcus*, viridans group (primarily S. *mitis*, *S. oralis*, *S. sanguis*, and *S. mutans*)
- *Streptococcus bovis* group (especially *S. gallolyticus* subsp. *gallolyticus*)
- *Streptococcus pneumoniae*
- *Abiotrophia defectiva*
- *Granulicatella adiacens*
- *Rothia mucilaginosa*
- *Enterococcus* spp. (primarily *E. faecalis* and *E. faecium*)
- HACEK group organisms
  - *Haemophilus parainfluenzae*
  - *Aggregatibacter actinomycetemcomitans*
  - *Aggregatibacter aphrophilus* (formerly *Haemophilus aphrophilus*)
  - *Aggregatibacter paraphrolius*
  - *Cardiobacterium hominis*
  - *Eikenella corrodens*
  - *Kingella kingae*
- *Salmonella* spp.
- *Serratia* spp. (and other enteric Gram-negative rods)
- *Pseudomonas aeruginosa*
- *Brucella* spp.
- *Bartonella* spp. (primarily *B. henselae*)
- *Corynebacterium* spp. (primarily in damaged or prosthetic valves)
- *Erysipelothrix rhusiopathiae*
- *Coxiella burnetii*
- *Chlamydophila psittaci*

Fungi
- *Candida* spp. (*C. parapsilosis*, *C. albicans*, *C. tropicalis*, and others)
- *Aspergillus* spp.
- *Histoplasma capsulatum*

Myocarditis

- Bacteria
  - *Corynebacterium diphtheriae*
  - *Clostridium perfringens*
  - *Streptococcus pyogenes*
  - *Borrelia burgdorferi*
  - *Neisseria meningitidis*
  - *Staphylococcus aureus*
  - *Salmonella* spp.
  - *Mycoplasma pneumoniae*
  - *Chlamydophila* spp. (*C. pneumoniae* and *C. psittaci*)
  - *Rickettsia rickettsii*
  - *Orientia tsutsugamushi*
- Viruses
  - Coxsackievirus groups A and B
  - Echoviruses
  - Poliovirus
  - Mumps virus
  - Rubeola virus
  - Influenza A and B viruses
  - Herpesvirus group
  - Adenoviruses
  - Flaviviruses
  - Arenaviruses
- Fungi
  - *Aspergillus* spp.
  - *Candida* spp.
  - *Cryptococcus neoformans*
- Parasites
  - *Trypanosoma* spp.
  - *Trichinella spiralis*
  - *Toxoplasma gondii*

Pericarditis

- Bacteria
  - *Streptococcus pneumoniae*
  - *Staphylococcus aureus*
  - *Neisseria* spp. (primarily *N. meningitidis* and *N. gonorrhoeae*)
  - *Mycoplasma pneumoniae*
  - *Mycobacterium tuberculosis* (and other *Mycobacterium* spp.)
- Viruses
  - Coxsackievirus groups A and B
  - Echovirus
  - Adenovirus
  - Mumps virus

Influenza A and B viruses
Herpesvirus group

Fungi
Histoplasma capsulatum
Coccidioides immitis
Blastomyces dermatitidis
Cryptococcus neoformans
Candida spp.
Aspergillus spp.

Parasites
Toxoplasma gondii
Entamoeba histolytica
Schistosoma spp.

Sepsis

Bacteria
Staphylococcus aureus (and other *Staphylococcus* spp.)
Enterobacteriaceae (commonly: *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Proteus mirabilis, Serratia* spp., *Citrobacter* spp., *Salmonella* spp.)
Enterococcus spp. (primarily *E. faecalis* and *E. faecium*)
Streptococcus pneumoniae
Pseudomonas aeruginosa
Streptococcus, beta-hemolytic (primarily groups A, B, C, and F)
Streptococcus, viridans group
Acinetobacter spp.
Mycobacterium avium complex
Mycobacterium tuberculosis

Fungi
Candida albicans
Candida glabrata
Candida parapsilosis
Candida tropicalis
Candida krusei
Cryptococcus neoformans
Trichosporon spp.
Malassezia spp.
Histoplasma capsulatum
Fusarium spp.

Transfusion-associated sepsis

Bacteria
Yersinia enterocolitica
Staphylococcus, coagulase-negative spp.
Pseudomonas fluorescens/putida
Salmonella spp.
Serratia marcescens (and other *Enterobacteriaceae*)

Campylobacter jejuni
Treponema pallidum
Bacillus cereus
Borrelia spp.

Viruses
Hepatitis viruses (primarily types A, B, C, and D)
Cytomegalovirus
Epstein-Barr virus
Human immunodeficiency virus
Human T-cell leukemia virus
Parvovirus B19
Colorado tick fever virus

Parasites
Plasmodium spp.
Babesia microti
Toxoplasma gondii
Trypanosoma cruzi
Leishmania spp.

Suppurative thrombophlebitis

Bacteria
Staphylococcus aureus
Klebsiella (and other *Enterobacteriaceae*)
Pseudomonas aeruginosa
Enterococcus spp. (primarily *E. faecalis* and *E.faecium*)
Bacteroides fragilis group
Campylobacter fetus

Fungi
Candida spp.
Malassezia spp.

CENTRAL NERVOUS SYSTEM INFECTIONS

Acute meningitis

Bacteria
Escherichia coli (Neonates)
Streptococcus agalactiae (group B) (Neonates and elderly)
Streptococcus pneumoniae
Neisseria meningitidis
Listeria monocytogenes (Neonates and elderly)
Haemophilus influenzae (rare in post-vaccine era)
Other Gram-negative rods (e.g., *Klebsiella* and *Pseudomonas* spp.)
Staphylococcus aureus (and other *Staphylococcus* spp.) (shunts, neurosurgical procedures)

- *Propionibacterium acnes* (shunts, neurosurgical procedures)
- *Nocardia* spp.
- *Treponema pallidum*
- *Borrelia burgdorferi*
- *Leptospira* spp.
- *Mycobacterium tuberculosis*
- *Mycobacterium avium* complex (and other *Mycobacterium* spp.)
- *Rickettsia* spp.
- *Orientia tsutsugamushi*
- *Ehrlichia* spp.

Viruses

- Enteroviruses (echovirus and coxsackievirus groups A and B)
- Orbivirus (Colorado tick fever virus)
- Mumps virus
- Measles virus
- Adenovirus
- Herpes simplex virus
- Human immunodeficiency virus

Fungi

- *Cryptococcus neoformans* (HIV infected)
- *Cryptococcus gattii*
- *Histoplasma capsulatum*
- *Coccidioides immitis*
- *Candida* spp.

Parasites

- *Naegleria fowleri*
- *Bayliasciaris* spp.
- *Acanthamoeba* spp.
- *Angiostrongylus cantonensis*

Chronic meningitis

Bacteria

- *Brucella* spp.
- *Borrelia burgdorferi*
- *Treponema pallidum*
- *Mycobacterium tuberculosis* (and other *Mycobacterium* spp.)
- *Nocardia* spp.

Fungi

- *Coccidioides immitis*
- *Histoplasma capsulatum*
- *Cryptococcus neoformans*
- *Sporothrix schenckii*

- Parasites
  - *Acanthamoeba* spp.
  - *Angiostrongylus cantonensis*

Encephalitis

- Bacteria
  - *Listeria monocytogenes*
  - *Treponema pallidum*
  - *Leptospira* spp.
  - *Actinomyces* spp.
  - *Nocardia* spp.
  - *Borrelia* spp. (associated with Lyme disease and relapsing fever)
  - *Rickettsia rickettsii*
  - *Coxiella burnetii*
  - *Mycoplasma pneumoniae*
  - *Mycobacterium tuberculosis*
- Viruses
  - Enteroviruses (poliovirus, coxsackievirus, echovirus, and hepatitis A virus)
  - Herpesvirus group
  - Alphaviruses (Eastern, Western, and Venezuelan equine encephalitis viruses)
  - Flaviviruses (St. Louis encephalitis virus, West Nile virus, Japanese encephalitis virus, and dengue virus)
  - Bunyaviruses (La Crosse virus and Rift Valley virus)
  - Arenaviruses (lymphocytic choriomeningitis virus, Machupo virus, Lassa virus, and Junin virus)
  - Filoviruses (Ebola virus and Marburg virus)
  - Rabies virus
  - Human immunodeficiency virus
  - Mumps virus
  - Measles virus
  - Rubella virus
  - Adenovirus
- Fungi
  - *Cryptococcus neoformans*
  - *Histoplasma capsulatum*
- Parasites
  - *Naegleria fowleri*
  - *Acanthamoeba* spp.
  - *Toxoplasma gondii*
  - *Plasmodium falciparum*
  - *Trypanosoma* spp.

Brain abscess

Bacteria

Staphylococcus aureus
Enterobacteriaceae (*Proteus*, *Escherichia*, *Klebsiella*, and other spp.)
Pseudomonas aeruginosa
Streptococcus, viridans group (*S. anginosus* group)
Bacteroides spp. (and other anaerobic Gram-negative rods)
Peptostreptococcus spp. (and other anaerobic Gram-positive cocci)
Actinomyces spp.
Clostridium spp.
Listeria monocytogenes
Nocardia spp.
Rhodococcus equi
Mycobacterium tuberculosis
Nocardia spp.

Fungi

Cryptococcus neoformans
Candida spp.
Coccidioides immitis
Aspergillus spp.
Mucorales
Cladiophialophora spp.
Scedosporium spp.
Exophiala spp.

Parasites

Acanthamoeba spp.
Toxoplasma gondii

EAR INFECTIONS

Otitis externa

Bacteria

Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes

Fungi

Aspergillus spp. (primarily *A. fumigatus* and *A. niger*)
Candida albicans
Pseudallescheria boydii
Malassezia spp.

Otitis media

Bacteria

Streptococcus pneumoniae
Haemophilus influenzae

- *Moraxella catarrhalis*
- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Turicella otitidis* (controversial—look for pure/predominant growth)
- Mixed anaerobes

Viruses
- Respiratory syncytial virus
- Influenza virus
- Enterovirus
- Rhinovirus

EYE INFECTIONS

Conjunctivitis

Bacteria
- *Streptococcus pneumoniae*
- *Streptococcus agalactiae*
- *Streptococcus*, viridans group
- *Staphylococcus aureus*
- *Moraxella catarrhalis*
- *Haemophilus aegyptius*
- *Neisseria gonorrhoeae*
- *Pseudomonas aeruginosa*
- *Corynebacterium diphtheriae*
- *Corynebacterium macginleyi*
- *Francisella tularensis*
- *Borrelia burgdorferi*
- *Bartonella henselae*
- *Chlamydia trachomatis*

Viruses
- Adenovirus
- Herpesvirus group
- Papillomavirus
- Rubella virus
- Influenza virus
- Measles virus

Fungi
- *Candida* spp.
- *Sporothrix schenckii*

Parasites
- *Onchocera volvulus*
- *Loa loa*
- *Wuchereria bancrofti*
- *Leishmania donovani*
- Microsporidia (most commonly *Encephalitozoon* spp.)
- *Toxocara canis*

Endophthalmitis
- Bacteria
  - *Staphylococcus aureus* (and other *Staphylococcus* spp.)
  - *Pseudomonas aeruginosa*
  - *Propionibacterium* spp.
  - *Corynebacterium* spp.
  - *Bacillus cereus* (and other *Bacillus* spp.)
  - Rapidly growing mycobacteria (primarily *M. chelonae* and *M. abscessus*)
- Viruses
  - Herpesvirus group
  - Rubella virus
  - Measles virus
- Fungi
  - *Candida albicans* (and other *Candida* spp.)
  - *Aspergillus* spp.
  - *Histoplasma capsulatum*
  - Opportunistic fungi
- Parasites
  - *Toxoplasma gondii*
  - *Toxocara* spp.
  - *Cysticercus cellulosae*

Keratitis
- Bacteria
  - *Staphylococcus aureus* (and other *Staphylococcus* spp.)
  - *Streptococcus pneumoniae*
  - *Streptococcus pyogenes*
  - *Enterococcus faecalis*
  - *Pseudomonas aeruginosa*
  - *Proteus mirabilis* (and other enteric Gram-negative rods)
  - *Bacillus* spp. (primarily *B. cereus*)
  - *Clostridium per:fringens*
  - *Neisseria gonorrhoeae*
- Viruses
  - Herpesvirus group
  - Adenovirus
  - Measles virus
- Fungi
  - *Fusarium* spp.
  - *Aspergillus* spp.
  - *Candida* spp.
- Parasites
  - *Onchocerca volvulus*
  - *Acanthamoeba* spp.

Leishmania braziliensis
Trypanosoma spp.
Microsporidia (primarily *Nosema* and *Encephalitozoon* spp.)

GASTROINTESTINAL INFECTIONS

Esophagitis
- Viruses
  - Cytomegalovirus
  - Herpes simplex virus
  - Human immunodeficiency virus
- Fungi
  - *Candida albicans* (and other *Candida* spp.)

Noninflammatory diarrhea
- Bacteria
  - *Escherichia coli*
  - *Staphylococcus aureus*
  - *Bacillus cereus*
  - *Clostridium perfringens*
  - *Vibrio* spp. (primarily *V. cholerae* and *V. parahaemolyticus*)
- Viruses
  - Rotaviruses
  - Caliciviruses (Norovirus)
  - Adenoviruses
  - Astroviruses
  - Coronaviruses

Inflammatory diarrhea
- Bacteria
  - *Escherichia coli*
  - *Salmonella* spp.
  - *Shigella* spp.
  - *Campylobacter* spp.
  - *Clostridium difficile*
  - *Yersinia enterocolitica*
  - *Vibrio parahaemolyticus*
  - *Plesiomonas shigelloides*
  - *Edwardsiella tarda*
  - *Aeromonas* spp.
- Viruses
  - Adenoviruses
  - Cytomegalovirus
- Fungi
  - Mucorales
- Parasites
  - *Giardia duodenalis*

Entamoeba histolytica
Neobalantidium (*Balantidium*) *coli*
Cryptosporidium parvum
Cryptoisospora (*Isospora*) *belli*
Microsporidia
Cyclospora cayetanensis
Diphyllobothrium tatum
Trichinella spiralis
Strongyloides stercoralis
Schistosoma spp. (primarily *S. mansoni* and *S. japonicum*)

GENITAL INFECTIONS

Cervicitis
- Bacteria
  - *Neisseria gonorrhoeae*
  - *Neisseria meningitidis*
  - *Chlamydia trachomatis*
  - *Actinomyces* spp.
- Viruses
  - Herpes simplex virus
  - Cytomegalovirus
  - Adenovirus
  - Measles virus
  - Papillomavirus

Genital ulcers and skin nodules
- Bacteria
  - *Treponema pallidum*
  - *Haemophilus ducreyi*
  - *Chlamydia trachomatis*
  - *Klebsiella granulomatis*
  - *Mycobacterium ulcerans*
  - *Mycobacterium tuberculosis*
- Viruses
  - Herpes simplex virus
  - Molluscipoxviruses
- Fungi
  - *Histoplasma capsulatum*

Urethritis
- Bacteria
  - *Neisseria gonorrhoeae*
  - *Chlamydia trachomatis*
  - *Ureaplasma urealyticum*
  - *Mycoplasma genitalium*
- Parasites
  - *Trichomonas vaginalis*

Vaginitis

Bacteria
- *Mobiluncus* spp.
- *Gardnerella vaginalis*
- *Mycoplasma hominis*
- Absence of *Lactobacillus* spp.

Fungi
- *Candida* spp.

Parasites
- *Trichomonas vaginalis*

GRANULOMATOUS INFECTIONS

Bacteria
- *Brucella* spp.
- *Francisella tularensis*
- *Listeria monocytogenes*
- *Burkholderia pseudomallei*
- *Actinomyces* spp.
- *Bartonella henselae*
- *Tropheryma whippelii*
- *Mycobacterium* spp.
- *Chlamydia trachomatis*
- *Coxiella burnetii*
- *Treponema pallidum*
- *Nocardia* spp.
- *Corynebacterium kroppenstedtii* (granulomatous breast abscesses)

Viruses
- Cytomegalovirus
- Measles virus
- Mumps virus
- Epstein-Barr virus

Fungi
- *Cryptococcus neoformans*
- *Candida* spp.
- *Sporothrix schenckii*
- *Histoplasma capsulatum*
- *Paracoccidioides brasiliensis*
- *Coccidioides immitis*
- *Blastomyces dermatitidis*
- *Aspergillus* spp.
- *Phialophora* spp.
- *Exophiala* spp.
- *Fonsecaea* spp.
- *Taloromyces marneffei* (formerly *Penicillium*)
- *Pseudallescheria boydii*

Parasites
- *Leishmania* spp.
- *Toxoplasma gondii*
- *Schistosoma* spp.
- *Toxocara* spp.

INTRA-ABDOMINAL INFECTIONS

Peritonitis

Bacteria
- *Escherichia coli*
- *Klebsiella pneumoniae* (and other enteric Gram-negative rods)
- *Pseudomonas aeruginosa*
- *Streptococcus pneumoniae*
- *Streptococcus anginosus*
- *Staphylococcus aureus*
- *Enterococcus* spp.
- *Bacteroides fragilis* group (and other *Bacteroides* spp.)
- *Fusobacterium* spp.
- *Clostridium* spp.
- *Peptostreptococcus* spp. (and other anaerobic Gram-positive cocci)
- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis*
- *Mycobacterium tuberculosis*

Fungi
- *Candida albicans*

Parasites
- *Strongyloides stercoralis*

Dialysis-associated peritonitis

Bacteria
- *Staphylococcus aureus* (and other *Staphylococcus* spp.)
- *Streptococcus* spp.
- *Corynebacterium* spp.
- *Propionibacterium* spp.
- *Escherichia coli* (and other *Enterobacteriaceae*)
- *Pseudomonas aeruginosa*
- *Acinetobacter* spp.

Fungi
- *Candida albicans*
- *Candida parapsilosis* (and other *Candida* spp.)
- *Aspergillus* spp.
- *Fusarium* spp.
- *Exophiala* spp.

Visceral abscesses

- Bacteria
  - *Escherichia coli* (and other *Enterobacteriaceae*)
  - *Enterococcus* spp.
  - *Staphylococcus aureus*
  - *Bacteroides fragilis* group
  - *Fusobacterium* spp.
  - *Actinomyces* spp.
  - Mixed aerobes and anaerobes
  - *Yersinia enterocolitica*
  - *Streptococcus anginosus*
  - *Mycobacterium tuberculosis*
  - *Mycobacterium avium* complex (and other *Mycobacterium* spp.)
- Fungi
  - *Candida albicans* (and other *Candida* spp.)
- Parasites
  - *Entamoeba histolytica* (primarily hepatic abscesses)
  - *Echinococcus* (hepatic abscesses)

RESPIRATORY TRACT INFECTIONS

Pharyngitis

- Bacteria
  - *Streptococcus pyogenes*
  - *Streptococcus dysgalactiae* (groups C and G)
  - *Fusobacterium necrophorum*
  - *Arcanobacterium haemolyticum*
  - *Chlamydophila pneumoniae*
  - *Neisseria gonorrhoeae*
  - *Corynebacterium diphtheriae*
  - *Corynebacterium ulcerans*
  - *Mycoplasma pneumoniae*
  - *Yersinia enterocolitica*
  - *Treponema pallidum*
- Viruses
  - Respiratory syncytial virus
  - Rhinovirus
  - Coronavirus
  - Adenovirus
  - Herpes simplex virus
  - Parainfluenza virus
  - Influenza virus
  - Coxsackievirus A
  - Epstein-Barr virus

- Cytomegalovirus
- Human immunodeficiency virus

Laryngitis
- Bacteria
  - *Mycoplasma pneumoniae*
  - *Chlamydophila pneumoniae*
  - *Streptococcus pyogenes*
- Viruses
  - Rhinovirus
  - Influenza virus
  - Parainfluenza virus
  - Adenovirus
  - Coronavirus

Laryngotracheobronchitis (croup)
- Bacteria
  - *Mycoplasma pneumoniae*
- Viruses
  - Parainfluenza virus
  - Influenza A and B viruses
  - Respiratory syncytial virus
  - Adenovirus
  - Rhinovirus
  - Enterovirus

Sinusitis
- Bacteria
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Moraxella catarrhalis*
  - Mixed anaerobes
  - *Staphylococcus aureus*
  - *Streptococcus pyogenes*
  - *Chlamydophila pneumoniae*
  - *Pseudomonas aeruginosa* (and other Gram-negative rods)
- Viruses
  - Rhinovirus
  - Influenza virus
  - Parainfluenza virus
  - Adenovirus
- Fungi
  - *Aspergillus* spp. (allergic sinusitis)
  - Hyphomycetes (allergic sinusitis)
  - Zygomycetes (invasive disease)

Bronchitis
- Bacteria
  - *Mycoplasma pneumoniae*

Chlamydophila pneumoniae
Bordetella pertussis
Moraxella catarrhalis
Haemophilus influenzae
Viruses
Rhinovirus
Coronavirus
Parainfluenza virus
Influenza virus
Respiratory syncytial virus
Adenovirus

Empyema
Bacteria
Staphylococcus aureus
Streptococcus pneumoniae
Streptococcus pyogenes
Bacteroides fragilis
Klebsiella pneumoniae (and other Gram-negative rods)
Actinomyces spp.
Nocardia spp.
Mycobacterium tuberculosis (and other *Mycobacterium* spp.)
Fungi
Aspergillus spp.

Community-acquired pneumonia
Bacteria
Streptococcus pneumoniae
Staphylococcus aureus
Klebsiella pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Neisseria meningitidis
Mycoplasma pneumoniae
Chlamydia trachomatis
Chlamydophila spp. (primarily *C. pneumoniae* and *C. psittaci*)
Pseudomonas aeruginosa
Legionella spp.
Bacteroides fragilis (and other anaerobes in mixed infections)
Nocardia spp.
Rhodococcus equi
Mycobacterium tuberculosis (and other *Mycobacterium* spp.)
Coxiella burnetii
Many other bacteria

- Viruses
  - Respiratory syncytial virus
  - Parainfluenza virus
  - Influenza virus
  - Adenovirus
  - Rhinovirus
  - Enteroviruses
  - Herpesviruses
  - Measles virus
- Fungi
  - *Pneumocystis jiroveci* (*carinii*)
  - *Cryptococcus neoformans*
  - *Cryptococcus gattii*
  - *Histoplasma capsulatum*
  - *Blastomyces dermatitidis*
  - *Coccidioides immitis*
  - *Paracoccidioides brasiliensis*
  - Zygomycetes (primarily *Rhizopus* and *Mucor* spp.)
- Parasites
  - *Ascaris lumbricoides*
  - *Strongyloides stercoralis*
  - *Toxoplasma gondii*
  - *Paragonimus* spp.

Hospital-acquired pneumonia

- Bacteria
  - *Streptococcus pneumoniae*
  - *Staphylococcus aureus*
  - *Haemophilus influenzae*
  - *Klebsiella pneumoniae*
  - *Enterobacter* spp.
  - *Escherichia coli*
  - *Serratia marcescens*
  - *Stenotrophomonas maltophilia*
  - *Acinetobacter* spp.
  - *Moraxella catarrhalis*
  - *Proteus mirabilis*
  - *Citrobacter* spp.
  - *Enterococcus* spp.
- Viruses
  - Cytomegalovirus
  - Respiratory syncytial virus
- Fungi
  - *Aspergillus fomigatus*
  - Mucorales (primarily *Rhizopus* and *Mucor* spp.)
- Parasites
  - *Toxoplasma gondii*

SKIN AND SOFT TISSUE INFECTIONS

Primary pyodermas

- Bacteria
  - *Staphylococcus aureus*
  - *Streptococcus pyogenes*
  - *Pseudomonas aeruginosa*
  - *Bacillus anthracis*
  - *Treponema pallidum*
  - *Haemophilus ducreyi*
  - *Francisella tularensis*
  - *Corynebacterium diphtheriae*
  - *Mycobacterium* spp. (primarily *M. ulcerans* and *M. marinum*)
- Fungi
  - *Candida* spp.
  - *Sporothrix schenckii*

Gangrenous cellulitis

- Bacteria
  - *Streptococcus pyogenes*
  - *Pseudomonas aeruginosa*
  - *Clostridium* spp. (primarily *C. perfringens*, *C. septicum*, *C. sordellii* and *C. novyi*)
  - *Vibrio vulnificus*
  - *Aeromonas hydrophila*
  - *Erysipelothris rhusiopathiae*
  - Mixed aerobes and anaerobes (e.g., *E. coli*, *Bacteroides* spp., and *Peptostreptococcus* spp.)
- Fungi
  - *Aspergillus* spp.
  - Zygomycetes (primarily *Rhizopus*, *Absidia*, and *Mucor* spp.)

Nodular lesions

- Bacteria
  - *Staphylococcus aureus*
  - *Nocardia* spp.
  - *Mycobacterium marinum*
  - *Bartonella* spp.
- Fungi
  - *Candida* spp.
  - *Sporothrix schenckii*
- Parasites
  - *Leishmania* spp.

Secondary skin infections

- Bacteria
  - *Staphylococcus aureus*
  - *Streptococcus pyogenes*

Pseudomonas aeruginosa
Enterobacter spp. (and other *Enterobacteriaceae*)
Anaerobic Gram-positive cocci
Pasteurella spp. (primarily *P. multocida* and *P. canis*)
Fungi
Candida spp.
Aspergillus spp.

Disseminated infections with cutaneous manifestations
Bacteria
Staphylococcus aureus
Streptococcus pyogenes
Neisseria spp. (primarily *N. meningitidis* and *N. gonorrhoeae*)
Pseudomonas aeruginosa
Salmonella enterica serovar Typhi
Listeria monocytogenes
Leptospira interrogans
Streptobacillus moniliformis
Burkholderia spp. (primarily *B. pseudomallei* and *B. mallei*)
Bartonella spp.
Mycobacterium tuberculosis (and other *Mycobacterium* spp.)
Nocardia spp.
Fungi
Candida spp.
Blastomyces dermatitidis
Aspergillus spp.
Coccidioides immitis
Fusarium spp.

URINARY TRACT INFECTIONS

Cystitis and pyelonephritis
Bacteria
Escherichia coli
Enterococcus spp. (primarily *E. faecalis* and *E. faecium*)
Proteus mirabilis
Klebsiella spp.
Pseudomonas aeruginosa
Corynebacterium urealyticum
Enterobacter spp.
Staphylococcus aureus
Staphylococcus saprophyticus (and other *Staphylococcus* spp.)
Streptococcus agalactiae (group B)

Aerococcus urinae
Aerococcus sanguinicola
Mycobacterium tuberculosis
Viruses
Adenovirus
Cytomegalovirus
BK virus
Fungi
Candida glabrata
Candida albicans (and other *Candida* spp.)
Parasites
Schistosoma haematobium

Renal calculi
Bacteria
Proteus spp.
Morganella morganii
Klebsiella pneumoniae
Corynebacterium urealyticum
Staphylococcus saprophyticus
Ureaplasma urealyticum

Prostatitis
Bacteria
Escherichia coli
Klebsiella spp.
Proteus mirabilis
Enterobacter spp.
Enterococcus spp.
Neisseria gonorrhoeae
Mycobacterium spp.
Fungi
Candida spp.
Cryptococcus neoformans

Summary of Notifiable Infectious Diseases: United States, 2015[a]

Bacterial (2015 totals in parentheses)
Anthrax (*Bacillus anthracis*) (2)
Botulism, food-borne (*Clostridium botulinum*) (37)
Botulism, infant (138)
Botulism, other (20)
Brucellosis (*Brucella* spp.) (126)
Campylobacteriosis (54,556)
Chancroid (*Haemophilus ducreyi*) (11)
Chlamydia (*Chlamydia trachomatis*) (1,526,658)
Cholera (*Vibrio cholerae*) (5)

Diphtheria (*Corynebacterium diphtheriae*) (0)
Ehrlichiosis and Anaplasmosis
Anaplasma phagocytophilum (3,656)
Ehrlichia chaffeensis (1,288)
Ehrlichia ewingii (14)
Gonorrhea (*Neisseria gonorrhoeae*) (395,216)
Haemophilus influenzae, invasive (4,138)
Serotype B (29)
Hemolytic uremic syndrome, post diarrheal
Hansen disease, leprosy (*Mycobacterium leprae*) (89)
Invasive pneumococcal disease (*Streptococcus pneumoniae*) (16,163)
Legionellosis (*Legionella* spp.) (6,079)
Leptospirosis (40)
Listeriosis (*Listeria monocytogenes*) (768)
Lyme disease (*Borrelia burgdorferi*) (38,069)
Meningococcal disease (*Neisseria meningitidis*) (372)
Serogroup B (111)
Pertussis (*Bordetella pertussis*) (20,762)
Plague (*Yersinia pestis*) (16)
Psittacosis (*Chlamydophila psittaci*) (4)
Q fever (*Coxiella burnetii*), acute (122)
Q fever (*Coxiella burnetii*), chronic (34)
Salmonellosis (*Salmonella* spp.) (55,108)
Shiga toxin-producing *Escherichia coli* (STEC) (7,059)
Shigellosis (*Shigella* spp.) (23,590)
Spotted fever rickettsiosis (*Rickettsia rickettsii*) (4,198)
Streptococcal toxic shock (*S. pyogenes*) (335)
Syphilis, all stages (*Treponema pallidum*) (74,702)
Syphilis, congenital (487)
Tetanus (*Clostridium tetani*) (29)
Toxic shock syndrome (other than streptococcal) (64)
Tuberculosis (*Mycobacterium tuberculosis*) (9,557)
Tularemia (*Francisella tularensis*) (314)
Typhoid fever (*Salmonella enterica* serovar Typhi) (367)
Vancomycin-intermediate *Staphylococcus aureus* (VISA) infection (183)
Vancomycin-resistant *Staphylococcus aureus* (VRSA) infection (3)
Vibriosis (any species of the family *Vibrionaceae,* other than toxigenic *Vibrio cholerae* 01 or 0139) (1,323)

(continued)

Summary of Notifiable Infectious Diseases: United States, 2015[a] (continued)

Viral

- Chickungunya virus disease
  - Neuroinvasive (4)
  - Nonneuroinvasive (892)
- Dengue virus infection
  - Dengue (929)
  - Dengue-like illness (16)
  - Severe dengue (6)
- Encephalitis, California (0)
- Encephalitis, Eastern equine (6)
- Encephalitis, Powassan
  - Neuroinvasive (6)
  - Nonneuroinvasive (1)
- Encephalitis, St. Louis
  - Neuroinvasive (19)
  - Nonneuroinvasive (4)
- Encephalitis, West Nile
  - Neuroinvasive (1455)
  - Nonneuroinvasive (720)
- Encephalitis, Western equine (0)
- Hantavirus infection, non-Hantavirus pulmonary syndrome (3)
- Hantavirus pulmonary syndrome (21)
- Hepatitis A, acute (1,390)
- Hepatitis B, acute (3,370)
- Hepatitis B, chronic (14,147)
- Hepatitis B, perinatal infection (37)
- Hepatitis C, acute (2,447)
- Hepatitis C, past or present (179,584)
- HIV diagnoses (33,817)
- Influenza-associated pediatric mortality (130)
- Jamestown Canyon virus
  - Neuroinvasive (6)
  - Nonneuroinvasive (5)
- La Crosse virus disease
  - Neuroinvasive (51)
  - Nonneuroinvasive (4)
- Measles
  - Indigenous (162)
  - Imported (26)
- Mumps (1,329)
- Novel influenza A virus infections (7)
- Poliomyelitis, paralytic (0)
- Poliovirus infection, nonparalytic (0)
- Rabies, animal (5,491)
- Rabies, human (2)
- Rubella (5)
- Rubella (congenital) (1)
- Severe acute respiratory syndrome associated coronavirus disease (SARS-CoV) (0)
- Smallpox (0)
- Varicella (Morbidity) (9,789)
- Varicella (Morality) (6)
- Viral hemorrhagic fever
  - Crimean-Congo hemorrhagic fever virus (0)
  - Ebola virus (0)
  - Lassa virus (1)
  - Lujo virus (0)
  - Marburg virus (0)
  - New World arenavirus
    - Guanarito virus (0)
    - Junin virus (0)
    - Machupo virus (0)
    - Sabia virus (0)
- Yellow fever (1)

Fungal

Coccidioidomycosis (*Coccidioides immitis*) (11,072)

Parasitic

Babesiosis (2,100)

Cryptosporidiosis (*Cryptosporidium parvum*) (9,735)

Cyclosporiasis (*Cyclospora cayetanensis*) (645)

Giardiasis (*Giardia lamblia*) (14,485)

Malaria (*Plasmodium* spp.) (1,390)

Trichinosis (*Trichinella spiralis*) (14)

[a]Data from *Morb. Mortal. Wkly. Rep.* Summary of Notificable Infectious Diseases and Conditions—United States, 2015(64):1-143, 2017. Numbers in parentheses represent the number of cases reported in 2015.

Table 2.2 Arthropod vectors of medically important diseases[a]

Arthropod	Etiologic agent	Disease
Crustacea		
Decapods (i.e., crayfish)	*Paragonimus* spp.	Paragonimiasis
Copepods (i.e., crustaceans)	*Diphyllobothrium* spp.	Diphyllobothriasis
	Dracunculus medinensis	Guinea worm disease
	Gnathostoma spinigerum	Gnathostomiasis
Insecta		
Anopleura		
Pediculus (i.e., louse)	*Rickettsia prowazekii*	Epidemic typhus
	Bartonella quintana	Trench fever
	Borrelia recurrentis	Epidemic relapsing fever
Siphonaptera (i.e., flea)		
Xenopsylla, Nosophyllus	*Yersinia pestis*	Plague
	Rickettsia typhi	Murine typhus
	Hymenolepis diminuta	Rat tapeworm
Pulex, Oropsylla	*Yersinia pestis*	Plague
Ctenocephalides	*Dipylidium caninum*	Dog tapeworm disease
Hemiptera		
Panstrongylus, Rhodnius, Triatoma (i.e., kissing bug)	*Trypanosoma cruzi*	Chagas disease
Diptera (i.e., flies)		
Aedes	*Wuchereria, Brugia*	Filariasis

	Flaviviruses	Dengue, yellow fever, zika
	Other arboviruses	Encephalitis
Anopheles	*Plasmodium* spp.	Malaria
	Wuchereria, Brugia	Filariasis
	Arboviruses	Encephalitis
Culex	*Wuchereria*	Filariasis
	Arboviruses	Encephalitis
Culicoides	*Mansonella* spp.	Filariasis
Glossina	*Trypanosoma brucei*	African sleeping sickness
Chrysops	*Loa loa*	Loiasis
	Francisella tularensis	Tularemia
Simulium	*Onchocerca volvulus*	Onchocerciasis
	Mansonella ozzardi	Filariasis
Phlebotomus, Lutzomyia	*Leishmania* spp.	Leishmaniasis
	Bartonella bacilliformis	Bartonellosis
	Phlebovirus	Sandfly fever
Arachnida		
Acari (ticks)		
Ixodes	*Borrelia burgdorferi*	Lyme disease
	Anaplasma phagocytophilum	Anaplasmosis
	Rickettsia conorii	Boutonneuse fever
	Babesia spp.	Babesiosis

(continued)

Table 2.2 Arthropod vectors of medically important diseases[a] *(continued)*

Arthropod	Etiologic agent	Disease
Dermacentor	*Rickettsia rickettsii*	Rocky Mountain spotted fever
	Francisella tularensis	Tularemia
	Coltivirus	Colorado tick fever
	Flavivirus	Omsk hemorrhagic fever
Amblyomma	*Rickettsia rickettsii*	Rocky Mountain spotted fever
	Francisella tularensis	Tularemia
	Ehrlichia chaffeensis	Human monocytic ehrlichiosis
Hyalomma	Nairovirus	Crimean-Congo hemorrhagic fever
Rhipicephalus	*Rickettsia conorii*	Boutonneuse fever
	Rickettsia rickettsii	Rocky Mountain spotted fever
Ornithodoros	*Borrelia* spp.	Relapsing fever
Acari (mites)		
Leptotrombidium	*Orientia tsutsugamushi*	Scrub typhus
Liponyssoides	*Rickettsia akari*	Rickettsialpox

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

Table 2.3 Fungal pathogens and geographic distribution

Fungi	Human body sites	Geographic distribution	Environmental reservoirs
Yeasts			
Candida spp.	Opportunistic pathogen involving any part of body, commensal of mucocutaneous membranes	Worldwide	Plants, alimentary tracts of mammals
Cryptococcus neoformans	Opportunistic pathogen primarily involving lungs and central nervous system; other body sites can be infected	Worldwide	Most commonly pigeon and other bird droppings and soil contaminated with these droppings
Cryptococcus gattii	Primarily lung disease without dissemination. Disease in immunocompetent	Worldwide	Gum and other varied trees
Cryptococcus, other spp.	Opportunistic pathogen rarely implicated in disease	Worldwide	
Blastoschizomyces capitatus	Opportunistic pathogen uncommonly implicated in systemic infections	Worldwide, but most commonly Southern Europe	Climates with hot, dry summers and mild, wet winters
Geotrichum spp.	Opportunistic pathogen uncommonly implicated in systemic infections	Worldwide	Widely distributed in environment, soil, beach sand, dairy products

(continued)

Table 2.3 Fungal pathogens and geographic distribution *(continued)*

Fungi	Human body sites	Geographic distribution	Environmental reservoirs
Malassezia spp.	Opportunistic pathogen involving skin surface (tinea versicolor, atopic dermatitis, folliculitis) and systemic disease (associated with lipid therapy)	Worldwide	Skin of warm-blooded mammals
Pseudozyma spp.	Opportunistic cause of fungemia	Worldwide	Plants
Rhodotorula spp.	Opportunistic pathogen uncommonly implicated in systemic infections	Worldwide	Moist skin, shower curtains, bathtub grout, toothbrushes
Trichosporon spp.	Opportunistic pathogen involving capital, axillary, or crural hairs (white piedra); systemic infections in immunocompromised patients	Worldwide	Soil, animals
Pneumocystis (*carinii*) *jiroveci*	Opportunistic pathogen primarily involving respiratory tract	Worldwide	Mammalian lung
Dimorphic fungi			
Blastomyces dermatitidis	Blastomycosis is primarily a pulmonary infection with dissemination to skin (cutaneous and subcutaneous lesions), genitourinary tract, bone, and central nervous system	Ohio and Mississippi River valleys, as well as Missouri and Arkansas River basins; southern Canada, South America, and portions of Africa	Probably soil (not well defined)

Coccidioides immitis, *Coccidioides posadasii*	Coccidioidomycosis is primarily a pulmonary infection with dissemination to skin, bone, joints, lymph nodes, adrenal glands, and central nervous system	Southwestern United States, northwestern Mexico, Argentina, and other dry areas of Central and South America	Soil, favors hot, semiarid regions
Histoplasma capsulatum	Histoplasmosis is primarily a pulmonary infection with dissemination to central nervous system, adrenal glands, mucocutaneous surfaces, and other tissues	Temperate, tropical, and subtropical regions throughout the world, particularly Ohio, Missouri, and Mississippi River valleys, southern portions of Canada, and areas in Central and South America	Soil contaminated with fecal matter from birds and bats
H. capsulatum var. *duboisii*	African histoplasmosis; pulmonary infection less common with more frequent involvement of skin and bones	Central Africa (between 20°N and 20°S)	
Paracoccidioides brasiliensis	Paracoccidioidomycosis (South American blastomycosis) is primarily a pulmonary infection with dissemination commonly to nose and mouth, less commonly to lymph nodes, spleen, liver, gastrointestinal tract, and adrenal glands	Central and South America	Rarely isolated from environment, soil, intestinal tracts of animals
Talaromyces (*Penicillium*) *marneffei*	Disseminated infection involving bone, skin, lung, lymph nodes, genitourinary and gastrointestinal tracts, central nervous system, and other tissues	Mountainous provinces of northern Thailand, Laos, Myanmar, and southeastern China	Unknown

(continued)

Table 2.3 Fungal pathogens and geographic distribution *(continued)*

Fungi	Human body sites	Geographic distribution	Environmental reservoirs
Sporothrix schenckii	Sporotrichosis involving skin and subcutaneous tissues with dissemination commonly via lymphatics to lymph nodes and, less commonly, to other internal organs	Worldwide, primarily in soil and decaying plant material	Plants, soil, decaying organic matter
Cutaneous fungi			
Epidermophyton floccosum	Infection of nails and skin, particularly of the groin and feet	Worldwide	Humans
Microsporum audouinii	Infection of scalp in children; rarely infects adults	Worldwide but primarily in Africa (Nigeria), eastern Europe, and Haiti; rarely in North America or western Europe	Humans
Microsporum canis	Infection of scalp in children, infection of beard and glabrous skin in all ages	Worldwide	Cats and dogs
Microsporum ferrugineum	Infection of scalp	Africa, East Asia, eastern Europe	Humans
Microsporum gypseum	Infection of scalp and glabrous skin	Worldwide	Soil and small rodents

Microsporum persicolor	Infection of scalp, glabrous skin, and feet	Worldwide	Small rodents
Phaeoannellomyces (*Exophiala*) *werneckii*	Infection (tinea nigra) of palms of hands and, occasionally, the dorsa of the feet	Tropical areas of Central and South America, Africa, and Asia; southeastern United States	Water, decaying plant matter
Piedraia hortae	Infection (black piedra) of scalp hair; less commonly beard, axillary, or pubic hairs	Tropical regions of Africa, Asia, and Central and South America	Soil
Trichophyton concentricum	Infection of glabrous skin	Southwestern Pacific islands, Southeast Asia, Central and South America	Humans
Trichophyton megninii	Infection of glabrous skin, scalp, and beard	Europe (particularly Portugal, Spain, and Sardinia), African nation of Burundi; rare in Western Hemisphere	Humans
Trichophyton mentagrophytes	Infection of all body surfaces including nails, hair, and (particularly) feet	Worldwide	Humans and small mammals
Trichophyton rubrum	Infection of skin and nails (most common pathogenic dermatophyte)	Worldwide	Humans
Trichophyton schoenleinii	Infection of scalp (favus) and occasionally nails and skin	Primarily in Eurasia and Africa	Humans

(continued)

Table 2.3 Fungal pathogens and geographic distribution *(continued)*

Fungi	Human body sites	Geographic distribution	Environmental reservoirs
Trichophyton soudanense	Infection of scalp and hair	Central and West Africa	Humans
Trichophyton tonsurans	Infection of scalp (most common pathogen), as well as skin and nails	Worldwide, particularly in the United States and Latin America	Humans
Trichophyton verrucosum	Infection of scalp, beard, nails, and other skin surfaces	Worldwide distribution	Cattle and horses
Trichophyton violaceum	Infection of scalp, as well as glabrous skin, nails, and soles of feet	North Africa, Middle East, Europe, South America, and Mexico	Humans
Mucormycosis			
Apophysomyces elegans	Rare cause of traumatic mucormycosis	Worldwide	Ubiquitous in the environment. Soil, plants, decaying organic material
Lichtheimia (*Absidia*) *corymbifera*	Pulmonary infections, as well as infections of the skin, meninges, and kidneys	Worldwide	
Basidiobolus ranarum	Subcutaneous mucormycosis of limbs, chest, back, or buttocks	Worldwide	

Conidiobolus coronatus	Subcutaneous mucormycosis of nasal mucosa, with spread into adjacent tissues	Worldwide, primarily in tropical and subtropical areas	
Cunninghamella bertholletiae	Rare cause of pulmonary or disseminated mucormycosis	Primarily in Mediterranean or subtropical areas	
Mucor spp.	Uncommon cause of disseminated mucormycosis	Worldwide	
Rhizomucor pusillus	Pulmonary, disseminated, or cutaneous mucormycosis	Worldwide	
Rhizopus spp.	Primary cause of invasive mucormycosis, particularly involving spread from nasopharynx to brain. *R. oryzae* most common.	Worldwide	
Saksenaea vasiformis	Occasional cause of rhinocerebral mucormycosis, as well as involvement of bone, skin, and subcutaneous tissues	Worldwide	
Eumycotic mycetoma			
Acremonium spp. (*A. falciforme*, *A. kiliense*, *A. recifei*)	Mycetoma (*A. falciforme* is the second most common cause in the United States)	India, Thailand, United States, Africa, Romania, Venezuela, Brazil	Environmental, soil, plants
Curvularia spp. (*C. geniculata*, *C. lunata*)	Mycetoma (*C. genicu/ata* in dogs)	United States (*C. geniculata*); Senegal (*C. lunata*)	Plants

(continued)

Table 2.3 Fungal pathogens and geographic distribution *(continued)*

Fungi	Human body sites	Geographic distribution	Environmental reservoirs
Exophiala jeanselmei	Mycetoma; subcutaneous phaeohyphomycosis; peritonitis	United States, Europe, India, Malaya, Thailand, Argentina	Water, decaying plant matter
Trematosphaeria (*Madurella*) spp.	Mycetoma	Venezuela, Argentina, Paraguay, Chile, Brazil, British West Indies, India, Zaire (*M. grisea*); Venezuela, Argentina, Romania, India, Sudan, Senegal, Somalia (*M. mycetomatis*)	Soil, hard plant material (i.e., thorns)
Neotestudina rosatii	Mycetoma	Australia, Cameroon, Guinea, Senegal, Somalia	
Pseudallescheria boydii	Mycetoma (most common cause in United States)	United States, Mexico, Venezuela, Argentina, Uruguay, India, Romania	
Pyrenochaeta romeroi	Mycetoma	Somalia, Senegal, India, South America	
Moniliaceous fungi			
Aspergillus spp	*A. fumigatus*, *A. flavus*, and *A. niger* are the most common pathogens; capable of colonization, invasive disease, toxicoses, or allergy	Worldwide	Environmental, decaying vegetation, soil, water, food, air

Fusarium spp.	*F. solani*, *F. oxysporum*, and *F. moniliforme* are the most common pathogens; cause eye infection and, less commonly, systemic infection, sinusitis, skin and nail infection, and mycetoma	Worldwide	Environmental, contaminated grain, water
Paecilomyces spp.	*P. variotii* and *P. lilacinus* are the most common pathogens; cause keratitis and, less commonly, endocarditis, sinusitis, nephritis, pulmonary infection, and skin and soft tissue infection	Worldwide	Soil, decaying plant material
Penicillium spp.	With the exception of *Talormyces* (*Penicillium*) *marneffei*, most isolates are contaminants	Worldwide	Ubiquitous in environment, deteriorating food products
Scopulariopsis spp.	*S. brevicaulis* is the most common pathogen, as well as a frequent lab contaminant; infection of toenails and (less commonly) fingernails	Worldwide	Soil, insects
Dematiaceous fungi			
Alternaria spp.	Phaeohyphomycosis of bone, skin, ears, eyes, sinuses, and urinary tract (this genus and other dematiaceous fungi are frequently isolated as lab contaminants)	Worldwide	Plants, soil

(continued)

Table 2.3 Fungal pathogens and geographic distribution *(continued)*

Fungi	Human body sites	Geographic distribution	Environmental reservoirs
Bipolaris spp.	Phaeohyphomycosis, sinuses, eyes, bone, skin, central nervous system. Opporunistic. Common contaminant	Worldwide	Plants
Cladosporium spp.	Common contaminant	Worldwide	Soil, plants
Cladophialophora spp. (*C. bantiana* and *C. carrionii*)	Neurotropic disease, high mortality (*C. bantiana*) Chromoblastomycosis (*C. carrionii*)	Worldwide, most common in tropical and subtropical areas	Soil, plants

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

Table 2.4 Parasitic pathogens and geographic distribution

Parasites	Human body sites	Geographic distribution
Protozoa: amoebae		
Acanthamoeba spp.	Brain, skin, eye, lung	Worldwide
Balamuthia mandrillaris	Brain, CSF[a]	Worldwide
Endolimax nana	Lumen of colon and cecum	Worldwide
Entamoeba bangladeshi	Newly described, probably the same as other *Entamoeba* spp.	Unknown
Entamoeba histolytical/dispar	Lumen of colon and cecum; extraintestinal sites include liver, lung, brain, skin	Worldwide
Entamoeba hartmanni	Lumen of colon and cecum	Worldwide
Entamoeba gingivalis	Mouth	Worldwide
Entamoeba coli	Lumen of colon and cecum	Worldwide
Entamoeba polecki	Lumen of colon and cecum	Worldwide
Iodamoeba butschlii	Lumen of colon and cecum	Worldwide
Naegleria fowleri	Brain, CSF	Worldwide
Protozoa: flagellates		
Chilomastix mesnili	Primarily large intestine	Worldwide
Dientamoeba fragilis	Colon	Worldwide

(continued)

Table 2.4 Parasitic pathogens and geographic distribution *(continued)*

Parasites	Human body sites	Geographic distribution
Giardia (aka *G. intestinalis* or *G. lamblia*) *duodenalis*	Small intestine	Worldwide
Leishmania chagasi, *L. donovani*, *L. infantium*	Visceral leishmaniasis: amastigotes in bone marrow or aspirates from spleen, lymph nodes, or liver	*L. chagasi* in Central and South America; *L. donovani* in China, India, Middle East, Africa; *L. infantum* in North Africa, Southwest Asia, Mediterranean, Europe, Central and South America
Leishmania tropica, *L. braziliensis*, *L. major*, other *Leishmania* spp.	Cutaneous leishmaniasis: amastigotes in cutaneous lesions	Many species worldwide
Leishmania waltoni	Cutaneous leishmaniasis	Dominican Republic
Pentatrichomonas (*Trichomonas*) *hominis*	Cecum	Worldwide
Trichomonas tenex	Mouth	Worldwide
Trichomonas vaginalis	Vagina, urethra, prostate, epididymis	Worldwide
Trypanosoma brucei gambiense	Trypomastigotes in blood, CSF, brain, lymph nodes, and spleen	West Central Africa south of Sahara Desert
Trypanosoma brucei rhodesiense	As with *T. b. gambiense*	East Central Africa south of Sahara Desert
Trypanosoma cruzi	Trypomastigotes in blood; amastigotes and epimastigotes in pseudocysts in cardiac and smooth muscle, glial cells, and phagocytes	Western Hemisphere from southern United States south to Argentina

Protozoa: ciliates		
Neobalantidium (*Balantidium*) *coli*	Colon	Widespread in temperate and warm climates
Protozoa: apicomplexans		
Babesia spp.	Parasite of erythrocytes	*B. microti* in North America and Europe; otherspecies (e.g., *B. divergens*, *B. equi*) with worldwide distribution in tropics and subtropics
Cryptosporidium parvum	Intracellular parasite of intestinal epithelial cells; also in respiratory tract and biliary system	Worldwide
Cryptosporidium viatorum	Newly described, probably same as other *Cryptosporidium* spp.	Africa and Asia
Cyclospora cayetanensis	Intracellular parasite of jejunum enterocytes	North, Central, and South America; Caribbean, Africa, Southeast Asia, Eastern and Western Europe, Australia
Cystoisospora (*Isospora*) *belli*	Intracellular parasite of duodenum and jejunum	Worldwide
Plasmodium falciparum	Ring forms and gametocytes infect erythrocytes of all ages; trophozoites and schizonts not typically seen in peripheral blood; no persistent exoerythrocytic stage	Widely distributed in tropics and subtropics, particularly Africa and Asia; chloroquine-resistant strains reported in all areas except Central America and the Caribbean

(continued)

Table 2.4 Parasitic pathogens and geographic distribution *(continued)*

Parasites	Human body sites	Geographic distribution
Plasmodium knowlesi	Early trophozoites appear as delicate ring forms, occasionally with double chromatin dots; late trophozoites resemble *P. malariae* with band formation	Southeast Asia, most common in Malaysia and Thailand
Plasmodium malariae	Trophozoites, schizonts, and gametocytes parasitize mature erythrocytes; no persistent exoerythrocytic stage, but low-level parasitemia can persist for years	Present in tropics and subtropics (e.g., tropical Africa, India, Myanmar, Sri Lanka, Malaysia, Indonesia) but less common than other plasmodia
Plasmodium ovale	Trophozoites, schizonts, and gametocytes parasitize reticulocytes; hypnozoites persist in hepatic parenchymal cells	Present in tropical Africa (particularly in West Africa), New Guinea, Philippines; also reported in Southeast Asia
Plasmodium vivax	Trophozoites, schizonts, and gametocytes in erythrocytes, with preference for reticulocytes; hypnozoites persist in hepatic parenchymal cells	Worldwide; predominant species in temperate areas: less commonly in tropics such as West Africa; chloroquine-resistant strains reported in Indonesia, Papua New Guinea, Myanmar, and Guyana
Sarcocystis spp.	Intracellular parasite of intestinal epithelium; cysts in skeletal and cardiac muscle	Worldwide
Toxoplasma gondii	Cysts in skeletal muscle, myocardium, brain; tachyzoites in blood, CSF, ocular fluid, bronchoalveolar lavage fluid	Worldwide

Nematodes		
Ancylostoma duodenale	Adults: small intestine; eggs: feces	Southern Europe, northern Africa, China, India, Japan
Parastrongylus (*Angiostrongylus*) *cantonensis*	Larvae and young adults in CSF	Thailand, Tahiti, Taiwan, Indonesia, Hawaii; less commonly in Cuba, Central America, and Louisiana
Parastrongylus (*Angiostrongylus*) *costaricensis*	Adults: terminal ileus, cecum, colon, regional lymph nodes, mesenteric arteries; larvae and eggs in surrounding tissue	Central and South America
Anisakis spp.	Larvae: wall of stomach or intestine; occasionally in extraintestinal sites	Worldwide where uncooked fish is consumed
Ascaris lumbricoides	Adults: small intestine; larvae: small intestine, liver, lungs; eggs: feces	Worldwide (particularly in warm, moist regions)
Brugia malayi	Adults: lymphatic system; microfilaria: blood	Southeast Asia, Philippines, Korea, southern China, India
Brugia timori	Adults: lymphatic system; microfilaria: blood	Lesser Sunda lslands of eastern lndonesian archipelago
Capillaria hepatica	Adults: liver	Worldwide
Capillaria philippinensis	Adults: intestine; eggs: feces; larvae: occasionally found in feces	Philippines, Thailand, Japan, Taiwan, Egypt, Iran, Colombia

(continued)

Table 2.4 Parasitic pathogens and geographic distribution *(continued)*

Parasites	Human body sites	Geographic distribution
Dirofilaria immitis	Larvae in pulmonary nodules	Worldwide in tropical, subtropical, and warm temperate regions; southern coastal and southeastern United States
Dracunculus medinensis	Adults in cutaneous lesions	Worldwide
Enterobius vermicularis	Adults: cecum, appendix, colon, rectum; eggs: deposited in perianal area	Worldwide
Eustrongyloides spp.	Adults: abdominal cavity, intestines	Worldwide where uncooked fish is consumed (rare)
Gnathostoma spp.	Larvae: tissues	China, Philippines, Thailand, Japan
Loa loa	Adults: subcutaneous tissue; microfilaria: blood	Equatorial rain forests of Central and West Africa south of Sahara Desert
Mansonella ozzardi	Adults: subcutaneous tissue; microfilaria: blood	Central America (e.g., Mexico, Panama) and northern part of South America, West Indies
Mansonella perstans	Adults: abdominal cavity, mesenteries, peritoneal tissues; microfilaria: blood	West and Central Africa south of Sahara Desert, South America, some Caribbean islands
Mansonella streptocerca	Adults: subcutaneous tissues; microfilaria: skin snips	Rain forests of Central and West Africa (e.g., Zaire, Ghana, Nigeria, Cameroon)
Necator americanus	Adults: small intestine; eggs: feces	Western Hemisphere, Central and South Africa, southern Asia, India, Melanesia, Polynesia

Onchocerca volvulus	Adults: subcutaneous nodules; microfilaria: skin snips; occasionally blood or urine	West and Central Africa south of Sahara Desert; Yemen, Central America (southern Mexico, Guatemala), South America (Venezuela, Colombia, Ecuador, Brazil)
Strongyloides stercoralis	Adults: small intestine; larvae: feces	Worldwide (particularly in warm, moist regions)
Toxocara spp.	Visceral larva migrans; larvae found in various tissues including liver, eye, and central nervous system	Worldwide (particularly in warm, moist regions)
Trichinella spiralis	Adults: intestines; larvae: encyst in muscle tissue	Worldwide (primarily in Europe and North America; less commonly in tropical countries)
Trichostrongylus spp.	Adults: small intestine; eggs: feces	Worldwide (associated with herbivorous animals)
Trichuris trichiura	Adults: large intestine, cecum, appendix; eggs: feces	Worldwide (particularly in warm, moist regions)
Wuchereria bancrofti	Adults: lymphatic system; microfilaria: blood	Widespread in tropics and subtropics (India, Bangladesh, China, Indonesia, Malaysia, Papua New Guinea, Philippines, Sri Lanka, Thailand, Vietnam, South Pacific islands, Africa, Egypt, Costa Rica, Brazil, West Indies)

(continued)

Table 2.4 Parasitic pathogens and geographic distribution *(continued)*

Parasites	Human body sites	Geographic distribution
Trematodes		
Clonorchis sinensis	Adults: bile ducts; eggs: feces	China, Taiwan, Japan, Korea, Vietnam
Dicrocoelium dendriticum	Adults: bile ducts; eggs: feces	Europe, former USSR, northern Africa, northern Asia, Far East, Western Hemisphere
Echinostoma hortense	Adults: small intestine; eggs: feces	Southeast Asia
Fasciola hepatica	Adults: bile ducts; eggs: feces	Worldwide (particularly in sheep-raising countries)
Fasciolopsis buski	Adults: small intestine; eggs: feces	China, Taiwan, Thailand, Indonesia, India, Bangladesh, Cambodia, Myanmar, Vietnam
Gastrodiscoides hominis	Adults: cecum, colon; eggs: feces	India, Southeast Asia, former USSR
Heterophyes heterophyes	Adults: small intestine; eggs: feces	Nile River delta, Turkey, East and Southeast Asia
Metagonimus yokogawai	Adults: small intestine; eggs: feces	China, Japan, Southeast Asia, Balkan states
Metorchis conjunctus	Adults: bile ducts; eggs: feces	Canada
Nanophyetus salmineola	Adults: small intestine; eggs: feces	Northwest North America
Neodiplostomum seoulense	Adults: small intestine; eggs: feces	Southeast Asia
Opisthorchis spp.	Adults: bile ducts; eggs: feces	*O. viverrini*: Thailand, Laos; *O. felineus*: Eastern Europe, former USSR

Paragonimus westermani	Adults: lung parenchyma; occasionally in abdominal wall, connective tissues, and organs; subcutaneous tissues; brain; eggs: feces or sputum	China, Japan, Korea; other species in Latin America, Southeast Asia, Africa
Phaneropsolus bonnei	Adults: small intestine; eggs: feces	Southeast Asia
Prosthodendrium molenkampi	Adults: small intestine; eggs: feces	Southeast Asia
Pygidiopsis summa	Adults: small intestine; eggs: feces	Southeast Asia
Schistosoma haematobium	Adults: venous plexuses of bladder and rectum; eggs: biopsy of bladder wall or rectum, feces	Africa, Madagascar, Arabian Peninsula, Iraq, Iran, Syria, Lebanon, Turkey, India
Schistosoma japonicum	Adults: venous plexuses of small intestine; eggs: feces, rectal biopsy	China, Philippines, Indonesia, Thailand
Schistosoma mansoni	Adults: venous plexuses of colon and lower ileum; hepatic portal system; eggs: feces, rectal biopsy	Africa, Madagascar, Arabian Peninsula, Caribbean islands, including Puerto Rico, South America (Brazil, Suriname, Venezuela)
Schistosoma mekongi	Adults: venous plexuses of small intestine; eggs: feces, rectal biopsy	Laos, Cambodia, Thailand
Cestodes		
Diphyllobothrium latum	Adults: small intestine; eggs and proglottids: feces	Fish tapeworm in cold lakes of northern Europe, Baltic countries, North America, Japan; other species found in Alaska, Peru, and Japan
Dipylidium caninum	Adults: small intestine; eggs and proglottids: feces	Worldwide (dog tapeworm)

(continued)

Table 2.4 Parasitic pathogens and geographic distribution *(continued)*

Parasites	Human body sites	Geographic distribution
Echinococcus granulosus	Unilocular hydatid disease; larvae form cysts in any tissue including liver, lung, and brain	Sheep-raising countries (e.g., Australia, New Zealand, southern Africa, southern South America); parts of Europe, North America, and the Orient
Echinococcus multilocularis	Multilocular hydatid disease; larvae form cysts in any tissue, particularly liver	Northern Europe, Japan, China, India, North America (Alaska, Canada, northern midwestern United States)
Echinococcus vogeli		Latin America
Hymenolepis diminuta	Adults: small intestine; eggs: feces	Worldwide (rat tapeworm)
Hymenolepis nana	Adults: small intestine; eggs: feces	Worldwide (dwarf tapeworm)
Spirometra mansoni	Larvae migrate to brain	China, Japan, Korea, Vietnam
Spirometra mansonoides	Larvae migrate in subcutaneous tissues	United States
Taenia multiceps	Larvae form cysts in subcutaneous tissues, muscle, eye, and central nervous system	Sheep-raising countries
Taenia saginata	Adults: small intestine; eggs and proglottids: feces	Worldwide (beef tapeworm)
Taenia solium	Adults: small intestine; eggs and proglottids: feces; larvae (*Cysticercus cellulosae*) form cysts in various tissues including brain and muscle	Worldwide (pork tapeworm), particularly in middle European countries, Mexico, Latin America, India, China

[a]CSF, cerebrospinal fluid.

SECTION 3

Specimen Collection and Transport

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The quality of any laboratory diagnostic test is directly dependent on the quality of the specimen that is submitted for analysis. This is particularly true for the diagnosis of infectious diseases, where specimen integrity is of paramount importance. Not only is it critical to collect an appropriate specimen that would contain the pathogen of interest, but it is also important that the specimen be transported to the laboratory in a timely manner and under conditions that preserve organism viability and ensure the reliability of the diagnostic procedure (e.g., culture, microscopy, and antigen or antibody tests). The following guidelines can be used for the most commonly submitted specimens. For further information, please consult the ASM *Manual of Clinical Microbiology,* 11th ed. As a general guideline for all specimens, the following considerations should be kept in mind.

1. Appropriate safety precautions must be used for the collection and transport of all specimens. Specimens should always be considered infectious. Therefore, gloves should always be worn when handling specimens, and all procedures should be performed behind barrier protection, preferably in a biosafety cabinet.

2. Many infections are caused by members of the patient's indigenous microbial population. For this reason, it is important to avoid contamination of the specimen with these organisms. In many cases, the presence of abundant epithelial (i.e., squamous or respiratory) indicate a suboptimal specimen that may contain commensal flor which may obscure culture results.

3. Specimens should be collected from the areas where organisms are present and replicating. Although it seems obvious, this principle is often ignored. For example, pus typically contains relatively few viable organisms. A more appropriate specimen would be scrapings or a biopsy specimen from the wall of an abscess. Likewise, the material collected from the surface of a wound is often *not* representative of the organisms present deep in the wound. Finally, the diagnosis of a lower respiratory tract infection requires collection of material from that site (e.g., sputum) and not from the mouth (e.g., saliva).

4. The quantity of specimen collected must be sufficient to ensure that all requested tests (cultures, microscopy, antigen tests, nucleic acid probes, and amplification tests) can be performed properly. If only a limited amount of specimen can be collected, tests should be performed selectively. If too many tests are attempted, no test will be performed adequately.

5. Traditionally, cotton-wrapped swabs have been discouraged for specimen collection. However, with the advent of the flocked swab with liquid Amies transport media, this is now an acceptable and in some cases a preferred specimen collection system. Flocked swab systems are particularly useful for those labs utilizing automated specimen processors.

6. Transport of specimens should maintain the viability of the etiologic agent (if culture is performed) and prevent overgrowth with contaminating organisms.

7. Specimens should always be transported in a leakproof container inserted in a leakproof plastic bag with a separate compartment for the requisition. Use of plastic bags allows the specimen to be examined before the bag is opened. Every effort should be made to collect a second specimen if the original specimen is received in a leaking container. However, if an additional specimen cannot be collected, the laboratory should attempt to process the specimen if it can be done safely.

8. For off-site specimen transport guidelines, refer to the International Air Transport Association (lATA) Dangerous Goods Regulations (http://www.iata.org/publications/dgr/Pages/index.aspx), the U.S. Department of Transportation (https://www.phmsa.dot.gov/hazmat), and the International Civil Aviation Organization (ICAO) regulations. When preparing a specimen for transport, always check the specimen transport guidelines of the receiving laboratory.

Table 3.1 Bacteriology: Collection and transport guidelines[a,b]

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
Abscess				
General	Remove surface exudate by wiping with sterile saline or 70% alcohol		Tissue or fluid is always superior to a swab specimen. If cotton swabs must be used, collect two, one for culture and one for Gram staining. Preserve swab material by placing in Stuart's or Amies medium. A single flocked swab in liquid media is appropriate for both culture and Gram stain.	
Open	Aspirate if possible or pass a swab deep into the lesion to firmly sample the lesion's "fresh border"	Swab transport system	≤2 h, RT	Samples of the base of the lesion and abscess wall are most productive.
Closed	Aspirate abscess material with needle and syringe; aseptically transfer all material into anaerobic transport device	Anaerobic transport system, 1 ml	≤2 h, RT	Contamination with surface material will introduce colonizing bacteria not involved in the infectious process.

Bite wound	See Abscess		Do not culture animal bite wounds ≤12 h old (agents are usually not recovered) unless signs of infection are present.	
Blood	Disinfect culture bottle; apply 70% isopropyl alcohol or phenolic to rubber stoppers and wait 1 min	Blood culture bottles for bacteria; adult, 20 ml/set (higher volume most productive) Pediatric, draw the maximum safe volume, many children (even neonates) have low-level bacteremia that requires maximal blood volume	≤2 h, RT	Acute febrile episode, antimicrobials to be started or changed immediately: two sets from separate sites, all within 10 min (before antimicrobials). Nonacute disease, antimicrobials will not be started or changed immediately: two or three sets from separate sites all within 24 h at intervals no closer than 3 h (before antimicrobials). Endocarditis, acute: three sets from separate sites, within 1–2 h, before antimicrobials if possible. from separate sites 1 h apart, within 24 h. If cultures are negative at 24 h, obtain two or three more sets.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
	Palpate vein before disinfection of venipuncture site		Fever of unknown origin: two or three sets from separate sites 1 h apart during a 24-h period. If negative at 24–48 h, obtain two or three more sets	
	Disinfection of venipuncture site: 1. Cleanse site with70% alcohol. 2. Swab concentrically, starting at the center, with an iodine preparation. 3. Allow the iodine to dry. 4. Do not palpate vein at this point without sterile glove. 5. Collect blood. 6. After venipuncture, remove iodine from the skin with alcohol.	Infant and child, 1–20 ml/set depending on weight of patient		Some data indicate that an additional aerobic or fungal bottle is more productive than the anaerobic bottle. Pediatric: Collect immediately; rarely necessary to document continuous bacteremia with hours between cultures. Mycobacteria: Use special culture systems (e.g., Isolator, Bactec 13A, Bactec Myco/F Lytic).

Bone marrow aspirate	Prepare puncture site as for surgical incision	Inoculate blood culture bottle or a lysis centrifugation tube; plated specimen delivered to laboratory immediately	≤24 h, RT, if in culture bottle or tube	Small volumes of bone marrow may be inoculated directly onto culture media. Routine bacterial culture of bone marrow is rarely useful.
Burn	Clean and debride the burn	Tissue is placed into a sterile screw-cap container; aspirate or swab exudate; transport in sterile container or swab transport system Quantitative cultures should be discouraged due to low reproducibility	≤24 h, RT	A 3- to 4-mm punch biopsy specimen is optimum when quantitative cultures are ordered. Process for aerobic culture only. Quantitative culture may or may not be valuable. Cultures of surface samples of bums may be misleading.
Catheter i.v.	1. Cleanse the skin around the catheter('> site with alcohol. 2. Aseptically remove catheter and clip 5 cm of distal tip directly into a sterile tube. 3. Transport immediately to microbiology laboratory to prevent drying.	Sterile screw-cap tube or cup	≤15 min, RT	Catheter cultures are of questionable clinical relevance. Acceptable i.v. catheters for semiquantitative culture (Maki method): central, CVP Hickman, Broviac, peripheral arterial, umbilical, hyperalimentation, Swan-Ganz.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
Foley	Do not culture, since growth represents distal flora			Not acceptable for culture.
Cellulitis, aspirate from area of	1. Cleanse site by wiping with sterile saline or 70% alcohol. 2. Aspirate the area of maximum inflammation (commonly the center rather than the leading edge) with a needle and syringe; irrigation with a small amount of sterile saline may be necessary. 3. Aspirate saline into syringe, and expel into sterile screw-cap tube.	Sterile tube (syringe transport not recommended	≤15 min, RT	Yield of potential pathogens in minority of specimens cultured
CSF	1. Disinfect site with iodine preparation. 2. Insert a needle with stylet at L3-IA, L4-L5, or L5-S1 interspace.	Sterile screw-cap tubes Minimum amount required: bacteria, 21 ml; AFB, 25 ml	Bacteria: never refrigerate; 15 min, RT	Obtain blood for culture also. If only one tube of CSF is collected, it should be submitted to microbiology first; otherwise submit tube two to microbiology. Aspirate of brain

	3. Upon reaching the subarachnoid space, remove the stylet and collect 1–2 m1 of fluid into each of three leakproof tubes.			abscess or a biopsy specimen may be necessary to detect anaerobic bacteria or parasites.
Decubitus ulcer	A swab is not the specimen of choice 1. Cleanse surface with sterile saline. 2. If a sample biopsy is not available, aspirate inflammatory material from the base of the ulcer.	Sterile tube (aerobic) or anaerobic system (for tissue)	≤2 h, RT	Since a swab specimen of a decubitus ulcer provides no clinical information, it should not be submitted. A tissue biopsy sample or needle aspirate is the specimen of choice.
Dental culture: gingival, periodontal, periapical, Vincent's stomatitis	1. Carefully cleanse gingival margin and supragingival tooth surface to remove saliva, debris, and plaque. 2. Using a periodontal scaler, carefully remove subgingival lesion material and transfer it to an anaerobic transport system.	Anaerobic transport system	≤2 h, RT	Periodontal lesions should be processed only by laboratories equipped to provide specialized techniques for the detection and enumeration of recognized pathogens.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
	3. Prepare smear for staining with specimen collected in the same fashion.			
Ear				
Inner	Tympanocentesis reserved for complicated, recurrent, or chronic persistent otitis media 1. For intact eardrum, clean ear canal with soap solution and collect fluid via syringe aspiration technique (tympanocentesis). 2. For ruptured eardrum, collect fluid on flexible shaft swab via an auditory speculum.	Sterile tube, swab transport medium, or anaerobic system	≤2 h, RT	Results of throat or nasopharyngeal swab cultures are not predictive of agents responsible for otitis media and should not be submitted for that purpose.

Outer	1. Use moistened swab to remove any debris or crust from the ear canal. 2. Obtain a sample by firmly rotating the swab in the outer canal.	Swab transport	≤2 h; RT	For otitis externa, vigorous swabbing is required since surface swabbing may miss streptococcal cellulitis.
Eye				
Conjunctiva	1. Sample each eye with separate swabs (premoistened with sterile saline) by rolling over each conjunctiva. 2. Medium may be inoculated at time of collection. 3. Smear may be prepared at time of collection; roll swab over 1–2-cm area of slide.	Direct culture inoculation: BAP and CHOC; laboratory inoculation: swab transport	Plates: ≤15 min, RT; swabs: ≤2 h, RT	If possible, sample both conjunctiva, even if only one is infected, to determine the indigenous microflora. The uninfected eye can serve as a control with which to compare the agents isolated from the infected eye. If cost prohibits this collection approach, rely on the Gram stain to assist in interpretation of culture.
Corneal scrapings	1. Specimen is collected by ophthalmologist. 2. Using sterile spatula, scrape ulcers or lesions, and inoculate scraping directly onto medium.	Direct culture inoculations: BHI with 10% sheep blood, CHOC, and inhibitory mold agar	≤15 min, RT	If conjunctival specimen is collected, do so before anesthetic application, which may inhibit some bacteria. Corneal scrapings are obtained after anesthesia. Include fungal media.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
	3. Prepare two smears by rubbing material from spatula onto 1–2-cm area of slide.			
Vitreous fluid aspirates	Prepare eye for needle aspiration of fluid.	Sterile screw-cap tube or direct inoculation of small amount of fluid onto media	≤15 min, RT	Include fungal media. Anesthetics may be inhibitory to some etiologic agents.
Feces				
Routine culture	Pass specimen directly into a clean, dry container; transport to microbiology laboratory within 1 h of collection or transfer to Cary-Blair holding medium.	Clean, leakproof; wide-mouth container or use Cary-Blair holding medium (>2 g)	Unpreserved: ≤1 h, RT Holding medium: ≤24 h, RT	Do not perform routine stool cultures for patients whose length of hospital stay is >3 days and the admitting diagnosis was not gastroenteritis, without consultation with physician. Tests for *Clostridium djfficile* should be considered for these patients. Swabs for routine pathogens are not recommended except for infants (see Rectal swabs).

C. difficile culture	Pass liquid or soft stool directly into a clean, dry container; soft stool is defined as stool assuming the shape of its container	Sterile, leakproof, wide-mouth container, >5 ml	≤1 h, RT; 1–24 h, 4°C; ≤24 h, —20°C or colder	Patients should be passing three to five liquid or soft stools per 24-h period. Testing of formed or hard stool is not recommended. Freezing at –20°C results in rapid loss of cytotoxin activity.
E. coli 0157:H7 and other Shiga-toxin-producing serotypes	Pass liquid or bloody stool into a clean, dry container	Sterile, leakproof, wide-mouth container, or Cary-Blair holding medium (>2 g)	Unpreserved: ≤1 h, RT Swab transport system: ≤24 h, RT or 4°C	Bloody or liquid stools collected within 6 days of onset among patients with abdominal cramps have the highest yield. Shiga toxin assay for all EHEC serotypes is better than sorbitol MacConkey culture for 0157:H7 only.
Leukocyte detection (not recommended for use with patients who have acute infectious diarrhea)	Pass feces directly into a clean, dry container; transport to microbiology laboratory within 1 h of collection, or transfer to ova and parasite transport system (10% formalin or PVA)	Sterile, leakproof, wide-mouth container or 10% formalin and/or PVA; >2 mi	Unpreserved: ≤l h, RT Formalin/PVA: indefinite, RT	Controversial: many believe this is of little clinical value.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
Rectal swab	1. Carefully insert a swab ca. 1 in. beyond the anal sphincter. 2. Gently rotate the swab to sample the anal crypts. 3. Feces should be visible on the swab for detection of diarrheal pathogens.	Swab transport	≤2 h, RT	Reserved for detecting *Neisseria gonorrhoeae*, *Shigella*, *Campylobacter*, and herpes simplex virus and anal carriage of group B *Streptococcus* and other beta-hemolytic streptococci, or for patients unable to pass a specimen.
Fistula	See Abscess			
Fluids: abdominal, amniotic, ascites, bile, joint, paracentesis, pericardial, peritoneal, pleural, synovial, thoracentesis	1. Disinfect overlying skin with iodine preparation. 2. Obtain specimen via percutaneous needle aspiration or surgery. 3. Always submit as much fluid as possible; never submit a swab dipped in fluid.	Anaerobic transport system, sterile screw-cap tube, or blood culture bottle for bacteria; transport immediately to laboratory Bacteria, >1 ml	≤15 min, RT	Amniotic and culdocentesis fluids should be transported in an anaerobic system and need not be centrifuged prior to Gram staining. Other fluids are best examined by Gram staining of a cytocentrifuged preparation. One aerobic blood culture bottle inoculated at bed site may be performed.

Gangrenous tissue	See Abscess			Discourage sampling of surface or superficial tissue. Tissue biopsy or aspiration should be performed.
Gastric				
Wash or lavage for mycobacteria	Collect in early morning before patients eat and while they are still in bed. 1. Introduce a nasogastric tube into the stomach. 2. Perform lavage with 25–50 ml of chilled, sterile distilled water. 3. Recover sample and place in a leakproof, sterile container.	Sterile, leakproof container	≤15 min, RT, or neutralize within 1 h of collection	The specimen must be processed promptly because mycobacteria die rapidly in gastric washings. Neutralize with sodium bicarbonate when holding for >1 h.
Biopsy for *H. pylori*	Collected by gastroenterologist during endoscopy	Sterile tube with transport medium	<1 h, RT	Culture may be needed for antimicrobial testing.
Genital, female				
Amniotic fluid	Aspirate via amniocentesis, or collect during cesarean delivery	Anaerobic transport system, ≥1 ml	≤2 h, RT	Swabbing or aspiration of vaginal secretions is *not* acceptable because of the potential for contamination with the commensal vaginal flora.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
Bartholin gland secretions	1. Disinfect skin with iodine preparation. 2. Aspirate fluid from ducts.	Anaerobic transport system, ≥1 ml	≤2 h, RT	
Cervical secretions	1. Visualize the cervix using a speculum without lubricant. 2. Remove mucus and secretions from the cervical or with swab, and discard the swab. 3. Firmly yet gently sample the endocervical canal with a new sterile swab.	Swab transport	≤2 h, RT	See the text for collection and transport need for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.
Cul-de-sac fluid	Submit aspirate or fluid	Anaerobic transport system, >1 ml	≤2 h, RT	
Endometrial tissue and secretions	1. Collect transcervical aspirate via a telescoping catheter. 2. Transfer entire amount to anaerobic transport system.	Anaerobic transport system, ≥1 ml	≤2 h, RT	

Products of conception	1. Submit a portion of tissue in a sterile container. 2. If obtained by cesarean delivery, immediately transfer to an anaerobic transport system.	Sterile tube or anaerobic transport system	≤2 h, RT	Do not process lochia, culture of which may give misleading results.
Urethral secretions	Collect at least 1 h after patient has urinated. 1. Remove old exudate from the urethral orifice. 2. Collect discharge material on a swab by massaging the urethra; for females, massage the urethra against the pubic symphysis through the vagina.	Swab transport	≤2 h, RT	If no discharge can be obtained, wash the periurethral area with Betadine soap and rinse with water. Insert a small swab 2–4 cm into the urethra, rotate it, and leave it in place for at least 2 s to facilitate absorption.
Vaginal secretions	1. Wipe away old secretions and discharge. 2. Obtain secretions from the mucosal membrane of the vaginal wall with a sterile swab or pipette. 3. If a smear is also needed, use a second swab.	Swab transport	≤2 h, RT	For intrauterine devices, place entire device into a sterile container and submit at RT. Gram stain, not culture, is recommended for the diagnosis of bacterial vaginosis. For detection of group B streptococcal colonization, submit a rectal swab.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
Genital, female or male lesion	1. Clean with sterile saline, and remove lesion's surface with a sterile scalpel blade. 2. Allow transudate to accumulate. 3. While pressing the base of the lesion, firmly rub base with a sterile swab to collect fluid.	Swab transport	≤2 h, RT	For dark-field examination to detect *T. pallidum*, touch a glass slide to the transudate, add cover- slip, and transport immediately to the laboratory in a humidified chamber (petri dish with moist gauze). *T. pallidum* cannot be cultured on artificial media.
Genital, male Prostate	1. Cleanse urethral meatus with soap and water. 2. Massage prostate through rectum. 3. Collect fluid expressed from prostate on a sterile swab.	Swab transport or sterile medium for >1 ml of specimen.	≤2 h, RT	Pathogens in prostatic secretions may be identified by quantitative culture of urine before and after massage. Ejaculate may also be cultured.

Urethra	Insert a small swab 2–4 cm into the urethral lumen, rotate swab, and leave it in place for at least 2 s to facilitate absorption.	Swab transport	≤2 h, RT	
Pilonidal cyst	See Abscess			
Respiratory, lower				
Bronchoalveolar lavage, brush or wash, endotracheal aspirate	1. Collect washing or aspirate in a sputum trap. 2. Place brush in sterile container with 1 ml of saline.	Sterile container, >1 ml	≤2 h, RT	A total of 40–80 ml of fluid is needed for quantitative analysis of BAL fluid. For quantitative analysis of brushings, place brush into 1.0 ml of saline.
Sputum, expectorated	1. Collect specimen under the direct supervision of a nurse or physician. 2. Have patient rinse or gargle with water to remove excess oral flora. 3. Instruct patient to cough deeply to produce a lower respiratory specimen (not postnasal fluid).	Sterile container, >1 ml Minimum amount: bacteria, >1 ml	≤2 h, RT	For pediatric patients unable to produce a sputum specimen, a respiratory therapist should collect a specimen via suction. The best specimen should have ≤10 squamous cells/100× field (10× objective and 10× ocular). Mycobacteria: submit an early morning specimen on 3 consecutive days.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
	4. Collect in a sterile container.			
Sputum, induced	1. Have patient rinse mouth with water after brushing gums and tongue. 2. With the aid of a nebulizer, have patients inhale approximately 25 ml of 3–10% sterile saline. 3. Collect in a sterile container.	Sterile container, >1 ml	≤2 h, RT	Same as above for sputum, expectorated.
Respiratory, upper				
Oral	1. Remove oral secretions and debris from the surface of the lesion with a swab; discard this swab.	Swab transport or sterile container	≤2 h, RT	Discourage sampling of superficial tissue for bacterial evaluation. Tissue biopsy specimens or needle aspirates are the specimens of choice.

	2. Using a second swab, vigorously sample the lesion, avoiding any areas of normal tissue.			
Nasal	1. Insert a swab, premoistened with sterile saline (or use flocked swab), approximately 1–2 cm into the nares. 2. Rotate the swab against the nasal mucosa.	Swab transport	≤2 h, RT	Anterior nose cultures are reserved for detecting staphylococcal carriers or for nasal lesions.
Nasopharynx	1. Gently insert a small swab (e.g., calcium alginate or flocked swab) into the posterior nasopharynx via the nose. 2. Rotate swab slowly for 5 s to absorb secretions.	Direct medium inoculation at bedside or examination table, swab transport	Plates: 15 min, RT; swabs: 2 h, RT	
Throat or pharynx	1. Depress tongue with a tongue depressor.	Swab transport	≤2 h, RT	Throat swab cultures are contra- indicated in patients with epiglottitis. Swabs for

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
	2. Sample the posterior pharynx, tonsils, and inflamed areas with a sterile swab.			*Neisseria gonorrhoeae* should be placed in charcoal-containing transport medium and plated 12 h after collection. JEMBEC, Biobags, and the GonoPak are better for transport at RT.
Tissue	Collected during surgery or cutaneous biopsy procedure	Anaerobic transport system or sterile, screw-cap container; add several drops of sterile saline to keep small pieces of tissue moist.	≤15 min, RT	Always submit as much tissue as possible. If excess tissue is available, save a portion of surgical tissue at −70°C in case further studies are needed. Never submit a swab that has been rubbed over the surface of a tissue.
Urine				
Male and female, first void (for *Chlamydia* and *N. gonorrhoeae*) NAAT		Sterile tube or transport medium specified by NAAT manufacturer	Unpreserved: ≤2 h, RT; ≤24 h, 4°C	Do not collect more than 30 ml for NAAT

Female, midstream	1. While holding the labia apart, begin voiding. 2. After several milliliters has passed, collect a midstream portion without stopping the flow of urine. 3. The midstream portion is used for bacterial culture.	Sterile, wide-mouth container, ≥1 ml, or urine transport tube with boric acid preservative	Unpreserved: ≤2 h, RT; preserved: ≤24 h, RT	
Male, midstream	1. While holding the foreskin retracted, begin voiding. 2. After several milliliters has passed, collect a midstream portion without stopping the flow of urine. 3. The midstream portion is used for culture.	Sterile, wide-mouth container, ≥1 ml, or urine transport tube with boric acid preservative	Unpreserved: ≤2 h, RT	
Straight catheter	1. Thoroughly cleanse the urethral opening with soap and water. 2. Rinse area with wet gauze pads.	Sterile, leakproof container or urine transport tube with boric acid preservative	Unpreserved: ≤2 h, RT; preserved: ≤24 h, RT	Catheterization may introduce members of the urethral flora into the bladder and increase the risk of iatrogenic infection.

(continued)

Table 3.1 Bacteriology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Transport device and/or minimum vol	Transport time and temp	Comments
	3. Aseptically, insert catheter into the bladder. 4. After allowing approximately 15 ml to pass, collect urine to be submitted in a sterile container.			
Indwelling catheter	1. Disinfect the catheter collection port with 70% alcohol. Clamp catheter below port and allow urine to collect in tubing for 10–20 min. 2. Use needle and syringe to aseptically collect 5– 10 ml of urine. 3. Transfer to a sterile tube or container.	Sterile leakproof container or urine transport tube with boric acid preservative	Unpreserved: ≤2 h, RT; preserved: ≤24 h, RT	Patients with indwelling catheters always have bacteria in their bladders. Do not collect urine from these patients unless they are symptomatic.
Wound	See Abscess			

[a]Adapted from Jorgensen J.H, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. (ed.), *Manual of Clinical Microbiology*, 11th ed., ASM Press, Washington, D.C., 2015.

[b]Abbreviations: AFB, acid-fast bacilli; BAL, bronchoalveolar lavage; BAP, blood agar plate; BHI, brain heart infusion; CHOC, chocolate agar; CSF, cerebrospinal fluid; CVP, central venous pressure; i.v., intravenous; PVA, polyvinyl alcohol fixative; RT, room temperature.

Table 3.2 Specimen collection and transport guidelines for infrequently encountered bacteria[a]

Organism (disease)	Specimen of choice	Transport issues	Diagnostic Test of Choice
Anaplasma (human granulocytic ehrlichiosis)	Blood smear, skin biopsy, blood (with heparin or EDTA anticoagulant), CSF,[b] serum	Material for culture sent on ice; keep tissue moist and sterile; hold at 4–20°C until tested or at—7°C for shipment; transport on ice or frozen for PCR test	Serology
Bartonella (cat scratch fever)	Blood, tissue, lymph node aspirate	1 week at 4°C; indefinitely at—70°C	Serology and/or tissue PCR
Borrelia burgdorferi (Lyme disease)	Skin biopsy at lesion periphery, blood, CSF	Keep tissue moist and sterile; hand carry to laboratory if possible	Serology
Borrelia (relapsing fever)	Blood smear (blood)	Hand carry to laboratory if possible	Wright-Giemsa stain of blood smear, serology (available at public health laboratories and some private reference laboratories)
Brucella (brucellosis)	Blood, bone marrow	Transport at room temperature; pediatric lysis-centrifugation tube is helpful	Culture and serology
Klebsiella granulomatis (granuloma inguinale; donovanosis)	Tissue, subsurface scrapings	Transport at room temperature	Culture

(continued)

Specimen Collection

Table 3.2 Specimen collection and transport guidelines for infrequently encountered bacteria[a] *(continued)*

Organism (disease)	Specimen of choice	Transport issues	Diagnostic Test of Choice
Coxiella (Q fever), *Rickettsia* (spotted fevers; typhus)	Serum, blood, tissue	Blood and tissue are frozen at—70°C until shipped	Phase I (chronic) and Phase II (acute) serology
Ehrlichia (ehrlichiosis)	Blood smear, skin biopsy, blood (with heparin or EDTA anticoagulant), CSF, serum	Material for culture sent on ice; keep tissue moist and sterile; hold at 4–20°C until tested or at—70°C for shipment; transport on ice or frozen for PCR test	Blood PCR
Francisella (tularemia)	Lymph node aspirate, scrapings, lesion biopsy, blood, sputum	Rapid transport to laboratory or freeze; ship on dry ice	Culture and serology
Leptospira (leptospirosis)	Serum, blood (citrate-containing anticoagulants should not be used), CSF (first week), urine (after first week)	Blood,<1 h; urine,<1 h or dilute 1:10 in 1% bovine serum albumin and store at 4–20°C or neutralize with sodium bicarbonate	Serology
Streptobacillus (rat bite fever; Haverhill fever)	Blood, aspirates of joint fluid	High-volume bottle preferred	Culture

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW, (ed.), *Manual of Clinical Microbiology*, 11th ed., ASM Press, Washington, DC. 2015.

[b]CSF, cerebrospinal fluid.

Table 3.3 Guidelines for collection of specimens for anaerobic culture[a]

Acceptable material	Unacceptable material
Aspirate (by needle and syringe)	Bronchoalveolar lavage washing
Bartholin's gland inflammation or secretions	Cervical secretions
Blood (venipuncture)	Endotracheal secretions (aspirate)
Bone marrow (aspirate)	Lochia secretions
Bronchoscopic secretions (protected specimen brush)	Nasopharyngeal swab
Culdocentesis fluid (aspirate)	Perineal swab
Fallopian tube fluid or tissue (aspirate/biopsy)	Prostatic or seminal fluid
Intrauterine device, for *Actinomyces* spp.	Sputum (expectorated or induced)
Nasal sinus (aspirate)	Stool or rectal swab samples
Placenta tissue (via cesarean delivery)	Tracheostomy secretions
Stool, for *Clostridium difficile*	Urethral secretions
Surgery (aspirate, tissue)	Urine (voided or from catheter)
Transtracheal aspirate	Vaginal or vulvar secretions (swab)
Urine (suprapubic aspirate)	

[a]Adapted from Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (ed.), *Manual of Clinical Microbiology*, 8th ed., ASM Press, Washington, D.C., 2003.

Virology: General Specimen Guidelines

1. The timing of specimen collection is critical because the duration of viral shedding is influenced by the type of virus, the organ or tissue involved, and the immunocompetence of the patient. For optimal recovery of most viruses, specimens should be collected within 3 to 7 days after onset of symptoms.

2. The method of collection can have a profound effect on detection of viruses. If viral culture is attempted, the viability of the virus must be maintained in appropriate transport medium. If nucleic acid amplification is attempted, the swab composition and anticoagulants can affect the assay.

3. If only a limited amount of material can be collected, the number of tests requested should also be limited.

4. Because viruses are obligate intracellular pathogens, wound and skin specimens (e.g., vesicles) should contain cellular material.

5. Specimens should be transported to the laboratory as quickly as possible, particularly for specimens submitted for viral culture. Viability is not required for antigen or nucleic acid amplification tests.

6. Viral transport medium (VTM) should be used to protect specimens from drying. VTM is not required for cerebrospinal fluids, blood, urine, bronchoalveolar lavage specimens, amniotic fluid, and feces.

7. Specimens other than blood should be maintained at 4°C if held for more than 1 h after collection. Freezing should be avoided unless a delay of more than 24 h is anticipated. Recovery of some enveloped viruses (e.g., respiratory syncytial virus, herpes simplex virus, cytomegalovirus, and varicella-zoster virus) is compromised by freezing.

8. A variety of commercial VTM are available. Most contain protein to stabilize the virus, antibiotics to prevent bacterial and fungal growth, and a buffer to control pH. Culturette swabs with Stuart's medium can also be used.

Virology: Specific Specimen Guidelines

Blood

1. An 8 to 10ml volume of blood is collected using appropriate aseptic techniques.

2. Anticoagulant tubes (EDTA [purple top], heparin [green top], and acid-citrate-dextrose [yellow top]) are used for detection of viruses in plasma or leukocytes. Heparin inhibits PCR and the infectivity of some viruses.

3. Plasma can be obtained by centrifuging blood collected in tubes with anticoagulants. Plasma partitioning tubes can facilitate separation of plasma from cellular material.

4. Plasma for nucleic acid amplification of RNA viruses should be separated within 4 to 6 h of collection and refrigerated for up to 72 h or frozen at –70°C for longer periods.

5. For serologic testing, acute-phase serum should be collected within the first few days of clinical onset, and convalescent-phase serum should be collected 2 to 4 weeks later.

Bone marrow

1. Bone marrow aspirates are collected from the posterior iliac crest, the anterior iliac crest (infants and children), or the sternum or the tibia (infants younger than 18 months).

2. Leukocytes from bone marrow specimens can be cultured for cytomegalovirus (CMV). Varicella-zoster virus (VZV) and human herpesvirus 6 can also be cultured from bone marrow. PCR is used to diagnose parvovirus B19 infections.

CSF

NOTE: Viral culture of CSF is only rarely performed because higher sensitivity alternatives exist for most of the viruses that could be cultured from the CSF.

1. Enteroviruses and herpes simplex virus (HSV) are the most common viruses grown from cerebral spinal fluid (CSF). Arboviruses are important causes of sporadic encephalitis but are difficult to culture. These infections are most commonly diagnosed by nucleic acid–based tests. Likewise, CMV, VZV, Epstein-Barr virus (EBV), and JC virus infections are most commonly diagnosed by nucleic acid–based tests.

2. Viral titers are generally low in CSF; therefore, the specimen should not be diluted and tests should be selectively performed.

3. CSF should be collected in a sterile tube without transport medium.

4. CSF for viral culture does not require special processing.

Respiratory specimens (throat, nasopharyngeal swab, nasopharyngeal aspirate, nasal washings, and bronchoalveolar lavage [BAL]) specimens)

1. Influenza viruses, parainfluenza viruses, RSV, adenoviruses, and rhinoviruses are most frequently identified in respiratory specimens. Metapneumoviruses and coronaviruses are also important respiratory pathogens.

2. With the adoption of the flocked swab, it is now thought that nasopharyngeal aspirates are equivalent to nasopharyngeal swabs for specimen collection.

3. Nasal washes do not contain a large number of virus-infected cells; however, they are often used when nasal aspiration is contraindicated.

4. Nasopharyngeal aspirates are typically transported in appropriate viral transport medium (VTM). The use of VTM is optional for nasal washes and BAL specimens.

5. All specimens can be used for viral culture. However, nasopharyngeal aspirates and washes are preferred for antigen detection.

6. Mucus in specimens can affect antigen detection by fluorescent-antibody (FA) assay and enzyme immunoassays (EIA). Mucus can inhibit the fixation of cells to slides (FA assay) and can cause nonspecific fluorescence. It can also interfere with penetration of the specimen into EIA membrane devices. Therefore, specimens should be broken up by glass beads or aspiration through a small-bore pipette before processing.

Urine

1. Urine is an important specimen for detection of CMV, enteroviruses, adenoviruses, and BK virus. Mumps and rubella viruses are rarely isolated in urine because of the use of vaccines.

2. Urine should be cultured for CMV within 7 days of birth to detect congenital infection.

3. Midstream urine should be collected in a sterile container. VTM is not required.

4. Before urine is cultured, it should be neutralized with 7.5% sodium bicarbonate solution and filtered through a 0.2-μm-pore-size filter to remove contaminating bacteria.

Feces

1. Many viruses responsible for gastroenteritis (e.g., enteric adenovirus, calicivirus, astrovirus, and rotavirus) cannot be cultivated.

2. Enveloped viruses are not normally recovered in feces, with the exception of CMV in immunocompromised patients.

3. Fecal specimens (2 to 4 g) are preferred to fecal swabs because an inadequate amount of material is collected on swabs.

Eyes

1. HSV and adenovirus are the most commonly isolated viruses; enterovirus 70 and coxsackievirus A24 may be detected by PCR.

2. Conjunctival swabs are collected from the lower conjunctiva with a flexible, fine-shaft swab moistened with sterile saline and placed in VTM.

3. Scrapings of cornea or conjunctiva should also be placed in VTM.

4. Aqueous and vitreous fluids can inhibit PCR; therefore, the specimen must be diluted or extracted to remove inhibitors.

Tissue

1. Many viruses can be isolated from tissues.

2. Tissues should be transported in VTM.

3. As much tissue as possible should be collected and submitted to the clinical microbiology and surgical pathology laboratories.

4. Upon receipt in the laboratory, tissues should be ground and centrifuged and the supernatant should be used for processing.

5. Tissue for nucleic acid detection should be minced, treated with proteolytic enzymes, and extracted with chaotropic salts or organic solvents.

Genital specimens

1. HSV-2 and HSV-1 are the most commonly isolated viruses from external genital lesions. HSV-1, HSV-2, and CMV are frequently isolated from the cervix, vagina, and urethra. These viruses can be readily cultured from these sites. Human papillomavirus, an important cause of cervical cancer, can be detected by molecular tests.

2. Genital lesions should be swabbed vigorously to collect cellular material, and the specimen should be transported to the laboratory in VTM.

3. Cervical specimens are collected by inserting a clean swab 1 cm into the cervical canal and rotating it for 5 s. The swab is transported in VTM.

4. To collect urethral specimens, exudates should be expressed and discarded. The patient should not have urinated for at least 1 h prior to specimen collection. A flexible, fine-shafted swab is inserted 4 cm into the urethra, rotated two or three times, removed, and placed in VTM.

Skin

1. Rubella virus, measles virus, adenoviruses, and enteroviruses can cause dermal rashes and be isolated in culture. Parvovirus B19 can cause a rash but is recovered from other sources. HSV, VZV, and enteroviruses can be recovered from vesicular lesions.

2. Fresh dermal lesions (not crusted, healing lesions) should be used for recovery of viruses.

3. Vesicular fluid and cells from the base of the lesion should be collected and transported to the laboratory in VTM.

Table 3.4 Recommended blood volumes to collect for blood cultures[a]

Patient weight (lb)	Recommended blood volume per culture (ml)	Total blood volume for two cultures (ml)	Volume of blood equal to 1% of patient's total blood volume (ml)
<18	1	2	2
18–30	3	6	6–10
30–60	5	10	10–20
60–90	10	20	20–30
90–120	15	30	30–40
>120	20	40	>40

[a]Jorgensen J H, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology*, 11th ed., ASM Press, Washington, D.C., 2015.

Table 3.5 Mycology: Collection and transport guidelines[a,b]

Specimen type	Collection guidelines	Processing procedure	Time and temperature for transport	Comments
Abscess (drain, exudate, pus, wound)				
	Clean surface with 70% alcohol. Collect from active peripheral edge with a sterile needle and syringe. If open, use swab system or aspirate	If thick, pretreat similarly to sputum specimens.	If ≤2 h, RT.	Examine for grains or granules and note color if present.
Blood				
	Collect as for bacterial cultures; disinfect skin with tincture of iodine; use maximum amount of blood recommended	Manual	If ≤2 h, RT; if longer RT.	
		Automated (BacT/Alert, BACTEC, VersaTrek)	As above	
		Lysis-centrifugation	As above; process in ≤16 h.	Lysis-centrifugation systems are good for recovery of molds, especially those causing endemic mycoses. They give high contamination and false-positive rates.

		Biphasic bottles	If ≤2 h, RT; if longer RT.	
Dimorphic/ filamentous fungi	As above	Lysis-centrifugation	As above	
Bone marrow	Collect aseptically in a heparinized syringe or lysis-centrifugation tube	Clotted bone marrow is unacceptable.	≤15 min, RT, If longer, RT.	Pediatric Isolator tubes are best.
Cutaneous (hair, skin, nails)	Disinfect all types with 70% alcohol Hair: hair root is most important, plucking is best; submit 10 to 12 hairs in sterile dry container or envelope. Skin: scrape with dull edge of a scalpel or glass slide or vigorously brush in a circular motion with a soft-bristle toothbrush. Nails: clip or scrape with a scalpel. Material under nail should also be scraped. Submit in a sterile container or clean dry paper envelope	Only the leading edge of a lesion should be sampled, as centers are often nonviable. All specimens should be pressed gently into the agar with a sterile swab; do not streak agar plates. If used, toothbrushes should be pressed gently into agar as well.	If ≤72 hr, RT (very stable), Never refrigerate, as dermatophytes are sensitive to cold.	Select hairs that fluoresce under a Wood's light. Hair and skin can be collected with a soft-bristle toothbrush. For pityriasis versicolor (*M. furfur*), olive oil or a paper disk saturated with olive oil should be placed on the first quadrant of agar plate.
Ear, external	Firmly rotate swab in outer ear canal		If ≤2 h, RT; if longer RT.	

(continued)

Specimen Collection

Table 3.5 Mycology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Processing procedure	Time and temperature for transport	Comments
Eye				
Corneal scrapings	Corneal scraping: taken by physicians and media/slides inoculated directly	Corneal: inoculate noninhibitory media in X or C-shaped motion	If ≤2 h, RT; if longer RT.	Very little material is usually available. Avoid media with cycloheximide.
Vitreous fluid	Needle aspiration	Concentrate by centrifugation; use sediment for media and smears	If ≤2 h, RT; if longer RT.	
Prostatic fluid	Have patient empty bladder, and then massage prostate gland to yield fluid	Inoculate media directly or transport in sterile wide-mouth container.	If ≤2 h, RT; if longer RT.	Fluid should always be examined microscopically. The first urine following massage has a high yield. This fluid is excellent for detection of endemic mycoses.
Respiratory, lower (BAL, wash, brush, aspirate, sputum)	Use first morning sputum collected after brushing teeth. Collect brushing and BAL fluid surgically. Place all samples in sterile containers. Inoculate media containing antimicrobial agents with and without cycloheximide.	Viscous lower respiratory specimens should be pretreated and centrifuged to concentrate their contents.	If ≤2 h, RT; if longer, 4°C.	Short survival time for dimorphic pathogens. Saliva or 24-h sputum is an unacceptable specimen. Methods for mycobacteria decontamination are not acceptable.

Respiratory, upper (oral, oropharyngeal, and sinus samples)	Swab oral lesions, avoiding tongue. Use a thin wire or flexible swab for oropharynx. Collect sinus contents surgically	Use swab transport system for oral and oropharyngeal samples. Place sinus contents in sterile container.	Oral: if ≤2 h, RT; if longer, 4°C. Sinus: If ≤2 h, RT; if longer, RT.	Selective and chromogenic media are best for recovery of *Candida*
Sterile body fluids (CSF and pericardial, peritoneal, and synovial fluids)	Collect as for bacteriology. Concentrate by centrifugation, and use sediment for inoculation. Clots should be ground	Except CSF, put sterile body fluids in sterile vacutainer tubes with heparin or in a lysis-centrifugation tube to prevent blood clotting. Except for CSF, blood culture bottle scan be used for recovery of yeast.	If ≤2 h, RT; if longer, RT. Never refrigerate.	Sterile fluid sediment should always be examined microscopically. With specimen volumes <2 ml, fluid should be plated directly using as much fluid on each plate as possible.
Stool				Specimen should be discouraged
Tissue biopsy specimen	Surgical collection, large quantity required than for bacteriology	Sterile container; add a few drops of sterile saline to keep moist	If ≤2 h, RT; if longer, RT.	Tissue biopsy recommended for invasive disease. Examine subcutaneous tissue for granules and see information for abscesses.

(continued)

Table 3.5 Mycology: Collection and transport guidelines[a,b] *(continued)*

Specimen type	Collection guidelines	Processing procedure	Time and temperature for transport	Comments
Urine	As for bacterial cultures; early-morning specimen preferred, or catheterized specimens; 24-h specimens are discouraged.	Sterile wide-mouth container or urine transport system. Concentrate specimens by centrifugation and inoculate sediment.	If ≤2 h, RT; if longer, 4°C. Urine transport systems can stay at RT for longer according to manufacturer recommendations.	Chromogenic media best for *Candida*. Use sediment for microscopic examination.
Vaginal	As for bacterial cultures	Swab transport system or sterile container for washings	If ≤2 h, RT; if longer, RT.	Antibacterial media or chromogenic agars are best for recovery of *Candida*.

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinica*$_l$ *Microbiology*, 11th ed., ASM Press, Washington, D.C., 2015.

[b]Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; RT, room temperature; h, hours.

Table 3.6 Parasitology: Specimen guidelines[a]

Body site	Specimen and procedures[b]	Recommended stain(s) and relevant parasites[b]
Blood	Microscopically: thin and thick blood films. Fresh blood (preferred) or EDTA-blood (fill EDTA tube completely with a blood and then mix).	Giemsa (all blood parasites); hematoxylin-based stain (sheathed microfilariae). Although Wright's stain (Wright-Giemsa combination stain) works, stippling in malaria may not be visible, and the organisms; colors may not match the descriptions. However, with other stains (those listed previously, in addition to some of the "quick" blood stains), the organisms should be detectable on the blood films.
	Concentration methods: EDTA-blood	Buffy coat, fresh blood films for detection of moving microfilariae or trypanosomes
	Antigen detection: EDTA-blood for malaria, serum or plasma for circulating antigens (hemolyzed blood can interact in some tests)	QBC, a screening method for blood parasites (hematocrit tube contains acridine orange), has been used for malaria, *Babesia,* trypanosomes, and microfilariae. It is usually impossible to identify malaria organisms to the species level: requires high levels of training. Commercial immunoassay test kits for malaria and some microfilariae. Sensitivity is not higher than for thick films for *Plasmodium* spp., much more sensitive for *Leishmania* spp. (peripheral blood is used from immunodeficient patients only).
	PCR: EDTA-blood, ethanol fixed or unfixed thin and thick blood films, coagulated blood, possibly with hemolyzed or frozen blood samples	Sequencing of PCR product is often used for species or genotype identification.

(continued)

Table 3.6 Parasitology: Specimen guidelines[a] *(continued)*

Body site	Specimen and procedures[b]	Recommended stain(s) and relevant parasites[b]
	Specific antibody detection: serum or plasma, anticoagulated or coagulated blood (hemolyzed blood can cause problems in some tests)	Most commonly used are EIA (many test kits commercially available), EITB (commercially available for some parasites), and IFA.
Bone marrow	Biopsy samples or aspirates Microscopy: thin and thick films with aspirate collected in EDTA Cultures: sterile material in EDTA or culture medium	Giemsa (all blood parasites). Sterile specimen required for cultures. Culture for *Leishmania* spp. (or *T. cruzi*) PCR for blood parasites
Central nervous system	Microscopy: spinal fluid and CSF (wet examination, stained smears), brain biopsy (touch or squash preparations, stained)	Giemsa (trypanosomes, *Toxoplasma gondii*); Giemsa, trichrome, or Calcofluor (amebae [*Naegleria*—PAM, *Acanthamoeba, Balamuthia*— GAE]); Giemsa, acid-fast, PAS, modified trichrome, silver methenamine (microsporidia) (tissue Gram stains also recommended for microsporidia in routine histologic preparations); H&E, PAS, routine histology (larval cestodes: *Taenia solium* cysticerci, *Echinococcus* spp.). Bright-field microscopy, look for motile amoeba in unstained slide (wet prep). Slides warmed to 35°C encourage motility.
	Culture: sterile aspirate or biopsy material (in physiological saline)	Free-living amebae (exception: *Balamuthia* spp. do not grow in the routine agar/bacterial overlay method). *Toxoplasma* spp. can be cultured in tissue culture media.

	PCR: aspirate or biopsy material, native, frozen, or fixed in ethanol	Protozoa and helminths: species and genotype characterization
Cutaneous ulcers	Microscopy: aspirate biopsy material (smears, touch or squash preparation, histologic sections) Culture: native material (see above) in PBS supplemented with antibiotics if possible to avoid bacterial growth PCR: native material in physiological NaCl or PBS, ethanol, or frozen	Calcofluor (*Acanthamoeba* spp. cysts only); Giemsa (amebic trophozoites, cysts); modified trichrome (preferred) or silver methenamine stain, PAS, acid-fast (micropsoridial spores; H&E, routine histology (cysticerci, *Loa Loa, Toxoplasma* spp.) Cultures: free-living amebae and *Toxoplasma* spp. Free-living amebae, *Toxoplasma,* microsporidial species and genotype identification.
Eye	Microscopy: biopsy material (smears, touch or squash preparation), scrapings, contact lens, sediment of lens solution Culture: native material (see above) in PBS supplemented with antibiotics if possible with antibiotics, if possible to avoid bacterial growth PCR: native material in physiological NaCl or PBS, ethanol, or frozen	Calcofluor for cysts only (amebae [*Acanthamoeba*]); Giemsa for trophozoites and cysts (amebae); H&E for routine histology (cysticerci, *Loa loa, Toxoplasma gondii*); silver methamine stain, PAS, acid-fast, EM (for microsporidia) Cultures: free-living amebae and *Toxoplasma* spp. Free-living amebae, *Toxoplasma,* microsporidial species and genotype identification

(continued)

Table 3.6 Parasitology: Specimen guidelines[a] *(continued)*

Body site	Specimen and procedures[b]	Recommended stain(s) and relevant parasites[b]
Intestinal tract	Stool and other intestinal material Microscopy: stool, sigmoidoscopy material, duodenal contents (all fresh or preserved (see Table 4), direct wet smear, concentration methods Anal impression smear Adult worms or tapeworm segments (proglottids)	Concentration methods: formalin-ethyl acetate sedimentation of formalin- or SAF-fixed stool samples (most protozoa); flotation or combined sedimentation flotation methods (helminth ova); agar or Baermann concentration (larvae of *Strongyloides* spp.; unpreserved stool required) Direct wet smear (direct examination of unpreserved fresh material is also used); (motile protozoan trophozoites; helminth eggs and protozoan cysts may also be detected) Stain: trichrome or iron hematoxylin (intestinal protozoa); modified trichrome (microsporidia); modified acid-fast (*Cryptosporidium* spp., *Cyclospora* spp., *Cystoisospora* spp.) Adhesive cellulose tape, no stain (*E. vermicularis*) Carmine stains (rarely used for adult worms or cestode segments). Proglottids can usually be identified to the genus level (*Taenia* spp., *Diphyllobothrium* spp. *Hymenolpesis* spp.) without using tissue stains.
	Antigen detection: fresh native or frozen material; suitability of fixation is test dependent PCR: native material, fresh, frozen or ethanol-fixed Biopsy material Microscopy: fixed for histology or touch or squash preparations for staining	Commercial immunoassays (e.g., EIA, FA), cartridge formats (*E. histolytica, Giardia* spp., and *Cryptosporidium* spp.) In-house tests for *Taenia solium* and *Taenia saginata*. No commercial test available. Primers for genus or species identification of most helminths and protozoa are published. H&E, routine histology (*E. histolytica, Cryptosporidium* spp., *Cyclospora* spp., microsporidia); less common findings include *Schistosoma* spp., hookworm, or *Trichuris* spp.

Liver and spleen	Biopsy samples or aspirates Microscopy: unfixed material in physiological NaCl; fixed for histology Culture: sterile preparation of native material Animal inoculation: sterile preparation of native material Animal inoculation: sterile preparation of native material PCR: native material, frozen or ethanol fixed	Examination of wet smear for *E. histolytica* (trophozoites), protoscolices of *Echinococcus* spp. or eggs of *Capillaria hepatica*. Giemsa stain (*Leishmania* spp., other protozoa and microsporidia); H&I (routine histology) For *Leishmania* (not common) Intraperitoneal inoculation of *E. multilocularis* cyst material for viability test after long-term chemotherapy Species or genotype identification (e.g., *Echinococcus* spp.)
Respiratory tract	Sputum, induced sputum, nasal and sinus discharge, bronchoalveolar lavage fluid, transbronchial aspirate, tracheobronchial aspirate, brush biopsy, open-lung biopsy; sterile for bronchoalveolar lavage fluid and air-dried smears. Microscopy: unfixed material, treated for smear preparation PCR: unfixed native material, frozen or fixed in ethanol	Modified acid-fast stains *(Cryptosporidium* spp.); H&E, routine histology *(Strongyloides stercoralis, Paragonimus* spp., *Dirofilaria* spp., amebae); silver methenamine stain, PAS, acid-fast, tissue Gram stains, modified trichrome, EM (microsporidia); Giemsa, silver methenamine, toluidine blue stains available for *Pneumocystis jiroveci*. Some helminth larvae (*Ascaris lumbricoides, Strongyloides stercoralis*), eggs (*Paragonimus* spp.), or hooklets (*Echinococcus* spp.) can be recovered in unstained respiratory specimens.

(continued)

Table 3.6 Parasitology: Specimen guidelines[a] *(continued)*

Body site	Specimen and procedures[b]	Recommended stain(s) and relevant parasites[b]
Muscle	Biopsy material Microscopy: unfixed, touch and squash preparations or fixed for histology and EM PCR: unfixed or native, frozen or ethanol fixed	Larvae of *Trichinella* spp. can be identified unstained (species identification with single larvae by PCR). H&E, routine histology (*Trichinella* spp., cysticerci); silver methenamine stain, PAS, acid-fast, tissue Gram stains, EM (rare microsporidia). Microsporidial identification to the species level requires subsequent sequencing.
Skin	Aspirates, skin snip, scrapings, biopsy	See Cutaneous ulcer (above).
	Microscopy: wet examination, stained smear (or fixed for histology or EM) PCR: unfixed native, frozen or fixed in ethanol	Wet preparations (microfilariae) Giemsa-stained smears or H&E, routine histology (*Onchocerca volvulus, Dipetalonema streptocerca, Dirofilaria repens,* leishmaniae, *Acanthamoeba* spp., *Entamoeba histolytica,* microsporidia), other larvae causing cutaneous larva migrans (zoonotic *Strongyloides* spp., hookworms), and arthropods (scabies and other mites) Primers for most parasite available.

Amniotic Fluid	PCR (and/or culture): native material Animal inoculation (*Toxoplasma*)	PCR based on the detection of highly repetitive gene sequences is the method of choice.
Urogenital system	Vaginal discharge, saline swab, transport swab (no charcoal), culture medium, plastic envelope culture, air-dried smear for FA; urethral discharge, prostatic secretions; urine, single unpreserved specimen, 24-h unpreserved specimen, early-morning specimen. Microscopy: wet smears, smears of urine sediment, stained smears Cultivation: vaginal or urethral discharge or swab preparations Molecular: native material, frozen or fixed in ethanol	Giemsa, immunoassay reagents (FA) (*Trichomonas vaginalis*); Delafield's hematoxylin (microfilariae); modified trichrome (microsporidia); H&E, routine histology (*Schistosoma haematobium,* microfilariae); PAS, acid-fast, tissue Gram stains, EM or PCR (microsporidia). Identification and propagation of *T. vaginalis* (commercial plastic envelope culture systems available); moving trophozoites can be detected microscopically (or in Giemsa-stained smears).

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]Abbreviations: CSF, cerebrospinal fluid; EIA, enzyme immunoassay; EM, electron microscopy; FA, fluorescent antibody; GAE, granulomatous amebic encephalitis; GI, gastrointestinal; H&E, hematoxylin and eosin; PAM, primary amebic encephalitis; PAS, periodic acid–Schiff stain.

Specimen Collection

Table 3.7 Guidelines for processing stool specimens for parasites[a,b]

Option	Pros	Cons
Rejection of stools from inpatients who have been in hospital for >3 days.	Patients may become symptomatic with diarrhea after they have been inpatients for a few days; symptoms are usually attributed not to parasitic infections but generally to other causes.	There is always the chance that the problem is related to a nosocomial parasitic infection (rare), but *Cryptosporidium* and microsporidia are possible considerations.
Examination of a single stool (O&P examination). Data suggest that 40–50% of organisms present will be found by only a single stool exam; any patient remaining symptomatic would require additional testing. Examination of two specimens is acceptable, but three are more sensitive.	If parasites are diagnosed in the first sample or if the patient becomes asymptomatic after collection of the first stool, subsequent specimens may not be necessary. However, with some intestinal parasitic infections, patients may alternate with constipation and diarrhea.	Diagnosis from a single stool exam depends on the experience of the microbiologist, proper collection, and the parasite load in the specimen. In a series of three stool specimens, it is often the case that not all three specimens are positive and/or may be positive for different organisms.
Examination of a second stool specimen only after the first is negative and the patient is still symptomatic.	With additional examinations, the yield of protozoa increases (*Entamoeba histolytica,* 22.7%; *Giardia duodenalis,* 11.3%; and *Dientamoeba fragilis,* 31.1%).	Assumes the second (or third) stool specimen is collected within the recommended 10-day time frame for a series of stools; protozoa are shed periodically. May be inconvenient for the patient.

Examination of a single stool and an immunoassay (EIA, FA cartridge) (*Giardia*); this approach is a mix: one immunoassay is acceptable; one O&P exam is not the best approach.	If the exams are negative and the patient's symptoms subside, probably no further testing is required.	Patients may exhibit symptoms (off and on), so it may be difficult to rule out parasitic infections with only a single stool specimen and immunoassay. If the patient remains symptomatic, then even if the *Giardia* immunoassay is negative, other protozoa may be missed (*E. histolytica*, *E. dispar*, and *D. fragilis*).
Pooling of three specimens for examination; one concentrate and one permanent stain are performed. The laboratory pools the specimens.	Three specimens are collected over 7 to 10 days and may save time and expense.	Procedure not recommended. Organisms present in small numbers may be missed due to the dilution factor. May be difficult to coordinate the collection of three specimens in a reasonable time frame.
Pooling of three specimens for examination; a single concentrate and three permanent stained smears are performed.	Three specimens are collected over 7 to 10 days; this would maximize recovery of protozoa in areas of the country where these organisms are most common.	Light helminth infection (eggs, larvae) might be missed due to the pooling of the three specimens for the concentration; however, with a permanent stain performed on each of the three specimens, this approach would probably be the next best option after the standard approach (concentration and permanent stained smear performed on every stool). May be difficult to coordinate the collection of three specimens in a reasonable time frame.

(continued)

Table 3.7 Guidelines for processing stool specimens for parasites[a,b] *(continued)*

Option	Pros	Cons
Collection of three stools; samples of stool from all three collections are placed in a single vial (patient is given a single vial only).	Pooling of the specimens would require only a single vial.	Not recommended. This would complicate patient collection and very probably result in poorly preserved specimens, especially regarding the recommended ratio of stool to preservative and the lack of proper mixing of specimen and fixative.
Perform immunoassays on selected patients (children <5 years, children from day care centers, patients with immunodeficiencies, and patients from diarrheal outbreaks) for intestinal protozoa.	Would be more cost effective than performing immunoassay procedures on all specimens; however, information required to group patients is often not received with specimens. Client education required for appropriate test orders.	Labs rarely receive information that would allow them to place a patient in a particular risk group such as children <5 yr old, children from day care centers (who may or may not be symptomatic), patients with immune deficiencies, and patients from outbreaks. Performance of immunoassay procedures on every stool specimen is not cost effective, and the positive rate will be below unless an outbreak situation is involved.

[a]Adapted from Jorgensen JH, Pfaller M. Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology*, 11th ed., ASM Press, Washington, D.C., 2015.

[b]Abbreviations: EIA, enzyme immunoassay; FA, fluorescent antibody; O&P, ova and parasite.

SECTION 4

Bacterial Diagnosis

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This section provides guidelines for the selection and processing of specimens for the detection of specific bacteria. Testing can be subdivided into microscopy, culture, antigen tests (including immunoassays and molecular diagnostic tests), and antibody tests. Although it is impossible to provide guidelines for all possible infections, the most common bacteria associated with human disease are included. This section has been expanded to include summary tables of identification tests as well as a more detailed discussion of the immunological detection of organisms where appropriate.

Table 4.1 Detection methods from clinical specimens for clinically relevant bacteria[a]

Organism	Microscopy	Culture	Antigen detection	Antibody detection	Molecular diagnostics
Aerobic Gram-positive cocci					
Staphylococcus aureus	A	A	B	D	B
Streptococcus, group A	B	A	A	B	A
Streptococcus, group B	B	A	C	D	B
Streptococcus pneumoniae	A	A	C	D	C
Enterococcus spp.	A	A	D	D	A
Aerobic Gram-positive rods					
Bacillus anthracis	A	A	D	D	C
Listeria spp.	B	A	D	D	C
Erysipelothrix spp.	A	A	D	D	D
Corynebacterium diphtheriae	A	A	D	D	C
Corynebacterium, other spp.	A	A	D	D	D
Gardnerella vaginalis	A	B	D	D	D
Acid-fast and partially acid-fast Gram-positive rods					
Mycobacterium tuberculosis complex	A	A	D	D	A
Mycobacterium avium complex	A	A	D	D	C
Nocardia spp.	A	A	D	D	D
Rhodococcus spp.	A	A	D	D	D

(continued)

Bacterial Diagnosis

Table 4.1 Detection methods from clinical specimens for clinically relevant bacteria[a] *(continued)*

Organism	Microscopy	Culture	Antigen detection	Antibody detection	Molecular diagnostics
Aerobic Gram-negative cocci					
Neisseria gonorrhoeae	A	A	D	D	A
Neisseria meningitidis	A	A	B	D	D
Moraxella catarrhalis	A	A	D	D	D
Aerobic Gram-negative rods					
Actinobacillus spp.	A	A	D	D	D
Pasteurella spp.	A	A	D	D	D
Capnocytophaga spp.	A	A	D	D	D
Kingella spp.	A	A	D	D	D
Eikenella spp.	A	A	D	D	D
Cardiobacterium spp.	A	A	D	D	D
Streptobacillus spp.	A	A	D	D	D
Haemophilus influenzae	A	A	C	D	C
Haemophilus ducreyi	B	A	D	D	C
Escherichia coli	A	A	D	D	B
Salmonella enterica serovar Typhi	A	A	D	C	B
Salmonella, other serovars	A	A	D	D	B
Shigella spp.	A	A	D	D	B
Yersinia pestis	A	A	D	B	C
Yersinia enterocolitica	A	A	D	B	B
Other *Enterobacteriaceae*	A	A	D	D	D

Aeromonas spp.	A	A	D	D	D
Vibrio cholerae	A	A	D	C	B
Vibrio, other spp.	A	A	D	D	B
Pseudomonas aeruginosa	A	A	D	D	D
Burkholderia pseudomallei	A	A	D	C	C
Burkholderia cepacia complex	A	A	D	D	C
Stenotrophomonas spp.	A	A	D	D	D
Acinetobacter spp.	A	A	D	D	D
Bordetella pertussis	B	B	D	A	A
Francisella spp.	B	A	D	A	C
Brucella spp.	B	A	C	A	D
Legionella spp.	B	A	A	B	C
Bartonella spp.	C	B	D	A	B
Campylobacter spp.	B	A	C	D	B
Helicobacter pylori	B	B	A	A	C
Anaerobic bacteria					
Clostridium perfringens	A	A	D	D	D
Clostridium botulinum	B	B	A	D	D
Clostridium tetani	A	A	D	D	D
Clostridium difficile	A	B	A	D	A
Actinomyces spp.	A	A	D	D	D
Mobiluncus spp.	A	B	D	D	D
Bacteroides fragilis group	A	A	D	D	D
Fusobacterium spp.	A	A	D	D	D

(continued)

Bacterial Diagnosis

Table 4.1 Detection methods from clinical specimens for clinically relevant bacteria[a] *(continued)*

Organism	Microscopy	Culture	Antigen detection	Antibody detection	Molecular diagnostics
Curved and spiral-shaped bacteria					
Leptospira spp.	B	C	D	A	C
Borrelia burgdorferi	C	C	D	A	B
Borrelia, other spp.	A	C	D	C	D
Treponema pallidum	A	D	D	A	D
***Mycoplasma* spp. and obligate intracellular bacteria**					
Mycoplasma pneumoniae	D	C	C	A	A
Chlamydia trachomatis	B	B	A	B	A
Chlamydophila psittaci	D	B	D	A	D
Chlamydophila pneumoniae	D	C	D	B	A
Rickettsia rickettsii	B	D	D	A	B
Ehrlichia spp.	B	D	D	A	A
Anaplasma spp.	B	D	D	A	B
Coxiella spp.	D	D	D	A	B

[a]A, test generally useful; B, test useful under certain circumstances or for the diagnosis of specific forms of infection; C, test seldom useful for general diagnostic purposes but may be available in reference labs; D, test not generally useful.

Table 4.2 Recommendations for Gram stain and plating media[a,b]

Specimen or organism	Gram stain	Aerobic media	Anaerobic media	Comments[c]
Body cavity fluids				Blood culture bottles should be used to incubate large volumes of specimens for all body cavity fluids. Following manufacturers recommendations
CSF (routine)	x	B C		
CSF (shunt)	x	B C Th		
Pericardial	x	B C	BBA	
Pleural	x	B C	BBA	
Peritoneal	x	B C Mac CNA	BBA LKV BBE	
CAPD	x	B C Th	BBA	
Synovial	x	B C		
Bone marrow	x	B C	BBA	
Catheter tip		B		
Ear external fluid/swab	x	B C Mac		
Ear internal fluid	x	B C	BBA	
Eye	x	B C		
Gastrointestinal tract				
Feces		B Mac HE Ca EB (optional); sorbitol-MAC/chromogenic agar/Shiga toxin testing		*C. jejuni/coli* in 5% O_2–10%, CO_2–85% N_2 at 42°C for all gastrointestinal tract specimens
Rectal swab		B Mac HE Ca EB		

(continued)

Bacterial Diagnosis

Table 4.2 Recommendations for Gram stain and plating media[a,b] *(continued)*

Specimen or organism	Gram stain	Aerobic media	Anaerobic media	Comments[c]
Genital tract				
Vaginal/cervix	X	B TM		Gram stain and NOT culture is the method of choice for the diagnosis of BV
Urethra/penis	X	TM		
Other	X	B C Mac TM	BBA LKV BBE	
Group B streptococcal screen		Selective broth, subculture to B		
Lower respiratory tract				
Sputum	x	B C Mac (Cystic Fibrosis); BCSA Mannitol Salt		
Tracheal aspirate	x	B C Mac		
Bronchoalveolar lavage fluid	x	B C Mac CNA		
Bronchoscopy brushing, washing	x	B C Mac CNA	Protected bronchoscope brushing required for anaerobic culture BBA LKV	
Tissue	x	B C Mac Th CNA	BBA LKV BBE	

Upper respiratory tract				
Nasopharynx		B C		
Nose		B		
Throat		B or SSA		Add chocolate agar for epiglottitis
Urine		B Mac or Chromogenic agar		
Wound or abscess				
Swab	x	B C Mac CNA	BBA LKV BBE	Anaerobic culture only if separate swab transported in appropriate system
Aspirate	x	B C Mac CNA	BBA LKV BBE	
Selected organisms				
Bordetella pertussis and *B. parapertussis*		Regan Lowe		
Brucella spp.		B C		
Corynebacterium diphtheriae		Cysteine-tellurite or Loeffler's serum or CNA if above not available		
Clostridium difficile		CCFA		NAAT more sensitive
E. coli O157:H7 (EHEC)		Sorbitol-Mac Chromogenic agar		Shiga toxin EIA or NAAT more sensitive
Francisella tularensis		C or BCYE		

(continued)

Bacterial Diagnosis

Table 4.2 Recommendations for Gram stain and plating media[a,b] *(continued)*

Specimen or organism	Gram stain	Aerobic media	Anaerobic media	Comments[c]
Haemophilus ducreyi		C + vancomycin (3 μg/ml)		Gram stain resembling "school of fish"
Helicobacter pylori	*x*	B or BHI		*Campylobacter* gaseous atmosphere at 35–37°C
Legionella spp.		BCYE		
Leptospira spp.		Fletcher's medium or EMJH		30°C for up to 13 wk
Neisseria gonorrhoeae		TM		
Nocardia		BCYE		
Vibrio spp.		TCBS		
Yersinia enterocolitica		CIN		

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Yolken RH (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]CAPD, fluid from chronic ambulatory peritoneal dialysis; B, blood agar; C, chocolate blood agar, Mac, MacConkey agar; Th, thioglycolate broth; Ca, Campylobacter agar; HE, Hektoen enteric; EB, enrichment broth; SSA, group A *Streptococcus* selective agar, TM, Thayer-Martin; BCYE, buffered charcoal yeast extract; TCBS, thiosulfate citrate bile salt sucrose; CIN, cefsulodin-Irgasan-novobiocin; BBA, brucella blood agar; LKV, laked blood with kanamycin and vancomycin; BBE, bacteroides bile esculin; CNA, anaerobic colistin-nalidixic acid; CCFA, cycloserine-cefoxitin-fructose agar; EMJH, Ellinghausen-McCullough-Johnson-Harris medium.

[c]Set up anaerobic culture upon request, if specimen is collected and transported appropriately. Call physician if appropriate specimen does not have request for anaerobic culture.

Table 4.3 Screening specimens for routine bacterial culture[a]

Specimen	Screening method	Results of screen[b]	
		Acceptable	**Unacceptable**
Sputum	Microscopic examination of Gram-stained smear	<10 SEC/average 10x field	>10 SEC/average 10x field
Endotracheal aspirate	Microscopic examination of Gram-stained smear	<10 SEC/average 10x field and bacteria detected in at least 1 of 20 fields (100x)	>10 SEC/average 10x field and no bacteria detected in 20 fields (100x)
Bronchoalveolar lavage fluid	Microscopic examination of Gram-stained smear	<1% of cells present are SECs	>1% of cells present are SEC
Superficial wound	Microscopic examination of Gram-stained smear	<2 + SEC, PMNs present	>2 + SEC and no PMNs
Stool for bacterial pathogens	Days in hospital	≤3 days	>3 days (exception: physician provides good rationale)
Urine	Urinalysis, Gram stain or urine sediment	Positive dipstick leukocyte esterase test result or seeing >10 PMNs/mm^3 is an indicator or possible infection, but no method has yet proved truly reliable. One bacterium per oil power field corresponds to 10,000 CFU/ml in the urine.	Growth of three or more potential pathogens usually indicates biofilm on indwelling catheter or fecal contamination. Mixed fecal morphologies on Gram stain may indicate fistula into bladder from GI tract.

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Yolken RH (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]LE, leukocyte esterase; SEC, squamous epithelial cells.

Bacterial Diagnosis

Table 4.4 Processing specimens for mycobacterial identification

		Solid and liquid media at:	
Specimen type[a]	**Smear**[b]	**35°C**	**30°C**
Abscess	R	X	
Blood/bone marrow	N	X	
Biopsy specimen			
Lung	R	X	
Not lung, lymph node, skin, or synovium	O	X	
Skin, synovium, and lymph node[c]	O	X	X
Superficial skin, wound, or tissue[c]	R	X	X
Eye	O	X	X
Fluids			
Not joint or synovial fluid	O	X	
Joint and synovial fluid	O	X	X
Gastric washing	R	X	
Respiratory (not mouth)	R	X	
Stool	O	X	
Urine	O	X	

[a]Sources not generally recommended for mycobacterial culture include genital sites, ears, catheter, mouth, and rectal swabs. Consult laboratory prior to making a request.

[b]R, staining should be performed routinely; 0, staining is optional and should be performed if requested; N, staining should not be performed unless the request is discussed with the physician.

[c]Suspect rapidly growing mycobacteria, *M. haemophilum,* or *M. marinum.*

Microscopy

Acid-Fast Stain

Acid-fast staining is useful for select group of bacteria that includes the *Mycobacteria* spp. (both rapidly and slowly growing), *Nocardia, Rhodococcus, Tsukamurella, Gordonia,* and *Legionella micdadei.* What differentiates these bacteria is that they have long-chain fatty acids, or mycolic acids, that make up their cell walls and make them resistant to decolorization. Thus, the principle of the acid-fast stain is the use of a robust decolorizing agent (3% acid-alcohol [Kinyoun and Ziehl-Neelsen], 0.5–1% sulfuric acid [modified acid-fast]). The primary difference between the Ziehl-Neelsen (ZN) stain and the Kinyoun modification is that the ZN procedure requires a lengthy heating procedure and a slow cooling process. In the Kinyoun procedure, there is no heating. In all acid-fast stains, the primary staining reagent is carbol fuchsin and the counter stain is methylene blue.

Acridine Orange Stain

Acridine orange is a fluorescent dye that intercalates into nucleic acid (native and the denatured). At neutral pH, bacteria, fungi, and cellular material (e.g., leukocytes and squamous epithelial cells) stain red-orange. At acidic pH (pH 4.0), bacteria remain red-orange but the background material stains green yellow. Optimal detection of fluorescence requires the use of a 420- to 490-nm excitation filter and a 520-nm barrier filter. This stain can be useful in many situations. It can be useful to assess whether ambiguous objects seen on Gram stain are real, and it can be used to screen low organism density specimens for organisms at a lower magnification (i.e., sterile body fluids, or positive blood culture bottles with no organisms visualized on Gram stain).

Auramine-Rhodamine Stain

Auramine and rhodamine are nonspecific fluorochromes that bind to mycolic acids and are resistant to decolorization with acid-alcohol (acid-fast stain). Acid-fast organisms appear orange-yellow. If the secondary stain is not used, organisms will fluoresce a yellow-green color. Potassium permanganate is used as a counterstain. It is a strong oxidizing agent that inactivates the unbound fluorochrome dyes, producing a black background for the stained specimens. Fluorochrome-stained smears can be restained by the Kinyoun or Ziehl-Neelsen methods. Optimal detection of fluorescence requires use of a 420- to 490-nm excitation filter and a 520-nm barrier filter.

Direct Fluorescent-Antibody Stain

A variety of organisms (e.g., *Legionella* spp., and *Chlamydia trachomatis*) can be directly detected in clinical specimens by using specific fluorescein-labeled antibodies. The labeled antibodies bind to the organisms and fluoresce green under UV light. The sensitivity and specificity of the stain are determined by the quality of the antibodies used in the reagents. Optimal detection of fluorescence requires the use of either a 420- to 490-nm (wide-band) or 470- to 490-nm (narrow-band) excitation filter and a 510- to 530-nm barrier filter.

Gram Stain

Gram stain is the most commonly used stain in clinical microbiology laboratories. It is used to separate bacteria into Gram-positive (blue) and Gram-negative (red) groups. Variations in the performance of this stain are commonplace; however, the staining principle is constant. After the specimen is fixed to a glass slide (by either heating or treatment with 95% methanol), it is exposed to the basic dye crystal violet. Iodine is added and forms a complex with the primary dye. During the decolorization step, this complex is retained in Gram-positive organisms but lost in Gram-negative organisms. The Gram-negative organisms are detected with a counterstain (e.g., safranin). The degree to which an organism retains the stain is a function of the species, culture conditions, and staining skills of the microbiologist. Older cultures tend to decolorize readily. Some notable Gram stain reactions to be aware of include the following:

- *Treponema, Mycoplasma, Chlamydia,* and *Rickettsia* either lack a cell wall or are too small and cannon be visualized by Gram stain.
- *Bacillus* spp. and *Clostridium* spp. frequently decolorize and appear Gram negative.
- Faintly staining Gram-negative rods, such as *Campylobacter, Francisella, Brucella, Legionella, Helicobacter,* and others, can be counter-stained with carbol fuchsin rather than safranin for better visualization.
- Organisms that have been treated with antibiotics (particularly with cell-wall acting antibiotics, i.e. beta-lactams) can have distorted appearance, which may complicate their identification.
- Bipolar staining is a feature most commonly attributed to *Yersinia*; however, a number of other members of the *Enterobacteriaceae* can display this feature as well.

Spore Stain

The Wirtz-Conklin spore stain is a differential stain for detection of spores. This is very useful for the identification of *Bacillus* and

Clostridium species. Using this procedure, spores stain green while the rest of the cell stains pink. Non-spore-forming bacteria are pink. In this procedure, the slide is flooded with 5 to 10% aqueous malachite green. The stain is left on the slide for 45 min. Alternatively, the slide can be heated gently to steaming for 3 to 6 minutes. Heating to steaming enhances the uptake of the stain into the spores. The slide is then rinsed with water. Aqueous safranin (0.5%) is used as a counterstain for 30 s. The slide is then washed, blotted dry, and examined by light microscopy at x1,000 magnification.

Primary Plating Media: Bacteria

Ashdown Medium

Ashdown medium is used for the selective isolation and characterization of *Burkholderia pseudomallei* from clinical specimens such as sputum. The medium contains crystal violet and gentamicin as selective factors. It is enriched with glycerol and contains neutral red. *B. pseudomallei* produces flat, wrinkled, purple colonies on this medium. Both Ashdown agar and Ashdown broth can be modified by the addition of antimicrobial for the selective culture of *B. pseudomallei.* Modified Ashdown broth remains the standard for isolation of *B. pseudomallei* from throat swabs in patients with suspected melioidosis.

Bacteroides Bile-Esculin (BBE) Agar

Bacteroides bile-esculin agar is a selective, differential agar medium used for the recovery of the *Bacteroides fragilis* group and *Bilophila wadsworthia.* The medium contains oxgall (bile), esculin, ferric ammonium citrate, hemin, vitamin, and gentamicin in a casein and soybean agar base. Growth of non-*B. fragilis* group organisms is inhibited by the bile and the gentamicin. Supplementation of the agar with hemin and vitamin K_1 stimulates the growth of *Bacteroides* spp. Esculin hydrolysis is detected when esculin is converted to esculin and reacts with ferric ammonium citrate to produce black colonies.

Bile-Esculin (Enterococcal Selective) Agar

Bile-esculin agar can be made selective for the recovery of vancomycin-resistant enterococci by adding 6 μg of vancomycin per milliliter to it. Enterococci are able to grow in the presence of bile and hydrolyze esculin. Vancomycin-resistant strains produce black colonies on this agar, but susceptible strains fail to grow. In addition, vancomycin resistant lactobacilli can break through and grow on this media.

Bismuth Sulfite Agar

Bismuth sulfite agar is a differential, selective medium used for the isolation and identification of *Salmonella enterica* server Typhi and other enteric rods. The medium contains digests of casein and animal tissue, beef extract, glucose, ferric sulfate, and bismuth sulfite. Most commensal organisms are inhibited by the bismuth sulfite. *S. enterica* server Typhi colonies appear black with a metallic sheen. This medium may be inhibitory for some species of *Shigella*.

Blood Agar

Many types of blood agar media are used in clinical laboratories. The medium is used for the isolation and detection of hemolytic organisms. Alpha hemolytic organisms will produce a greenish color in the media surrounding the colony, whereas beta-hemolytic organisms produce a clear zone around the colony. The nature of beta-hemolysis can be characteristic of certain organisms. The two basic components are the basal medium (e.g., brain heart infusion, brucella, Columbia, Shaedler's, tryptic soy) and blood (e.g., sheep, horse, rabbit). Additional supplements are commonly used to enhance the growth of specific organisms or to suppress the growth of unwanted organisms.

Bordet-Gengou Agar

Recovery of *Bordetella pertussis* and *Bordetella parapertussis* is inhibited by factors such as fatty acids, metal ions, sulfides, and peroxides that are commonly present in media. Starch, charcoal, serum albumin, blood, or similar components are added to the medium to neutralize these inhibitors. Bordet-Gengou agar is a potato infusion–glycerol-based agar medium supplemented with 20 to 30% sheep, horse, or rabbit blood. Potato infusion is required for the growth of *Bordetella* spp., and glycerol is added to conserve moisture in the medium. Antibiotics such as methicillin or cephalexin are commonly added to suppress the growth of bacteria such as staphylococci, which inhibit the growth of *Bordetella* spp. Because this medium must be made fresh (it has a shelf life of less than 1 week), it has largely been replaced by Regan-Lowe agar.

Brain Heart Infusion Agar and Broth

Brain heart infusion agar is a general-purpose medium used for the isolation of a wide variety of pathogens. The basic formula includes infusion from brains and beef heart, as well as meat peptones, yeast extract, and dextrose. Vitamin K and hemin can be added for the enriched growth of anaerobes. The anaerobic formulation is infe-

rior for the isolation of anaerobic gram negative organisms. Broth formulations supplemented with 6.5% sodium chloride are used for the isolation of salt-tolerant streptococci and enterococci. The medium is particularly useful for culturing streptococci, pneumococci, and meningococci.

Brilliant Green Agar

Brilliant green agar is a selective, differential medium used for the isolation of *Salmonella* serovars, except *S. enterica* server Typhi. The nutritive base contains meat and casein peptones. Brilliant green dye at a high concentration inhibits most Gram-positive and Gram-negative bacteria, including *Shigella* spp. and *S. enterica* server Typhi. Phenol red is the pH indicator. Yeast extract provides additional nutrients. Acid production from the fermentation of sucrose or lactose produces yellow-green colonies with a yellow-green zone around the colony. Nonfermenters (i.e., *Salmonella*) may range in color from white to reddish pink with a red zone.

Brucella Agar and Broth

Brucella agar is a medium designed originally for the isolation of *Brucella* spp. Brucella agar supplemented with 5% horse blood can be used as a general-purpose medium for the isolation of both aerobic and anaerobic organisms. The nutritive base includes meat peptones, dextrose, and yeast extract. The agar formulation can be supplemented with hemin and vitamin K for recovery of fastidious anaerobes or with cefoxitin and cycloserine for the selective recovery of *Clostridium difficile.* The broth contains sodium bisulfite as a reducing agent and has been used for cultivation of *Campylobacter* spp.

Buffered Charcoal-Yeast Extract (BCYE) Agar

Buffered charcoal-yeast extract agar is selective for the recovery of *Legionella*, *Nocardia*, and *Francisella* spp. It contains agar, yeast extract, charcoal, and salts and is supplemented with L-cysteine, ferric pyrophosphate, ACES [Ar-(2-acetamido)-2- aminoethanesulfonic acid] buffer, and a-ketoglutarate. The charcoal detoxifies the medium; the yeast extracts are rich in nutrients; and the L-cysteine, ferric pyrophosphate, and alpha-Ketoglutarate stimulate the growth of *Legionella* spp. The addition of ACES is required to buffer the medium because *Legionella* spp. have a narrow pH tolerance (growth is optimal at pH 6.9). Various antibiotics such as polymyxin B, anisomycin, cefamandole, vancomycin, and cycloheximide are added to inhibit the growth of other bacteria when nonsterile clinical and environmental specimens are cultured.

Burkholderia cepacia Selective Agar (BCSA)

Burkholderia cepacia selective agar is an enriched, selective medium used for the isolation of *B. cepacia*. Trypticase peptone, yeast extract, sodium chloride, sucrose, and lactose form the nutritive base; and polymyxin B, gentamicin, vancomycin, and crystal violet are added as selective agents. This agar is the most sensitive and selective medium for the recovery of *B. cepacia*.

Campylobacter Selective Medium

A large number of media have been developed for the selective isolation of ***C****ampylobacter* spp. from stool specimens. Most contain a brucella basal medium, which preferentially supports the growth of *Campylobacter* spp. Blood is added, as are various combinations of antibiotics (e.g., cephalothin, vancomycin, trimethoprim, amphotericin, and polymyxin in the Blaser-Wang formulation; cycloheximide, cefazolin, novobiocin, bacitracin, and colistin in the Butzler formulation; cycloheximide, cefoperazone, and vancomycin in the Karmali formulation; and cycloheximide, rifampin, trimethoprim, and polymyxin in the Preston formulation).

Cefsulodin-Irgasan-Novobiocin (CIN) Agar

Cefsulodin-Irgasan-novobiocin agar is a selective, differential agar medium used for the isolation of *Yersinia enterocolitica*, most commonly from stool specimens. The medium consists of digests of animal tissue and gelatin, beef and yeast extracts, sodium pyruvate, sodium deoxycholate, neutral red, crystal violet, cefsulodin, Irgasan, and novobiocin. The antibiotics and sodium deoxycholate inhibit the growth of most organisms in stool specimens. However, *Yersinia* spp. are resistant and can ferment mannitol in the medium. This fermentation produces colonies with a bull's-eye appearance (i.e., deep red centers with transparent edges sometimes referred to as a "bull's eye").

Chromogenic Media

A large number of chromogenic media exist for the selective and differential isolation of bacteria and yeast. These media can include antibiotics and can be used to isolate drug-resistant bacteria such as vancomycin resistant enterococci, methicillin resistant *Staphylococcus aureus,* and resistant Gram-negative organisms. In many cases, the components of the media are proprietary and cannot be discussed here. The list of chromogenic media includes, but is not limited to, the isolation and identification of ESBL producing *Enterobacteriaceae,* carbapenemase resistant *Enterobacteriaceae,* MRSA, VRE, *P. aeruginosa, Salmonella, Streptococcus*

agalactiae, Vibrio, C. difficile, Enterobacteriaceae, Yersinia enterocolitica, Acinetobacter, and others.

Chocolate Agar

Chocolate agar is an enriched medium that derives its name from its color. Blood or hemoglobin is added immediately after the medium is heated, and the heat causes the added component to lyse and turn brown. This medium supports the growth of most bacteria and is required for the recovery of many *Haemophilus* spp. and some pathogenic *Neisseria* strains. The so-called nutritionally deficient streptococci (*Abiotrophia* and *Granulicatella*) will also grow on chocolate, but not on blood agar. A variety of formulations of this medium have been used, but the most common consists of a peptone base enriched with 2% hemoglobin or IsoVitaleX. Catalase-negative bacteria (e.g., *Streptococcus pneumoniae*) grow less well on this medium than on blood agar because catalase from ruptured erythrocytes in blood agar is not available to protect the bacteria from peroxides that accumulate in the medium.

Chopped-Meat Broth

Chopped-meat broth is an enriched broth used for the recovery of a variety of bacteria, particularly anaerobes, from clinical specimens. Extracts as well as solid particles of beef or horse meat are suspended in broth with peptones, yeast extract, sugars, starch, and L-cysteine. The L-cysteine helps maintain a low E_h (oxidation-reduction potential), which supports the growth of anaerobes.

Colistin-Nalidixic Acid (CNA) Agar

Colistin-nalidixic acid agar is a selective medium used for the recovery of aerobic and anaerobic Gram-positive bacteria. The medium consists of Columbia agar base supplemented with nalidixic acid, colistin, and blood. Nalidixic acid inhibits most aerobic Gram-negative rods, as does colistin. The *B. fragilis* group is usually resistant to these antibiotics, but other anaerobic Gram-negative rods can be inhibited by colistin.

Columbia Agar and Broth

Columbia agar with 5% sheep blood is a general-purpose medium used for the isolation of common bacteria. The medium contains meat and casein peptones, beef extract, yeast extract, and cornstarch as the nutritive base. Sheep blood allows the determination of hemolytic reactions and provides X factor. However, the substantial carbohydrate content may make beta hemolytic streptococci

appear to be alpha-hemolytic or take on a greenish hue. Use of horse or rabbit blood improves the hemolysis. NADase in sheep blood destroys the V factor (NAD); therefore, organisms that require this factor do not grow. Salt and Tris buffers are added to the broth formulation to enhance the growth of organisms and increase the buffering capacity, respectively.

Cycloserine-Cefoxitin-Egg Yolk-Fructose Agar (CCFA)

Cycloserine-cefoxitin-egg yolk-fructose agar is a selective, differential medium used for the recovery of *Clostridium difficile.* The medium consists of animal tissue digest, fructose, cycloserine, cefoxitin, and neutral red. Cycloserine and cefoxitin inhibit most intestinal bacteria. *C. difficile* can ferment fructose, producing a more acidic pH, which is detected by the indicator dye neutral red (shift from red to yellow medium surrounding the colonies). Various modifications of this medium are used, including supplementation with egg yolk to stimulate the growth of Clostridia.

Cystine Tellurite Blood Agar

Cystine tellurite blood agar medium is a selective, differential medium used for the recovery of *Corynebacterium diphtheriae.* The medium consists of heart infusion agar, potassium tellurite, L-cystine, and rabbit blood. Potassium tellurite inhibits the growth of most commensal organisms and allows *C. diphtheriae* to grow. The organism produces hydrogen sulfide from cystine, and the reaction of tellurite with hydrogen sulfide results in brown halos surrounding the colonies of *C. diphtheriae.*

Egg Yolk Agar

Egg yolk agar (modified McClung-Toabe agar) is a selective, differential medium used for the isolation and differentiation of *Clostridium* spp. Degradation of lecithin results in an opaque precipitate around the bacterial colony, and lipase destroys fats in the egg yolk, resulting in an iridescent sheen on the colony surface. Proteolysis can also be determined on the basis of a translucent clearing of the medium around the colony. Addition of neomycin makes the egg yolk agar moderately selective by inhibiting some facultative anaerobic gram-negative rods.

Ellinghausen-McCullough-Johnson-Harris Medium

The modified bovine albumin Tween 80 medium is selective for the growth of *Leptospira* spp. The basal medium, consisting of glycerol, sodium pyruvate, and thiamine, is supplemented with bovine

albumin, Tween 80, vitamin Bi2, and salts of iron, calcium, magnesium, zinc, and copper.

Enterococcosel agar

This medium is used for the rapid, selective isolation of enterococci. It is also used for the cultivation of staphylococci and *L. monocytogenes.* The medium contains digest of casein, iron, Esculin, and yeast extract. The addition of vancomycin can allow for the selective detection of vancomycin resistant enterococci.

Eosin-Methylene Blue (EMB) Agar

Eosin-methylene blue agar is a differential, selective medium used for the isolation and differentiation of lactose-fermenting and nonfermenting Gram-negative rods. The agar medium consists of casein digests, lactose, sucrose, eosin Y, and methylene blue. The Levine formulation does not include sucrose. Growth of Gram-positive bacteria is suppressed by the methylene blue, which, together with eosin Y, also serves as an indicator for carbohydrate fermentation (dyes precipitate at an acidic pH). Bacteria that ferment lactose (e.g., *Escherichia*, *Klebsiella*, and *Enterobacter* spp.) form colonies that have a green metallic sheen or that are blue-black to brown. Nonfermentative bacteria (e.g., *Proteus*, *Salmonella*, and *Shigella* spp.) have colorless or light purple colonies.

Fletcher Medium

Fletcher medium is a semisolid medium used for the recovery of *Leptospira* spp. The medium consists of 0.15% agar, salt, peptones, beef extract, and rabbit serum. *Leptospira* spp. usually grow within 1 to 2 weeks in this medium.

Gram-Negative (G N; Hajna) Broth

This medium is used for the selective cultivation of *Salmonella* and *Shigella* spp. The medium contains digest of casein and animal tissue, citrate, mannitol, glucose, and deoxycholate. Commensal organisms will overgrow the enteric pathogens if the broth is incubated for more than 4 to 6 h.

Haemophilus Test Medium (HTM) Agar and Broth

Haemophilus test medium is an enriched medium used for susceptibility testing of *Haemophilus* spp. The medium contains beef and casein extracts. Yeast extract, hemin, and NAD provide the necessary growth factors and enrichments. Antagonists to sulfonamides

and trimethoprim are removed by thymidine phosphorylase. The advantage of the agar medium is that it is a clear agar base so that sharp growth end-point interpretations can be made. The calcium and magnesium concentrations are adjusted to the concentrations recommended by the CLSI.

Hektoen Enteric Agar

Hektoen enteric agar is a selective medium used for the isolation of *Salmonella* and *Shigella* spp. and differentiation of these organisms from other Gram-negative rods that may be recovered on this medium. It consists of a peptone base agar supplemented with bile salts, lactose, sucrose, salicin, ferric ammonium citrate, and the pH indicators bromthymol blue and acid fuchsin. The bile inhibits all Gram-positive bacteria and many Gram-negative rods. Acids produced by fermentation of lactose, sucrose, or salicin react with bromthymol blue to produce a yellow color and with acid fuchsin to produce a red color. Hydrogen sulfide (H_2S) produced by the metabolism of sodium thiosulfate is detected when a black precipitate forms after the addition of ferric ammonium citrate. Lactose-fermenting bacteria (e.g., *E. coli*) are slightly inhibited on this agar and appear as orange or salmon pink colonies. *Salmonella* colonies typically appear blue-green with black centers. *Shigella* colonies appear green with no black center. *Proteus* spp. are inhibited; their colonies are colorless.

Kanamycin-Vancomycin Laked Blood (LKV) Agar

Kanamycin-vancomycin laked blood agar is a selective, differential medium used for the recovery of anaerobic Gram-negative rods, especially *Bacteroides* and *Prevotella* spp. The medium consists of casein and soybean meal agar supplemented with kanamycin, vancomycin, vitamin K, and lysed (laked) sheep blood. Kanamycin inhibits most facultative, Gram-negative rods; and vancomycin inhibits most Gram-positive organisms and *Porphyromonas* spp.; Vitamin K stimulates the growth of some *Prevotella* strains, which also develop a black pigment in the presence of lysed blood.

LIM Broth

LIM broth is a selective enrichment broth used for the recovery of group B streptococci. The medium consists of Todd-Hewitt broth supplemented with yeast extract, colistin, and nalidixic acid. Most aerobic and anaerobic Gram-negative rods are inhibited by the antibiotics, whereas group B streptococci grow well in this broth.

Loffler Medium

Loffler medium is an enriched medium used for the recovery of *Corynebacterium diphtheriae.* The medium consists of animal digests, heart muscle infusion, beef serum, egg, and glucose. *C. diphtheriae* grows rapidly on this medium, and Gram stains of colonies demonstrate characteristic metachromatic granules.

MacConkey (MAC) Agar

MacConkey agar is a selective agar medium used for the isolation and differentiation of lactose-fermenting and nonfermenting Gram-negative rods. The medium consists of digests of peptones, bile salts, lactose, neutral red, and crystal violet. Bile salts and crystal violet inhibit the growth of Gram-positive bacteria and some fastidious Gram-negative bacteria. Colonies that ferment lactose (e.g., *Escherichia*, *Klebsiella*, and *Enterobacter* spp.) produce acid, which causes a red color shift in the neutral red pH indicator and precipitates the bile salts. Colonies appear red to pink, while nonfermenting colonies (e.g., *Proteus*, *Salmonella*, and *Shigella spp.*) appear yellow, colorless, or translucent. MacConkey agar will typically inhibit *Proteus* from swarming over the plate.

MacConkey Agar with Sorbitol—see Sorbitol-MacConkey Agar

Mannitol Salt Agar

Mannitol salt agar is a selective medium used for the isolation of staphylococci. The medium consists of digests of casein and animal tissue, beef extract, mannitol, salt, and phenol red indicator. If the organism can grow in the presence of 7.5% salt and ferment mannitol, the acid turns the indicator yellow. Most strains of *Staphylococcus aureus* produce yellow colonies, whereas coagulase-negative staphylococci do not ferment the mannitol and thus remain red. Most other organisms are inhibited by the high salt concentration.

Martin-Lewis Agar

Martin-Lewis agar, a formulation of the modified Thayer-Martin (MTM) agar, is an enriched selective medium for the isolation of *Neisseria gonorrhoeae.* The nutritive base is chocolate agar. The specific differences from MTM agar are a higher concentration of vancomycin (4 versus 3 μg/ml) and replacement of nystatin with anisomycin. Trimethoprim and colistin are also incorporated. Some pathogenic *Neisseria* strains have been reported to be inhibited by vancomycin and trimethoprim.

McBride *Listeria* agar

This medium is used for the selective isolation of *L. monocytogenes* from clinical specimens containing mixed biotas. The medium contains glycine, digest of casein and animal tissue, beef extract, phenylethyl alcohol, and lithium chloride.

Mueller-Hinton Agar and Broth

Mueller-Hinton agar and broth are recommended by CLSI for the routine susceptibility testing of non-fastidious organisms. Supplementation of this agar with 5% sheep blood is used for susceptibility testing of fastidious organisms such as *Streptococcus pneumoniae.* Beef and casein extracts and soluble starch form the nutritive base. Calcium and magnesium concentrations are controlled.

New York City Agar

New York City agar is a selective medium used for the isolation of pathogenic *Neisseria* spp. The medium consists of peptones, cornstarch, yeast dialysate, glucose, hemoglobin, horse plasma, and a mixture of antibiotics (vancomycin, colistin, amphotericin B, and trimethoprim). It can be used instead of Thayer-Martin agar.

Oxidative-Fermentative Polymyxin B-Bacitracin-Lactose (OFPBL) Agar

Oxidative-fermentative polymyxin B-bacitracin-lactose agar is a selective, differential medium used for the isolation of *Burkholderia cepacia*. The nutritive base is an oxidative-fermentative medium with peptones. When acid is produced from the utilization of lactose, as with *B. cepacia*, the bromthymol blue indicator changes the colony from green to yellow. Polymyxin B and bacitracin are selective agents that inhibit some Gram-negative and Gram-positive organisms, respectively. Other organisms may grow on this medium and are differentiated from *B. cepacia* by the inability to produce acid from lactose.

Phenylethyl Alcohol (PEA) Blood Agar

Phenylethyl alcohol blood agar is a selective medium that consists of casein and soybean agar supplemented with phenylethyl alcohol and blood. Facultative Gram-negative rods are inhibited by the phenylethyl alcohol (e.g., the growth of swarming *Proteus* spp. is suppressed). Most Gram-positive and Gram-negative anaerobes, as well as aerobic Gram-positive bacteria, will grow on this medium. *Pseudomonas* spp. are not inhibited.

Regan-Lowe Agar Medium

Regan-Lowe agar medium, for the selective isolation of *Bordetella* spp., contains beef extract, gelatin digest, starch, charcoal, niacin, 10% horse blood, and cephalexin (40 μg/ml). The charcoal and horse blood are required to neutralize fatty acids and other inhibitory factors present in the medium. Sheep but not human blood can replace horse blood. Cephalexin can delay the detection of *Bordetella* spp. on this medium, but the use of an additional nonselective medium is not considered necessary. The shelf life of this medium is 6 to 8 weeks.

Salmonella-Shigella (SS) Agar

Salmonella-shigella agar is a highly selective medium for the recovery of *Salmonella* spp. The medium is not recommended for the primary isolation of *Shigella* spp. The medium consists of beef extract and peptone digests, lactose as a carbohydrate source, bile salts, sodium citrate, sodium thiosulfate, neutral red, brilliant green, and ferric citrate. Bile salts, sodium citrate, and brilliant green are inhibitory for all Gram-positive and selected Gram-negative bacteria. Bacteria that grow on the medium and produce hydrogen sulfide from the metabolism of sodium thiosulfate are detected by the black precipitate formed with ferric citrate. Acid produced from lactose fermentation is detected with the pH indicator neutral red. All lactose-fermenting bacteria form pink or red colonies, while nonfermenting bacteria form either colorless (e.g., *Shigella* spp.) or black (e.g., *Salmonella* spp.) colonies.

Schaedler's Agar

Schaedler's agar is a general-purpose medium used for the isolation of anaerobic bacteria. The nutritive base includes vegetable and meat peptones, dextrose, and yeast extract. Sheep blood, vitamin *Ku*, and hemin provide other additives that stimulate the growth of fastidious anaerobes. Because of the high carbohydrate content, colonies with beta-hemolytic reactions may have a greenish hue. Acid production may also lead to rapid cell death.

Selenite Broth

Selenite broth is a selective enrichment broth used for the isolation of *Salmonella* spp. from stools and other contaminated specimens. It consists of peptones, sodium phosphate, lactose, and sodium selenite. *E. coli* and other Gram-negative rods are inhibited by sodium selenite. The broth should be subcultured within 8 to 12 h after inoculation with the specimen, or else the enteric pathogens will be overgrown with commensal organisms.

Skirrow Brucella Medium

Skirrow medium is an enriched selective blood agar medium used for the isolation of *Campylobacter* spp. The nutritive agar base is brucella agar. Hematin is provided by sheep blood. The selective agents are trimethoprim, vancomycin, and polymyxin B, which inhibit the normal flora found in fecal specimens.

Sorbitol-MacConkey Agar

Sorbitol-MacConkey agar is a selective differential agar used for the isolation of *E. coli* 0157. Lactose is replaced with sorbitol. Most *E. coli* strains ferment sorbitol; however, *E. coli* 0157 does not, and therefore its colonies are colorless on this agar.

Streptococcus selective medium

This medium is used for the selective isolation of streptococci from clinical specimens. The medium contains peptone, starch, and blood. It also contains Colistin sulfate and oxolinic acid as selective factors. It is a commonly used medium for throat cultures.

StrepB Carrot Broth

This is a proprietary broth that is used for detecting the presence of GBS infections in pregnant women. The medium contains peptone, starch, morpholinepropanesfulfonic acid, glucose, pyruvate, growth factors, and selective factors. It is a modification of Granada medium consisting of a one-step method for screening pregnant women for the presence of GBS. Tubes show an orange-to-red color change, typical of GBS. The production of orange, red, or brick red pigment is a unique characteristic of hemolytic GBS due to reactions with substrates such as starch, proteose peptone, serum, and folate pathway inhibitors.

Tetrathionate Broth, Hajna

Tetrathionate broth is a selective enrichment broth used for the recovery of *Salmonella* spp. (except *Salmonella* Typhi and *S. enterica* subsp. *arizonae*) from stool and urine specimens. It consists of a peptone base supplemented with yeast extract, mannitol, glucose, sodium deoxycholate, sodium thiosulfate, calcium carbonate, and brilliant green. The sodium deoxycholate, sodium thiosulfate, and brilliant green inhibit Gram-positive and Gram-negative bacteria. The broth should be subcultured for 12 to 24 h after inoculation to prevent overgrowth of *Salmonella* spp. with commensal organisms.

Thayer-Martin (Modified) Agar

Many modifications of Thayer-Martin medium have been developed for the isolation of pathogenic *Neisseria* spp. The blood agar base medium is enriched with hemoglobin and supplements. The growth of unwanted bacteria can be suppressed by the addition of antibiotics such as colistin (which inhibits most Gram-negative bacteria except *Proteus* spp.), trimethoprim (which inhibits *Proteus* spp.), vancomycin (which inhibits most Gram-positive bacteria), and nystatin (which inhibits yeasts). Some strains of *N. gonorrhoeae* are inhibited by vancomycin, and so nonselective media (e.g., chocolate agar) should be used for primary isolation.

Thioglycolate Broth

Thioglycolate broth is an enrichment broth used for the recovery of aerobic and anaerobic bacteria. Various formulations are used, but most include casein digest, glucose, yeast extract, cysteine, and sodium thioglycolate. Supplementation with hemin and vitamin K will enhance the recovery of anaerobic bacteria.

Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar

Thiosulfate citrate bile salts sucrose agar is a selective, differential medium used for the recovery of *Vibrio* spp. The medium consists of digests of casein and animal tissue, yeast extract, sodium citrate, sodium cholate, oxgall (bile), sucrose, ferric citrate, thymol blue, and bromthymol blue. Sodium citrate, sodium cholate, and bile inhibit commensal organisms. *Vibrio cholerae* colonies are yellow on this medium due to fermentation of sucrose with the acid, resulting in a yellow color shift of the indicator, bromthymol blue. *Vibrio parahaemolyticus* fails to ferment sucrose, and the colonies are therefore blue-green. Some enteric rods and enterococci may grow, but the colonies are usually small and translucent. Sucrose-fermenting *Proteus* strains produce yellow colonies that are similar to *Vibrio* colonies.

Tinsdale Agar

Tinsdale agar is a selective differential medium used for the isolation of *Corynebacterium diphtheriae* from upper respiratory specimens. The medium consists of peptones, salt, yeast extract, L-cysteine, potassium tellurite, and serum. The potassium tellurite inhibits the growth of most commensal organisms in the upper respiratory tract and allows the growth of *C. diphtheriae* and related *Corynebacterium* species. *C. diphtheriae* colonies can be distinguished by the brown halo that develops around the black colonies. These halos result from the

reaction of tellurite with hydrogen sulfide, which *C. diphtheriae* produces from the cysteine in the medium.

Tryptic or Trypticase Soy Agar (TSA) and Broth (TSB)

Tryptic(ase) soy agar with 5% sheep blood is a general-purpose medium used for the isolation of a wide variety of organisms. The medium contains soybean and casein peptones as the nutritive base. The addition of sheep blood enriches the medium and allows the growth of more fastidious organisms by providing hemin (X factor). V factor (NAD) is inactivated by enzymes in the sheep blood. Sheep blood is used for the interpretation of hemolytic reactions. The broth formulation is recommended for preparation of inocula for susceptibility testing. Addition of 6.5% sodium chloride to the broth formulation can be used for isolation of salt-tolerant organisms, and Fildes enrichment can be added to the broth for recovery of fastidious organisms such as *Haemophilus* spp.

Xylose-Lysine-Deoxycholate (XLD) Agar

Xylose-lysine-deoxycholate agar is a moderately selective medium used for the isolation and differentiation of enteric pathogens. The medium consists of yeast extract with xylose, lysine, lactose, sucrose, sodium deoxycholate, sodium thiosulfate, ferric ammonium citrate, and phenol red. The majority of the nonpathogenic enteric rods ferment lactose, sucrose, or xylose, producing yellow colonies (the phenol red indicator is yellow at acidic pH). Because *Shigella* spp. do not ferment these carbohydrates, the colonies are red. *Salmonella* and *Edwardsiella* spp. ferment xylose, but they also decarboxylate lysine to an alkaline diamine, cadaverine. This diamine neutralizes the acid products of fermentation by decarboxylation of lysine and produces red colonies. If the organism produces hydrogen sulfide (e.g., *Salmonella* and *Edwardsiella* spp.), the center of the colonies will blacken. Sodium deoxycholate inhibits the growth of many nonpathogenic organisms (in the presence of acid, it precipitates, producing yellow, opaque colonies).

Primary Plating Media: Mycobacteria

American Trudeau Society Medium

American Trudeau Society medium contains coagulated egg yolks, potato flour, glycerol, and malachite green. The concentration of malachite green is lower than in Lowenstein-Jensen medium, allowing earlier detection of mycobacterial colonies (other than *M. leprae*), but the medium is also more easily overgrown by contaminants.

Dubos Broth (Dubos Tween albumin broth)

Dubos broth, a nonselective broth, contains casein digests, salt solutions, L-asparagine, ferric ammonium citrate, albumin or serum, and Tween 80. The growth of most species of mycobacteria is rapid in this medium, although the addition of antibiotics is required when specimens from contaminated sites are processed. Tween 80 is a surfactant that facilitates the dispersal of clumps of mycobacteria and results in more rapid, homogeneous growth.

Lowenstein-Jensen (LJ) Medium

Lowenstein-Jensen medium consists of glycerol, potato flour, defined salts, and coagulated whole eggs (to solidify the medium). Malachite green is added to inhibit contaminating bacteria, particularly Gram-positive bacteria. LJ medium has a long shelf life (several months) and supports the growth of most mycobacteria, in part because lecithin in the eggs neutralizes many toxic factors present in clinical specimens. A problem with LJ medium is that the contaminants (commonly *Pseudomonas aeruginosa* from Cystic Fibrosis sputa) that grow on this medium can completely hydrolyze it.

Lowenstein-Gruft Modification

The Gruft modification of LJ medium contains RNA, penicillin, and nalidixic acid, which further suppress the growth of contaminating organisms. Because the growth of mycobacteria can be delayed with this selective medium, it should always be used with a tube of nonselective medium. *Mycobacterium tuberculosis* appears as granular, rough, dry colonies. *M. kansasii* appears as smooth to rough, photochromogenic colonies. *M. gordonae* appears as smooth, yellow-orange colonies. *M. avium* appears as smooth, colorless colonies. *M. smegmatis* appears as wrinkled, creamy white colonies.

Lowenstein-Jensen Medium, Mycobactosel Modification

The Mycobactosel modification of LJ medium contains cycloheximide, lincomycin, and nalidixic acid to suppress the growth of contaminants.

Middlebrook 7H9 Broth

The 7H9 formulation of Middlebrook broth is the same as Middlebrook 7H10 agar, except that the agar and malachite green are absent. The growth of most mycobacteria is rapid in this medium, although antibiotics must be added to suppress the growth of

contaminants. It is also used for performing mycobacterial susceptibility testing.

Middlebrook 7H10 Agar

Middlebrook 7H10 agar is a nonselective medium that contains defined salts, vitamins, cofactors, oleic acid, albumin, catalase, glycerol, glucose, and malachite green. The addition of glycerol enhances the growth of *Mycobacterium avium/intracellulare.* Pyruvic acid can be added if *M. bovis* is suspected, and 0.25% L-asparagine or 0.1% potassium aspartate must be added for maximal production of niacin. The medium has a relatively short shelf life (approximately 1 month), and exposure to heat or light may result in its deterioration and in the release of formaldehyde. Growth of mycobacteria can be detected earlier on this medium than on egg-based media.

Middlebrook 7H11 Agar

Middlebrook 7H11 agar is preferred over 7H10 agar because the addition of casein hydrolysates improves the recovery of isoniazid-resistant strains of *M. tuberculosis,* which have become prevalent in some communities. It is also particularly useful for the cultivation of fastidious strains of tubercle bacilli that occur following treatment of tuberculosis with secondary antitubercular drugs.

Middlebrook 7H11 Agar, Mitchison's Modification

Mitchison's modification of 7H11 medium contains carbenicillin, polymyxin B, trimethoprim, and amphotericin B. The carbenicillin is particularly useful for suppressing the growth of *Pseudomonas* spp.

Middlebrook 7H13 Broth

Middlebrook 7H13 broth is based on the 7H9 broth formulation supplemented with casein hydrolysate, polysorbate 80, sodium polyanetholesulfonate, catalase, and [14C] palmitic acid. This broth is used in the BACTEC system.

Petragnani Medium

Petragnani medium is a nonselective mycobacterial medium that contains coagulated whole eggs, egg yolks, whole milk, potato, potato flour, glycerol, and malachite green. This medium is more inhibitory than LJ medium because it contains a higher concentration of malachite green. It should be restricted to use with heavily contaminated specimens. It is particularly useful for the cultivation and maintenance of *M. smegmatis.*

Specific Diagnostic Tests for Pathogen Detection

Aerobic Gram-Positive Cocci

***Enterococcus* spp.** Microscopy and culture are the most commonly used detection methods. Selective media can be used to recover the bacteria from specimens contaminated with Gram-negative bacteria, and selective media supplemented with vancomycin can be used to recover vancomycin-resistant enterococcal (VRE) strains. Direct detection from specimens using molecular methods may be used for infection control screening for VRE. In addition, commercially available molecular methods exist for the identification of enterococci and vancomycin resistance from positive blood culture broth.

***Staphylococcus aureus*.** Microscopy and culture are the most commonly used detection methods. Selective media (e.g., mannitol salt agar, colistin-nalidixic acid agar, and phenylethyl alcohol agar) can be used for recovery from heavily contaminated specimens (most commonly used for culture of Cystic Fibrosis sputa). Molecular assays are available for detecting methicillin-resistant *S. aureus* both for infection control screening purposes as well as from positive blood cultures.

Coagulase Negative Staphylococci (CONS). Microscopy and culture are the most commonly used detection methods. CONS will readily grow on routinely used media (blood agar, chocolate agar, CNA agar). However, *S. saccharolyticus* is a strict anaerobe and requires incubation in anaerobic conditions. Assess specimen Gram stain results for the presence of epithelial cells, which may indicate a poorly collected specimen and insignificance of CONS. Virtually any CONS can be pathogenic in the setting of a hardware-associated infection.

***Streptococcus,* Group A**. Group A *Streptococcus* grows readily in culture. *Streptococcus* selective media may be used to enhance isolation of GAS from clinical specimens. Microscopy is not useful for diagnosis of pharyngitis but is useful for cutaneous infections. Numerous direct antigen tests are available for streptococcal pharyngitis. Although the tests are highly specific (for *S. pyogenes* but not other species of group A *Streptococcus*), the sensitivity is <80% and the Centers for Disease Control (CDC) recommends that negative reactions must be confirmed by culture for pediatric, but not adult, patients. Antibody tests are used to confirm antecedent group A streptococcal pharyngitis or pyoderma in patients with suspected rheumatic fever or nephritis. The most popular tests are the antistreptolysin O (ASO) and anti-DNase B tests. Both tests have a sensitivity of 85%, and they should be performed together. The ASO

test is nonreactive in patients with nephritis following streptococcal pyoderma. False-positive ASO titers can occur in patients with liver disease and infections with streptococcal groups C or G. Anti-DNase B is specific for group A streptococci; there is no reactivity with other streptococcal groups. Peak ASO and anti-DNase B titers occur 2 to 3 weeks after the primary infection and persist for 6 months or more. Positive titers are two or more dilutions above the upper limit of normal. Other tests (e.g., Streptozyme) are less sensitive and reproducible.

***Streptococcus,* Group B.** Group B *Streptococcus* grows readily in culture, although 10% of strains are nonhemolytic and may not be detected in mixed cultures. Enrichment broth (i.e., LIM broth) can be used to recover small numbers of organisms. Microscopy is not helpful for detection of genital carriage. Numerous direct antigen tests have been developed for the detection of genital carriage, but the test sensitivity is too low to justify its use. Commercially available molecular tests are widely available and used for the screening of genital carriage in pregnant women. Of note, most molecular methods still require preliminary enrichment broth growth prior to testing. Multiplex PCR meningitis/encephalitis panels now include GBS for detection in CSF.

Streptococcus pneumoniae. Microscopy and culture are sensitive detection methods, although the bacteria can undergo spontaneous lysis and hence will not be recovered in specimens when processing is delayed. A variety of tests have been developed for detecting pneumococcal capsular antigens in cerebrospinal fluid (CSF) and urine. Urine tests are less sensitive than CSF tests, and all antigen tests are generally no more sensitive than a Gram stain. Use of direct specimen antigen tests has largely been discontinued due to poor performance. Type-specific anticapsular antibody titers can be measured to assess the response to vaccination but are not measured for diagnostic purposes. Multiplex PCR meningitis/encephalitis panels now include *S. pneumoniae* for detection in CSF.

Aerobic Gram-Positive Rods

Bacillus anthracis. Microscopy is useful if positive, but the test is insensitive and the capsule is generally not seen when the Gram stain is used. Fluorescein-labeled anticapsular antibodies have been developed. The organism grows rapidly in culture and has a characteristic colony morphology (large, irregular, sticky, nonhemolytic colonies). Antigen tests and molecular diagnostic tests have been developed and are available through state public health laboratories. These tests are generally specific but lack sensitivity, especially

for asymptomatic patients exposed to *B. anthracis.* The serologic response to anthrax toxin (i.e., protective antigen) can be used to assess the response to vaccination but not as a diagnostic tool.

Corynebacterium diphtheriae. Diagnosis is based on clinical parameters. Microscopy is generally not useful. The organism grows readily on nonselective sheep blood agar; selective media (cysteine-tellurite blood agar or Tinsdale medium) should also be used for primary cultures. The Centers for Disease Control and Prevention (CDC) offer a direct PCR test for diphtheria toxin; this test is recommended for confirming the diagnosis of diphtheria but should not be used alone. Immunoassays have been developed to measure the level of antibodies against C. *diphtheriae* toxin in patients immunized with toxoid. These tests assess immunity and cannot be used for the diagnosis of diphtheria. Antitoxin levels of ≥0.01 IU/ml are considered protective. Lower levels indicate that immunization with toxoid may be required.

Corynebacterium, **Other spp.** Microscopy and culture are the most commonly used detection methods. Some species are slow growing unless the isolation media are supplemented with lipids. Some pathogenic lipophilic strains, such as *C. jeikeium*, *C. macginleyi*, and *C. kroppenstedtii*, will grow better on sheep blood agar than on chocolate.

Erysipelothrix spp. Microscopy is generally insensitive, but the presence of long, slender, Gram-positive rods in the tissue of a patient suspected of having erysipeloid is helpful. Growth on blood agar plates is slow, and incubation should be extended for 7 days. Antigen and molecular diagnostic tests have not been developed, and serologic testing is not useful because patients do not develop antibodies after an episode of erysipeloid.

Gardnerella vaginalis. Gram stains of vaginal specimens are helpful if thin Gram-variable coccobacilli or rods are seen. The organism grows poorly in culture and may not be detected. It can often be isolated on CNA agar.

Listeria **spp.** Microscopy is insensitive for patients with meningitis (generally, small numbers of organisms are present in CSF) and nonspecific (the organism can be confused with ***C****orynebacterium* or *Streptococcus*). The organism grows well on most nonselective media, but hemolysis may not be obvious on sheep blood agar. Special selective agars have been developed for the recovery of *Listeria* from stools and food products. Antigen detection kits have also been developed for food products but are not licensed for

clinical specimens. Multiplex PCR meningitis/encephalitis panels now include *Listeria monocytogenes* for detection in CSF.

Acid-Fast and Partially Acid-Fast Gram-Positive Rods

Mycobacterium avium* complex *(MAC). Microscopy and culture are sensitive detection methods. Disseminated infections are common in immunocompromised patients, with organisms recovered in high concentrations in blood and many body tissues. MAC probes exist for organism identification from cultured isolates but are not used for direct detection from specimens.

***Mycobacterium tuberculosis* complex.** Tuberculosis is most commonly diagnosed by microscopy (acid-fast stain) and culture. Mycobacteria can also be detected directly by using molecular methods. The amplification methods are useful for use with smear-positive respiratory specimens but cannot be used with nonrespiratory specimens and have low sensitivity for smear-negative specimens (~60%), although some methods are now approved for smear-negative specimens. Serologic methods in addition to the skin test can be used for the detection of latent tuberculosis infection (LTBI). Interferron gamma release assays (IGRA) QuantiFERON-TB Gold (QFT) and T-Spot have received U.S. Food and Drug Administration approval for LTBI screening. IGRA tests measure the patient's immune reactivity to *M. tuberculosis.* Overall, performance of IGRAs is equivalent to the skin test. However, IGRAs have better specificity than the skin test in patients who have received the BCG vaccination or have had a prior mycobacterial infection.

***Nocardia* spp.** Diagnosis of nocardiosis relies on microscopic detection of the organism in clinical specimens and isolation in culture. Filamentous forms stain poorly with the Gram stain and weakly with the acid-fast stain (even when a weak decolorizing solution is used). Although the organism grows on most nonselective enriched media, recovery is best on buffered charcoal yeast extract (BCYE) agar and Thayer-Martin agar. Serological testing methods have been handicapped by the antigenic heterogeneity of pathogenic *Nocardia* spp., poor serologic response of the patient, high levels of immunoreactivity to *Nocardia* spp., in healthy individuals, and cross reactivity with other microbial antigens.

***Rhodococcus* spp.** *Rhodococcus* spp. are weakly acid-fast, with relatively few cells staining acid-fast unless the organism is cultured on Lowenstein-Jensen medium or Middlebrook agar. Bacteria grown in broth cultures incubated for a few hours will appear as long rods, while those grown for longer periods will appear as cocci or coccobacilli. Prolonged incubation of culture may be required for the isolation of this organism.

Aerobic Gram-Negative Cocci

Moraxella catarrhalis. Microscopy and culture are the most commonly used detection methods. Large numbers of organisms associated with polymorphonuclear leukocytes and mucus are typically observed in patients with respiratory tract infections.

Neisseria gonorrhoeae. Historically, microscopy and culture were the diagnostic tests of choice. For patients with genital infections, the Gram stain has a sensitivity of 90 to 95% and a specificity of 95 to 100% in symptomatic males but a sensitivity of only 50 to 70% in symptomatic females and much lower in asymptomatic females. The organism grows on chocolate agar, but selective media are used most commonly to suppress the urethral flora. For the most part, culture has now been replaced by molecular tests, initially with amplification-based molecular tests. Amplification assays are more sensitive than the molecular probes that have historically been used, but care must be taken to eliminate inhibitors present in specimens (particularly in urine) and prevent cross-contamination of specimens. Antibody testing is not useful. Molecular tests are not approved for use in non-genital tract specimens but data suggest there is utility to molecular testing of rectal, pharyngeal, and ocular specimens.

Neisseria meningitidis. Microscopy and culture are the most commonly used detection methods. *N. meningitidis* is a common colonizer of the respiratory tract and is a well-described cause of conjunctivitis. Tests are available for detecting meningococcal capsular polysaccharide antigens in CSF, serum, and urine. The tests detect serogroups A, B, C, Y, and W135 (serogroup B antibodies cross-react with *E. coli* Kl antigen), and false-positive urine antigen tests have been reported. The test sensitivity approaches 90% for serogroups A, C, Y, and Wl35 but is much lower for serogroup B. Multiplex PCR meningitis/encephalitis panels now include *N. meningitidis* for detection in CSF.

Aerobic Gram-Negative Rods

***Acinetobacter* spp.** The organisms are Gram-negative coccobacilli, occasionally appearing Gram positive, and are typically arranged in pairs. Growth on blood agar media is usually good. Most strains are able to grow on MacConkey agar (some common clinical species will not), but there is no growth anaerobically (strict aerobic growth).

Aeromonas spp. Most species grow readily in culture. Selective media (e.g., blood agar with ampicillin [20 μg/ml] and CIN agar) improve recovery from contaminated specimens. Enrichment broth (e.g., alkaline peptone water) enhances recovery, but its use

is generally not indicated. Serological assays are not commonly used. Multiplex PCR gastroenteritis panels now exist but thus far do not include *Aeromonas* detection.

***Bartonella* spp.** Bacilli may be observed in clinical specimens from diseased patients (e.g., those with cat scratch disease [CSD], bacillary angiomatosis, or peliosis) with the Warthin-Starry silver stain, although this is not commonly used in clinical microbiology laboratories. Some reference laboratories offer PCR assays for these bacteria and are most useful from tissue specimens in those being considered for CSD and/or culture negative endocarditis. PCR from peripheral blood is significantly less sensitive than from tissue for infectious endocarditis. Culture is not recommended for patients with CSD but has been successful in other settings. Blood should be processed in the Isolator system, although some success has been achieved with broth-based blood culture systems. Prolonged incubation is required. Tissues should be cultured on heart infusion agar supplemented with rabbit or horse blood. These media are preferred to blood or chocolate agar. Cultures should be maintained in a humid atmosphere for 3 to 4 weeks. Serologic testing is the mainstay of diagnosis, particularly in patients with CSD, with tests performed at the CDC, state laboratories, and some reference laboratories. Commercial test kits are not available in the United States. Cross-reactions are observed in patients with infections caused by *Coxiella* and *Chlamydia* spp.

Bordetella pertussis. Microscopy and culture are relatively insensitive detection methods compared with PCR-based assays. The organisms appear as small Gram-negative coccobacilli. They are best observed using a DFA test; commercial monoclonal and polyclonal (for *B. pertussis* and *B. parapertussis*) DFA tests (directed against cell wall lipooligosaccharides) are available. Both tests have a low sensitivity (30 to 70% compared with culture) and specificity. *B. pertussis* is a fastidious, strictly aerobic organism which does not grow on blood agar media or MacConkey agar (*B. parapertussis* grows on blood agar and has variable growth on MacConkey agar). Growth on Regan-Lowe medium is more reliable than on Bordet-Gengou medium. Detection generally requires a minimum of 3 to 4 days of incubation, which should be extended for a week or more. The PCR assay is clearly the most sensitive method for detecting *B. pertussis.* A variety of target genes have been used, including the pertussis toxin promoter region and the *IS481* gene. *IS481* is a multicopy gene, and assay targeting this gene appears to be more sensitive than those targeting the pertussis toxin promoter region. However, *IS481* is also found in *B. holmeseii* and can therefore lead to false positive results in patients colonized with this organism. Positive tests are observed even after 7 days of effective therapy. Many sero-

logic tests are available, with ELISA being the method of choice. Immunoglobulin G (IgG) and IgA responses to pertussis toxin (PT) or filamentous hemagglutinin (FHA) are reliable indicators of infection. Antibodies to PT are specific for *B. pertussis;* antibodies to FHA are specific to *B. pertussis* and *B. parapertussis* (with cross-reactions with other bacteria). Serologic testing (IgG versus PT) appears to be the most sensitive measure of *B. pertussis* infection in an unimmunized individual, but seroconversion must be demonstrated.

***Brucella* spp**. The organism is a small coccobacillus, with cells arranged typically singly or, less commonly, in pairs and small chains. DFA stains are not available. The organism is a strict aerobe, requiring complex media containing several amino acids, thiamine, nicotinamide, and magnesium ions. The presence of serum and a CO_2-enriched atmosphere enhance growth, and prolonged incubation is required, although modern blood culture media can grow *Brucella* spp. within the commonly used 5-day incubation period. Various serologic assays have been developed, with the serum agglutination test being the most commonly used. A single titer of >1:160 is suggestive of infection (with *B. abortus*, *B. suis*, or *B. melitensis*). Cross-reactions are observed with *F. tularensis*, *V. cholerae*, and *Y. enterocolitica*. A rapid dipstick assay for IgM antibodies has been developed.

***Burkholderia cepacia* complex**. Selective media have been used to improve recovery from contaminated specimens. *B. cepacia* selective agar is the most sensitive and selective medium. PCR assays have been developed but are used for organism identification rather than detection. Serologic tests are not available.

Burkholderia pseudomallei. The organisms appear on direct Gram stain as small Gram-negative rods with bipolar staining. The cells resemble "safety pins," and this characteristic can be used to make a presumptive identification. The organism grows on blood agar and MacConkey agar, but better recovery is found on Ashdown medium. An enrichment broth of Ashdown medium supplemented with colistin (incubated for 7 days before subculture) improves recovery. A noncommercial indirect hemagglutination assay has been developed; however, cross-reactions with *B. cepacia* complex occur, and high antibody titers are found in healthy individuals living in areas of endemic infection. A single titer cannot be interpreted, and so seroconversion must be demonstrated.

***Campylobacter* spp.** The bacteria are curved rods typically arranged in pairs (resembling "gull wings" or S-shaped). The bacteria are thin and may not be observed in clinical specimens. Growth of most species requires a microaerophilic atmosphere, and selective

medium is recommended for the recovery of *C. jejuni* and *C. coli* from fecal specimens. *C. upsaliensis* may be a common enteric pathogen, but its recovery is compromised because it is inhibited on most selective media for Campylobacters. *C. fetus* is more commonly recovered in the blood. Serologic testing is useful for epidemiologic investigations but not for diagnostic purposes. Multiplex PCR assays now exist for the testing of stool and include *Campylobacter jejuni*, *C. coli*, and *C. upsaliensis.*

***Capnocytophaga* spp.** (includes former DF-1 and DF-2). The organisms are fusiform; curved, coccoid, and spindle-shaped forms are also observed. Growth requires enriched media and CO_2; good growth occurs in 2 to 4 days; adherent colonies may be slightly yellow and have either a regular edge or a spreading edge. There is no growth on MacConkey agar; the organism is a facultative anaerobe.

***Cardiobacterium* spp.** Rods are arranged singly or in pairs, short chains, or rosettes. The organism is facultatively anaerobic, with no growth on MacConkey agar. It is fastidious and slow growing and requires CO_2.

Eikenella spp. The organisms are slender, straight rods that are facultatively anaerobic. There is no growth on MacConkey agar; growth requires hemin and is enhanced by CO_2.

Escherichia coli. Selective media (e.g., MacConkey agar with sorbitol) can be used to detect enterohemorrhagic *E. coli* strains, and immunoassays can be used to detect the Shiga toxins. Commercial assays are also available to detect the heat-labile and heat-stable toxins of enterotoxigenic *E. coli* strains. Molecular tests have been developed to detect virulence factors in these and other *E. coli* strains. Multiplex PCR assays now exist for the testing of stool and include the detection of Enteropathogenic, Enteroaggregative, Enteroinvsasive, Enterotoxigenic, and shiga-like toxin producing *E. coli.* In addition, multiplex tests have been developed for the detection of *E. coli* from CSF as well as positive blood cultures.

***Francisella* spp.** Microscopy is relatively insensitive. The organism is a very small coccobacillus that retains the safranin counterstain poorly. A polyclonal DFA reagent is available, although the sensitivity and specificity are not well characterized. *Francisella* spp. are fastidious, strict aerobes that fail to grow on blood agar or MacConkey agar. Growth requires media supplemented with sulfhydryl compounds (e.g., cysteine, cystine, thiosulfate, and IsoVitaleX). Growth is slow but good on chocolate agar, Mueller-Hinton agar, and BCYE agar. Media should be incubated for up to 2 weeks. Antigen and PCR tests have been developed, but their sensitivities are

low (antigen test, 106 bacteria/ml; PCR, 102 bacteria/ml). Serologic testing is the most commonly used diagnostic method. Antibodies are detected as early as 1 week after the onset of symptoms and may persist for years. Tube agglutination (TA) and microagglutination (MA) are the standard assays. A single TA titer of >1:160 or MA titer of >1:128 is considered a presumptive positive reaction. A four-fold titer change is considered diagnostic.

Haemophilus ducreyi. The Gram stain morphology of this organism has been described as "long chains ('schools of fish')," but this description is more characteristic of *in vitro* cultures than of specimens collected from genital ulcers. The Gram stain sensitivity is <50%. Direct fluorescent-antibody (DFA) assay reagents have been prepared, but polyclonal antisera have poor specificity and monoclonal antisera are not commercially available. Culture requires the use of selective media (e.g., GC agar supplemented with vancomycin, hemoglobin, fetal bovine serum, and IsoVitaleX) and has variable sensitivity. PCR-based assays have been developed but are not widely available.

Haemophilus influenzae. The organisms are pleomorphic rods (i.e., coccoid, coccobacillary, or short rods); they are facultatively anaerobic, with no growth on sheep blood agar or MacConkey agar. Detection of type-specific capsular antigen has been used to diagnose disseminated disease (i.e., meningitis); however, this test is no more sensitive than a Gram stain, and vaccination has dramatically reduced the incidence of *H. influenzae* disease. Multiplex PCR assays now exist for the detection of *H. influenzae* in CSF. Serologic testing is generally restricted to demonstration of a response to vaccination.

Helicobacter pylori. Microscopy is useful for examination of tissue biopsy specimens but is performed primarily in cytology laboratories. Diagnosis is most commonly made using antigen tests, particularly the detection of urease activity in tissue biopsy specimens or by breath analysis. Stool antigen testing is also widely available and commonly used. The tests vary widely in sensitivity and specificity but generally are >90% sensitive and specific. PCR assays are available but offer no more sensitivity than the antigen tests. Culture can also be performed (and is useful if drug resistance is suspected). The organism grows best on freshly prepared nonselective media (e.g., brucella or brain heart infusion agars supplemented with horse blood) incubated in a microaerophilic atmosphere for a minimum of 5 days. A variety of serologic tests are also available. Serum IgG assays are the tests of choice, although whole-blood assays are used with increasing frequency in physician offices (the tests have a sensitivity of 80 to 90% compared with serum assays). Serum IgA

assays have a low sensitivity but may prove useful as a follow-up assay for patients with negative IgG assays.

***Kingella* spp.** The organisms are nutritionally fastidious, facultatively anaerobic short rods with square ends, arranged in pairs or chains. They decolorize unevenly on Gram staining. They do not require CO_2, but growth is enhanced; there is no growth on MacConkey agar. Some evidence suggests that bedside inoculation of bone and/or joint specimens can increase the yield of *Kingella*. In addition, PCR has been shown to demonstrate superior sensitivity to culture for the diagnosis of *Kingella* septic arthritis. It is hypothesized that synovial fluid may be inhibitory to the *in vitro* growth of the organism.

***Legionella* spp.** *Legionella* spp. are small, poorly staining rods. If observed in clinical specimens, they typically appear as coccobacilli; long filamentous forms can be seen in culture. Monoclonal and polyclonal DFA stains are commercially available; their sensitivity compared with culture is poor (33 to 70%), and cross-reactions are observed with the monoclonal reagents (*Bacillus cereus*) and polyclonal reagents (*Bacteroides fragilis*, *Pseudomonas* spp., *Stenotrophomonas* spp., and *Bordetella pertussis*). Urinary antigen tests for *L. pneumophila* serogroup 1 are available (Wampole Laboratories, Bartels) and Binax and Biotest market urinary antigen tests for nonserogroup 1 *L. pneumophila* and other *Legionella* spp. The sensitivity of these assays varies, but sensitivities are reported in the range of 70 to 80%. The specificity is good, although the Bartels test reacts with *S. pneumoniae*, and false-positive reactions due to nonspecific protein binding have been reported for the Binax EIA. Reactivity persists in *Legionella*-infected patients for weeks to months after effective therapy. PCR assays are available but generally are not more sensitive than culture. The organism is fastidious, requiring media supplemented with L-cysteine and iron salts. BCYE, supplemented with antibiotics to suppress the growth of contaminating organisms, is the medium of choice. Incubation should be extended for a week or more. IFA tests and ELISA are available to measure an antibody response to infection. The test sensitivity and specificity are 75 and 96%, respectively. A titer of >1:256 is suggestive of current infection; however, titers at this level have been found in healthy individuals. A fourfold change in titer is presumptive evidence of recent disease.

***Pasteurella* spp.** The organisms are coccoid to coccobacillary, arranged singly, in pairs, or in short chains. Growth does not require hemin or CO_2, but some strains require V factor. The most commonly isolated species fail to grow on MacConkey agar; the organism is a facultative anaerobe.

Pseudomonas aeruginosa. *P. aeruginosa* grows readily on a variety of laboratory media. PCR amplification methods have been developed for direct detection of the organism in respiratory specimens, particularly from cystic fibrosis patients, but these are not commonly used or commercially available. The method is rapid but less sensitive than culture. Serologic testing is not useful. Multiplex PCR assays now exist for the testing of media from positive blood cultures and include *P. aeruginosa*.

Salmonella enterica **Serovar Typhi.** Selective media must be used to optimize detection in fecal specimens. The Widal test measures agglutinating antibodies to the O and H antigens of *S. enterica* server Typhi and is used for serodiagnosis; however, it lacks sensitivity and specificity. Tests using other antigens (e.g., Vi antigen) have been developed but are restricted to epidemiological studies.

Salmonella, **Other Serovars.** Selective media must be used to optimize detection in fecal specimens. Multiplex PCR assays now exist for the testing of stool and include *Salmonella*.

Shigella **spp.** Selective media must be used to optimize detection in fecal specimens. Serodiagnostic assays have been developed for epidemiological surveys but have not been used for diagnostic testing. Multiplex PCR assays now exist for the testing of stool and include *Shigella*.

Stenotrophomonas **spp.** Microscopy and culture are sensitive detection methods. PCR assays have been developed but are not used for organism detection.

Streptobacillus **spp.** The organisms are rod shaped, but on extended incubation they can form very long filaments (100 to 150 μm long) and bulbous forms. Colonies develop slowly. The organism is facultatively anaerobic; no growth occurs on MacConkey agar. Isolation of the organism is inhibited by the presence of SPS, which is found in most commercially available blood culture media.

Vibrio cholerae. The viability of *V. cholerae* is maintained at an alkaline pH but decreases in formed stools or at an acidic pH. If culture is delayed, the specimen should be stored in Cary-Blair transport medium but not in buffered glycerol saline. The organism grows well on blood agar, slowly on MacConkey agar (lactose-negative), and well on selective media (e.g., TCBS). A reverse passive latex agglutination test is commercially available for the detection of cholera toxin (reacts also with ***E. coli*** heat-labile enterotoxin). Reference laboratories are also able to measure antibody response to infection. Multiplex PCR assays now exist for the testing of stool and include *Vibrio cholerae.*

***Vibrio,* Other spp.** Most pathogenic *Vibrio* spp. grow well on blood agar and MacConkey agar. As with *V. cholerae,* specimens should be processed immediately or transported in Cary-Blair medium. *V. parahaemolyticus* hemolysin (Kanagawa toxin) can be detected directly in specimens by a commercial reverse passive latex agglutination test. Multiplex PCR assays now exist for the testing of stool and include *Vibrio parahaemolyticus* as well as *V. vulnificus.*

Yersinia enterocolitica. The use of enrichment methods (e.g., storage of specimens inoculated into phosphate-buffered saline for up to 21 days at 4°C) for recovery of *Y. enterocolitica* is generally not necessary for diagnosis of patients with diarrhea but has proven useful for diagnosis of patients with terminal ileitis or postinfectious arthritis. CIN agar is the preferred selective medium, and growth is better at 25 to 30°C than at 35°C. MacConkey agar (lactose-negative colonies) can also be used. PCR tests directed against plasmid and chromosomal virulence factors have been developed but are not widely available. Antibodies against serogroups 0:3, 0:9, 0:5, 27, and 0:8 can be detected by tube or microtiter agglutination tests. Titers of >1:40 or a fourfold rise in titer is considered significant. Cross-reactions occur with *Brucella* spp. These tests are available through specialty laboratories but are not commercially available. Multiplex PCR assays now exist for the testing of stool and include *Yersinia enterocolitica.*

Yersinia pestis. A variety of stains have been used (i.e., Giemsa, Wright, Wayson, methylene blue) in addition to the Gram stain. The characteristic "bipolar, safety pin" morphology is not observed with the Gram stain. A DFA stain directed against the capsular Fl antigen is performed by state health department laboratories but is not commercially available. The organism can be isolated on nonselective agar (sheep blood agar or brain heart infusion agar) or from contaminated specimens on MacConkey agar or CIN agar (with a reduced cefsulodin concentration [4 pg/ml]). Pinpoint growth is seen at 24 h. PCR directed against the genes for the plasminogen activator protein (*pla*) and capsular Fl antigen (*caf1*) have been developed but are less sensitive than culture and an ELISA for capsular Fl antigen. Passive hemagglutination tests and ELISAs, available through the CDC, have been developed to detect antibodies directed against the Fl antigen. A titer of >1:10 is presumptive evidence of disease, and a fourfold rise or fall in the antibody titer is confirmatory.

Anaerobic Bacteria

***Actinomyces* spp.** The organisms may grow slowly in an aerobic atmosphere, and some strains are difficult to isolate in culture.

Microscopic examination of "sulfur granules" (macroscopic colonies present in clinical specimens) is helpful for making the diagnosis of actinomycosis.

***Bacteroides fragilis* group.** The organisms typically appear as pleomorphic rods in clinical specimens. Growth is rapid on most anaerobic media, although selective media (e.g., laked kanamycin-vancomycin sheep blood agar and Bacteroides bile esculin agar) should be used with nonsterile specimens.

Clostridium botulinum. Microscopy is generally of little value except when examining implicated food products. The CDC provides tests measuring the level of antibodies against *C. botulinum* toxin (antitoxin levels) in patients immunized with toxoid. These tests assess immunity and cannot be used for the diagnosis of botulism. The appropriate diagnostic test for foodborne botulism is demonstration of botulinal toxin in serum, feces, gastric contents, or vomitus or recovery of the organism in the feces of the patient. Demonstration of the organism or toxin in suspected foods provides indirect evidence of botulism. The presence of the organism or detection of toxin in wound exudates confirms the diagnosis of wound botulism.

Clostridium difficile. Microscopic examination of fecal specimens is of no clinical utility. Culture on selective media is a sensitive method for detecting the organism but does not differentiate between colonization and clinically significant disease. The diagnosis of *C. difficile* infection (CDI) is controversial, and there are several nonculture-based diagnostic strategies that are employed. PCR that detects the toxin gene (usually toxin B) is considered to be the most sensitive of the commonly used diagnostics, but there is concern that it may be too sensitive and detect patients who are colonized with *C. difficile* but do not have CDI. EIA assays that detect toxin are less sensitive than PCR and may miss some patients with CDI, but is less likely to be positive in colonized patients without CDI. Glutamine dehydrogenase (GDH) is an antigen produced by *C. difficile* and can be used as a nonspecific marker to detect *C. difficile.* GDH is nonspecific for CDI because both toxin negative and toxin positive strains can be detected by GDH. These three assays (PCR, Toxin EIA, and GDH) are the foundation for various algorithms that can be used to diagnose CDI. Among the most common include the GDH/Toxin EIA algorithm, which reflexes to PCR if GDH is positive and the EIA is negative. An alternative to this method is a PCR screen with positive tests reflexing to EIA.

Clostridium perfringens. The microscopic morphology is characteristic (large, short, fat, rectangular cells with no spores observed).

Growth on anaerobic sheep blood agar is rapid, with a double zone of hemolysis typically observed. Antigen tests for detection of toxins have not been developed, and PCR assays are restricted to research laboratories.

Clostridium tetani. Microscopy and culture are typically of little use because relatively small numbers of organisms can cause clinical disease. However, observation of the organisms in clinical specimens or recovery in culture can be diagnostic in the appropriate clinical setting. Serologic testing is also not useful for diagnostic purposes because antibodies are not formed in patients with clinical disease. It can be useful for assessing the immune status of an individual, with antitoxin levels of >0.5 IU/ml generally considered protective.

***Fusobacterium* spp**. Some species (e.g., *F. nucleatum*) have a characteristic thin, fusiform morphology. Culture may require prolonged incubation (5 days or more). *F. necrophorum* is a common cause of Lemierre's syndrome, which is typically diagnosed with positive blood cultures. In addition, *F. necrophorum* is an underappreciated cause of pharyngitis because anaerobic throats cultures are not commonly performed.

***Mobiluncus* spp.** Routine culture of vaginal specimens for *Mobiluncus* spp. is generally not clinically useful. The preferred diagnostic test is microscopy, with curved rods observed in vaginal smears.

Curved and Spiral-Shaped Bacteria

Borrelia burgdorferi. Microscopic examination of blood, CSF, and other specimens is not useful for patients with Lyme disease because the level of spirochetes is below the detection level. Culture on media such as modified Kelly medium can be performed, although extended incubation must be used and the yield is low. ELISAs have been developed to detect antigen in tissues, but the method is not recommended. PCR assays have been developed for the detection of *B. burgdorferi.* The sensitivity of the assay depends on the testing method, target gene, and stage of illness. The following test sensitivities have been reported: skin, 50 to 70% for culture or PCR; synovial fluid, 50 to 70% for PCR, culture seldom positive; CSF, 10 to 30% for culture or PCR; and urine, 0%. Serologic testing has been the method most commonly used for the diagnosis of Lyme disease. The early antibody response is primarily an IgM response and is directed against outer membrane–associated protein OspC, p35, and flagellum subunits p37 and p41. The antibody titers peak within a week of clinical onset but may persist for months, even after effective treatment. IgG antibodies appear after the first weeks of disease,

with reactivity against p37, p41, and OspC early in disease; against p39 and p58 in the early disseminated stage; and against a wide variety of antigen in the late disseminated stage. Reactivity against other antigens is also commonly observed. Assay methods include IFA, EIA, and immunoblotting. The specificity of IFA is improved by adsorption of sera with *Treponema phagedenis* sonicate. IFA titers of >1:64 are regarded as positive. This assay is difficult to standardize, and EIA is the preferred testing method. Immunoblotting is used to identify which antigens are reactive. The recommended approach to serodiagnosis is to screen serum or CSF for IgG and IgM antibodies with EIA. If the reaction is positive or borderline, IgG and IgM immunoblotting is performed. The sensitivity of serologic testing is as follows: stage 1 (early, localized disease), 20 to 50% with IgM predominant; stage 2 (early, disseminated disease), 70 to 90% with IgG predominant; stage 3 (late, disseminated disease), nearly 100% with IgG predominant. Antibodies persist for months despite treatment.

***Borrelia,* Other spp.** Patients with relapsing fever have large numbers of borreliae in their blood during febrile attacks. The diagnostic method of choice is microscopy, with the blood examined by dark-field microscopy or stained with Giemsa. Small numbers of bacteria can be concentrated in buffy coat preparations. Culture on media such as modified Kelly medium can be performed, although the cultures should be incubated at 30°C for at least 6 weeks.

***Leptospira* spp.** The organisms may be detected by dark-field microscopy or DFA, although large numbers of organisms (10^4/ ml) must be present in the specimen. Culture of blood and CSF during the first week of illness and culture of urine beginning in the second week can be performed using Ellinghausen-McCullough-Johnson-Harris medium. Cultures are incubated at 28 to 30°C and examined weekly by dark-field microscopy for up to 13 weeks. PCR assays have been developed but are not used extensively. Serologic testing is the most commonly used diagnostic method. Antibodies are detected by using the microscopic agglutination test with blood 5 to 7 days after onset of symptoms. Paired sera are required to confirm the diagnosis, with a presumptive diagnosis being made if a single serum has a titer of >1:200. The test is technically complex, and other assays have been developed. The indirect hemagglutination assay was shown to have a sensitivity and specificity of 92 and 95%, respectively. Latex agglutination assays and ELISAs have also been developed but have not been adequately evaluated.

Treponema pallidum. When available, dark-field microscopy is very sensitive for diagnosis based on a freshly sampled genital chancre or secondary-stage exudates. The DFA test for *T. pallidum* (DFA-TP) does not require viable spirochetes and can differentiate

between *T. pallidum* and nonpathogenic spirochetes. The test sensitivity for primary or secondary syphilis approaches 100%. The organism has not been grown in vitro, and antigen tests are not available. PCR methods have been developed but currently are restricted to research laboratories. Serologic testing by nontreponemal and treponemal assays is the most common diagnostic method. The nontreponemal assays include the Venereal Disease Research Laboratory (VDRL) test, rapid plasma regain (RPR) card test, unheated serum regain (USR) test, and toluidine red unheated serum test (TRUST). Treponemal tests include fluorescent treponemal antibody-absorption (FTA-ABS) test, treponemal pallidum particle agglutination (TP-PA) test, and EIA. The test sensitivities vary with the method and stage of disease. In general, nontreponemal tests have a sensitivity of 72 to >90% for the primary stage, 100% for the secondary stage, 95 to 100% for latent disease, and <75% for the late stage, while treponemal tests have a sensitivity of 80 to >90% for the primary stage, 100% for the secondary and latent stages, and >95% for the late stage. A number of conditions affect the test specificity, with more false-positive reactions being observed with nontreponemal tests. Historically, patients have been screened with a nontreponemal test (typically RPR) with positive results reflexing to treponemal tests for confirmation. A so-called reverse algorithm has recently been adopted by many labs, which screens with a treponemal test and reflexes to the RPR for confirmatory testing of positive results.

Mycoplasma spp. and Obligate Intracellular Bacteria

***Anaplasma* spp.** Giemsa or Wright stains of peripheral blood or buffy coat cells have a sensitivity approaching 60%. As with *Ehrlichia* spp., PCR assays have been developed and have a wide range of sensitivities (50 to 86%). Their specificity is reported to be 100%. IFA is the serologic method of choice. The typical response during the acute phase of infection is a rapid rise in antibody levels, reaching titers of >1:640 within the first month of disease. Antibodies can persist for many months to years. The test sensitivity is >90%. False-positive reactions have been encountered for patients with infections caused by *Rickettsia* spp., *Coxiella* spp., and Epstein-Barr virus. Patients with high *Anaplasma* titers will also have elevated titers to *B. burgdorferi.*

Chlamydia trachomatis. Infections have historically been confirmed by culture, observation of elementary bodies in specimens by DFA, or detection of chlamydial antigens (i.e., lipopolysaccharide and major outer membrane proteins) by EIA. DFA has a sensitivity and specificity of 75 to 85 and 99%, respectively. The sensitivity of

EIAs is reported to be 60 to 70% compared with that of nucleic acid amplification assays. More recently, these methods have been replaced by molecular methods, initially probe tests and now amplification tests. Amplification tests are the most sensitive, although care must be taken to eliminate inhibitors (particularly in urine) and to avoid cross-contamination of specimens. Two serologic assays are in common use: CF and microimmunofluorescence (micro-IF). The CF test detects antibodies to *C. trachomatis* as well as to *Chlamydophila psittaci* and *Chlamydophila pneumoniae*. The micro-IF test is type specific. The CF test is positive in virtually all patients with lymphogranuloma venereum but generally not positive in patients with oculogenital infections and trachoma. A positive CF titer is >1:16. CF titers for patients with lymphogranuloma venereum generally exceed 1:128, whereas patients with inclusion conjunctivitis, cervicitis, or urethritis have antibody titers of <1:16. The micro-IF test is more sensitive than the CF test. Most patients with chlamydial infections show positive reactivity (90 to 100% of patients have detectable IgG antibodies). However, antibodies to past infections persist for years and are commonly detected in the assay.

Chlamydophila pneumoniae. Most infections are diagnosed by either PCR-based assays or serological testing. Multiplex PCR assays for upper respiratory tract infection are now commonly used and include *C. pneumophila*. Micro-IF testing is available for diagnosing acute infections. Cross-reactivity with other bacteria is uncommon. Serum specimens are generally screened for IgM and IgG antibodies at 1:8, and those giving positive reactions are tested at increasing twofold dilutions. The diagnostic criteria for acute infections include at least a fourfold rise in titer and a single serum IgM titer of >1:16 and/or IgG titer of 2*1:512.

Chlamydophila psittaci. Most infections are diagnosed by serological methods, with the CF test being the most common.

Coxiella spp. *C. burnetii* can be recovered in culture, although this is rarely attempted. PCR appears to be sensitive but is currently restricted to research laboratories. A variety of serologic methods, including microagglutination, CF, IFA, and ELISA, have been used, with IFA currently being the method of choice. ELISA appears to be more sensitive than IFA, but interpretive standards have not been defined. Antigenic phase variation occurs with *C. burnetii* infections. In acute self-limited infections, antibodies to the phase II antigen appear first and dominate the immune response. In chronic infections, antibodies to the phase I antigen predominate. Phase II antibodies appear first and peak within 1 month at 1:1,024 or greater. Phase I antibodies appear later and peak at 4 months. The ratio between phase I and phase II responses may be useful for distinguishing

between acute and chronic infections. A phase I titer of 1:800 or greater is diagnostic of chronic Q fever (e.g., endocarditis).

***Ehrlichia* spp.** *Ehrlichia* spp. have been successfully cultured from blood, although this test is rarely used for diagnosis. Likewise, infected monocytes can be detected by use of Giemsa or Wright-stained peripheral blood or buffy coat cells, but this test has a sensitivity of only <30%. PCR is a widely used diagnostic test, with the 16S rRNA gene being targeted; it has a sensitivity of 79 to 100% *compared* with serologic testing. Serological testing is also useful, with IFA being the test of choice. Although a specific antibody titer has not been defined for significant disease, a titer of >1:64 is considered presumptive evidence of past or current disease.

Mycoplasma pneumoniae. Microscopy is not useful, and isolation of *M. pneumoniae* in culture is slow and insensitive. For this reason, a variety of antigen-directed tests including DFA, EIA, and immunoblotting have been developed. These tests, as well as DNA probes, have poor sensitivity and specificity. In contrast, PCR assays are reported to be highly sensitive and specific. Specific serologic tests include complement fixation (CF), ELISA, IFA, and latex agglutination. CF detects primarily IgM antibodies. Seroconversion is observed for about 60% of culture-positive patients, while 80 to 90% of patients have a single titer of >1:32. ELISA detects both IgM and IgG antibodies and appears to be more sensitive than CF. Specificity can be improved by using purified PI adhesin protein as the capture antigen. Immunofluorescence IgG and IgM antibody titers of >1:10 are considered positive, with active disease being indicated by a fourfold change in titer. The latex agglutination assay detects IgG and IgM antibodies. A single agglutination antibody titer of >1:320 or a fourfold change in titer is indicative of active or recent infection. The specificity of each of these tests is a problem because cross-reactions with other *Mycoplasma* spp. have been observed. Upper respiratory tract infection can be diagnosed with multiplex PCR panels, many of which include *Mycoplasma pneumoniae.*

Rickettsia rickettsii. Members of the spotted fever group of rickettsiae can be detected in tissue specimens by immunofluorescence or PCR. The 17-kDa lipoprotein gene is the principal target of PCR, although other targets have also been used. The sensitivity of PCR is generally thought to be lower than serology. A variety of group-specific serologic tests have been developed (e.g., IFA, CF, ELISA, RIA, latex agglutination, and hemagglutination), with the IFA being considered the "gold standard." A diagnostic titer of >1:64 is usually detected in the second week of illness. The Proteus OX agglutination test was used historically but has been replaced by the more specific serologic tests.

Table 4.5 Differential characteristics of catalase-positive Gram-positive cocci

Genus	Strict aerobe	Adherent to agar	Growth in 6.5% NaCl	Oxidase	Motility	Lysostaphin (200 μg/ml)	Erythromycin (0.4 μg/ml)	Bacitracin (0.04-U disk)	Furazolidone (100-μg disk)
Staphylococcus	-	-	+	—[c]	—	S	R	R	S
Macrococcus	-	-	+	+	-	S	R	R	S
Kocuria	-	-	+	+	-	R	S	S	R
Micrococcus	+	—	+	+	—	R	S	S	R
Planococcus	+	—	+	NT[a]	+	R	NT	NT	S
Alloiococcus[b]	+	—	+	—	—	NT	NT	NT	NT
Rothia	—	+	—	—	—	R	NT	S	NT

[a]NT, not tested.

[b]*Alloiococcus* is weakly catalase positive or catalase negative.

[c]*Staphylococcus lentus, S. scuiri,* and *S. vitulus* are oxidase positive.

Bacterial Diagnosis

Table 4.6 Differential characteristics of most common clinically significant *Staphylococcus* species

***Staphylococcus* species**	**Coagulase**	**Clumping factor**	**Heat-stable nuclease**	**Alkaline phosphatase**	**PYR[a] hydrolysis**	**Ornithine decarboxytase**	**Urease**	**β-Galactosidase**	**Voges-Proskauer**	**Novobiocin**	**Polymyxin B**
S. aureus	+	+	+	+	—	—	V	–	+	S	R
S. epidermidis	–	–	–	+	–	V	+	–	+	S	R
S. haemolyticus	–	–	–	—	+	—	—	–	+	S	S
S. hyicus (veterinary)	V	–	+	+	—	—	V	–	–	S	R
S. intermedius	+	V	+	+	+	–	+	+	–	S	S
S. pseudintermedius (veterinary)	+	-	NT	V	+	NT	+	+	NT	S	R
S. lugdunensis	–	(+)	–	–	+	+	V	—	+	S	V
S. saprophyticus subsp. *saprophyticus*	–	—	—	–	–	–	+	+	+	R	S
S. schleiferi subsp. *schleiferi*	–	+	+	+	–	–	–	(+)	+	S	S

[a]PYR, pyrrolidonyl arylamidase.

NT, not tested; V, variable; parentheses indicate delayed reaction

Table 4.7 Differential characteristics of catalase-negative Gram-positive cocci

Genus	Pyrrolidonyl arylamidase	Leucine aminopeptidase	Morphology[a]	Growth in 6.5% NaCl	Satellite growth	Motility	Vancomycin	Arginine hydrolysis	β-Glucuronidase	Esculin hyrolysis
Enterococcus	+	+	Chains	+	—	V[d]	S/R			+
Abiotrophia	+	+	Clusters	—	+	—	S	+	—	
Gemella	+	+	Chains[b]	—	—	—	S			
Granulicatella	+	+	Chains	—	—	—	S	V[e]	V[e]	
Facklamia	+	+	Chains[c]	+	—	—	S		—	
Vagococcus	+	+	Chains	V	—	+	S			
Globicatella	+	—	Chains	+	—	—	S			
Aerococcus viridans	+	—	Clusters	+	—	—	S			
Aerococcus urinae	—	+	Clusters	+	—	—	S			
Pediococcus	—	+	Clusters	V	—	—	R			
Lactococcus	V	+	Chains	V	—	—	S			
Streptococcus	V	+	Chains	V	—	—	S			
Leuconostoc	—	—	Chains	+	—	—	R			

[a]Cellular morphology in broth culture.
[b]*Gemella haemolysans* is arranged in clusters.
[c]*Facklamia languida* is arranged in clusters.
[d]*Enterococcus casseliflavus* and *E. gallinarum* are motile.
[e]*Granulicatella adiacens* is ARG negative and BGUR positive; *G. elegans* is ARG positive and BGUR negative

Table 4.8 Differential characteristics of beta-hemolytic streptococci[a]

***Streptococcus* species**	**Lancefield group**	**Beta-hemolysis**	**Colony**	**PYR**	**Bacitracin**	**Voges-Proskauer**	**CAMP**	**Hippurate**
S. pyogenes	A	Large	Large	+	+	—	—	—
S. agalactiae	B	Small	Large	—	—	—	+	+
S. dysgalactiae subsp. *equisimilis*	C, G	Large	Large	—	—	—	—	—
S. dysgalactiae subsp. *dysgalactiae*	C	Alpha	Large	—	—	—	—	—
S. anginosus group	A, C, F, G, nongrp	Variable	Small	—	—	+	—	—
S. pseudoporcinus	E, P, NG1, nongrp, B (!)	Large	Large	V	-	NT	+	-
S. porcinus	E, P, U, V, nongrp	Large	Large	V	-	+	+	V
S. canis	G	Large	Large	-	NT	NT	+	-

[a]PYR, pyrrolidonyl arylamidase; BGUR, U-glucuronidase; NT, not tested; (!), *S. pseudoporcinus* do not possess the Lancefield B antigen, but can lead to false positive typing reactions.

Table 4.9 Differential characteristics of viridans streptococci

	Acid from:			Hydrolysis of:	
***Streptococcus* group**[a]	**Mannitol**	**Sorbitol**	**Voges-Proskauer**	**Arginine**	**Esculin**
S. mitis group	—	V	—	V	V
S. salivarius group	—	—	+	—	+
S. mutans group	+	+	+	—	+
S. anginosus group	—	—	+	+	+
S. bovis group	V	—	+	—	V

[a]*S. mitis* group: *S. mitis, S. sanguinis, S. parasanguinis, S. gordonii, S. cristatus, S. oralis, S. infantis, S. peroris, S. australis, S. sinensis, S. orisratti, S. oligofermentans,* and *S. massiliensis.*

S. salivarius group: *S. salivarius, S. vestibularis,* and *S. thermophiles.*

S. mutans group: *S. mutans, S sobrinus.*

S. anginosus group: *S. anginosus, S. intermedius, S. constellatus.*

S. bovis group: *S. equinus, S. gallolyticus, S infantarius,* and *S. alactoyticus.*

Bacterial Diagnosis

Table 4.10 Differential characteristics of common *Enterococcus* species

Group	Species	Acid from: Arabinose	Mannitol[a]	Sorbitol[a]	Raffinose	Sucrose	Pyruvate	Arginine hydrolysis[a]	Growth in 0.04% tellurite	Motility	Pigment production	Methyl-α-D-glucopyranoside
I	*E. avium*	+	+	+	—	+	—	—	—	—	+	
	E. raffinosus	+	+	+	+	+	+	—	—	—	—	+
II	*E. faecalis*	—	+	+	—	+	+	+	+	—	—	—
	E. faecium	+	+	V	V	+	—	+	—	—	—	—
	E. casseliflavus	+	+	V	+	+	V	+	—	+	+	+
	E. gallinarum	+	+	—	+	+	—	+	—	+	—	+
III	*E. durans*	—	—	—	—	—	—	—	—	—	—	—
	E. hirae	—	—	—	V	+	—	+	—	—	—	
	E. dispar	—	—	—	+	+	+	+	—	—	—	+
IV	*E. cecorum*	—	—	+	+	+	+	—	—	—	—	—
V	*Vagococcus fluvialis*	—	+	—	—	—	+	—	—	+	—	+

[a]Key tests for grouping enterococci.

Table 4.11 Differential characteristics of Gram-positive rods

Catalase negative

Beta-hemolytic — *Arcanobacterium*

Alpha-hemolytic — *Erysipelothrix, Lactobacillus*

Nonhemolytic — *Actinomyces*

Catalase positive

Regular shape, spore former — *Bacillus, Paenibacillus*

Regular shape, non-spore former — *Listeria*

Irregular shape, pink pigment — *Rhodococcus equi*

Irregular shape, orange pigment — *Rhodococcus, Microbacterium, Nocardia*

Irregular shape, yellow pigment

Oerskovia, Brevibacterium, Cellulomonas, Aureobacterium

Irregular shape, no pigment

Bacillary — *Corynebacterium*

Coccobacillary — *Arthrobacter, Brevibacterium, Corynebacterium, Dermabacter, Rhodococcus*

Branching — *Nocardia, Actinomyces, Propionibacterium, Rothia, Streptomyces, Turicella*

Table 4.12 Differential characteristics of common *Corynebacterium* species

Species	Fermentation/ oxidation	Lipophilism	Nitrate reduction	Urease	Esculin hydrolysis	Acid from:					Comments
						Glucose	Maltose	Sucrose	Mannitol	Xylose	
C. jeikeium	O	+	—	—	—	+	V	—	—	—	Strict aerobe
C. urealyticum	O	+	—	+	—	—	—	—	—	—	Strong urease production
C. pseudodiphtheriticum	O	—	+	+	—	—	—	—	—	—	
C. diphtheriae	F	—	+	—	—	+	+	—	—	—	
C. ulcerans	F	—	—	+	—	+	+	—	—	—	CAMP inhibited
C. amycolatum	F	+	V	V	—	+	V	V	—	—	
C. macginleyi	F	+	+	—	—	+	—	+	V	—	From eye; may have rose color
C. minutissimum	F	—	—	—	—	+	+	V	V	—	
C. striatum	F	—	+	—	—	+	—	V	—	—	
C. glucuronolyticum	F	—	V	V	V	+	V	+	—	V	CAMP and BGUR positive, floral odor
C. pseudotuberculosis	F	—	V	+	—	+	+	V	—	—	CAMP inhibited
C. kroppenstedtii	F	+	—	—	+	+	V	+	—	—	From breast abscesses
C. aurimucosum	F	—	—	—	V	+	+	+	—	—	Most strains yellowish, some gray/black

Table 4.13 Differential characteristics of selected coryneform bacteria

Organism	Fermentation/ oxidation	Catalase	Nitrate reduction	Esculin hydrolysis	Motility	Acid from: Glucose	Maltose	Sucrose	Mannitol	Xylose	Comments
Turicella otitidis	O	+	—	—	—	—	—	—	—	—	Ear; CAMP positive
Arthrobacter spp.	O	+	V	V	V	V	V	V	—	—	
Brevibacterium spp.	O	—	V	—	—	V	V	V	—	—	Cheese-like odor
Microbacterium spp.	O/F	V	V	V	V	+	+	V	+	+	Yellow pigment
Curtobacterium spp.	O	+	—	+	V	+	V	V	V	+	
Leifsonia aquatica	O	+	V	—	+	+	V	V	+	+	
Arcanobacterium bemardiae	F	—	—	—	—	+	+	—	—	—	
Arcanobacterium haemolyticum	F	—	—	—	—	+	+	V	—	—	CAMP inhibited
Arcanobacterium pyogenes	F	—	—	V	—	+	V	V	V	+	Weak hemolysis
Gardnerella vaginalis	F	—	—	—	—	+	+	V	—	—	Gram variable to Gram negative
Dermabacter hominis	F	+	—	+	—	+	+	+	—	V	Coccoid to coccobacillary
Rothia dentocariosa	F	V	+	+	—	+	+	+	—	—	
Oerskovia turbata	F	+	+	+	V	+	+	+	—	+	
Helcobacillus spp.	F	+	+	—	—	+	+	+	+	+	
Cellulomonas spp.	F	+	+	+	V	+	+	+	V	+	
Trueperella bernardiae	F	—	—	—	—	+	+	—	—	—	
Trueperella pyogenes	F	—	—	V	—	+	V	V	V	+	

Bacterial Diagnosis

Table 4.14 Differential characteristics of selected *Bacillus* species and related spore-forming genera

Organism	Growth:			Spore Position	Lecithinase (egg yolk)	Casein hydrolysis	Gelatin hydrolysis	Arginine dihydrolase	Acid from:					
	Anaerobic	50°C	60°C						D-Arabinose	Glycerol	Glycogen	Insulin	Mannitol	Salicn
B. anthracis	+	—	—	S	+	+	+	—	—	—	+	—	—	—
B. cereus	+	—	—	S, C	+	+	+	V	—	V	+	—	—	+
B. thuringiensis	+	—	—	S	+	+	+	+	—	+	+	—	—	+
B. mycoides	+	—	—	S, (C)	+	+	+	V	—	+	+	—	+	
B. subtilis	—	V	—	S, C	—	+	+	—	+	+	+	+	+	
B. licheniformis	+	+	—	S, C	—	+	+	+	—	+	+	V	+	+
B. circulans	+	—	—	S, T	—	—	—	—	—	V	+	+	+	+
Paenibacillus	—	—	—	S, C, T	—	V	+	—	V	+	+	V	+	V
Geobacillus spp.	V	+	+	S, T	—	V	V	—	—	V	V	V	V	V

S, subterminal; C, central; T, terminal; parentheses indicates rare occurrence.

Table 4.15 Differential characteristics of selected actinomycetes[a,b]

	Aerial hyphae	Conidia	Acid-fast nature	Mycolic acids	Growth at 50°C	Growth in lysozyme
Actinomadura	V	V	—	—	—	V
Amycolatopsis	V	V	—	—	—	V
Corynebacterium	—	—	—	V	—	—
Dermatophilus	+	—	—	—	—	NT
Dietzia	—	—	—	+	NT	NT
Gordonia	—	—	W	+	—	V
Mycobacterium	—	—	+	+	—	+
Nocardia	+	V	W	+	—	+
Nocardiopsis	+	+	—	—	—	—
Rhodococcus	—	—	W	+	—	—
Saccharomonospora	+	+	—	—	+	V
Saccharopolyspora	+	+	—	—	+	V
Streptomyces	+	+	—	—	—	V
Thermoactinomyces	+	+	—	—	+	+
Tsukamurella	—	—	W	+	—	+

[b] W, weak or partially acid fast; NT, not tested; V, variable.

Bacterial Diagnosis

Table 4.16 Differential characteristics of selected *Nocardia* species[a]

Species	Arylsulfiatase (14 day)	Hydrolysis of:				Susceptibility to:							
		Acetamide	Casein	Tyrosine	Xanthine	Amikacin	Linezolid	Ceftriaxone	Ciprofloxacin	Clarithromycin	Gentamicin	Imipenem	Tobramycin
N. abscessus	—	—	—	—	—	S	S	S	R	R	V	R	V
N. cyriacigeorgica	—	+	—	—	—	S	S	R	R	R	V	S	V
N. farcinica	—	+	—	+	—	S	S	R	S	R	R	S	R
N. nova complex	+	—	—	—	—	S	S	S	V	S	V	S	V
N. brasiliensis	—	—	+	+	—	V	S	V	R	R	V	V	V
N. pseudobrasiliensis	—	—	+	+	—	V	S	V	S	S	V	V	V
N. otitidiscaviarum	—	—	—	—	—	S	S	R	S	V	S	R	V

[a]The taxonomy of this genus has been extensively modified; molecular testing is required for identification of most species. V, variable.

Table 4.17 Differential characteristics of select, slow-growing *Mycobacterium* species

Descriptive term	Species	Optimal temp (°C)	Usual colony morphology[c]	Pigmentation[d]	Niacin	Growth on T2H (10 μg/ml)[b]	Susceptibility to Pyrazinamide	Nitrate reduction
TB complex	*M. tuberculosis*	37	R	–	+	+	+	+
	M. caprae	37	S	–	–	–	+	–
	M. bovis	37	S	–	–	–	–	–
	M. bovis BCG	37	R	–	–	–	–	–
Non- chromogens	*M. avium*	30–37	Smt/R	–	–	NA	NA	–
	M. intracellulare	30–37	Smt/R	–	–	NA	NA	–
	M. chimaera	25–37	S	–	–	NA	NA	–
	M. haemophilum[c]	28–32	R	–	–	NA	NA	V
	M. malmoense	30	Sm	– (88)	–	NA	NA	–
	M. shimoidei	37	R	–	–	NA	NA	–
	M. genavense	37	Smt	–	–	NA	NA	–
	M. celatum	33–42	S	–	–	NA	NA	–
	M. ulcerans	30	R	–	–	NA	NA	–
	M. terrae complex	35	S/R	– (7)	–	NA	NA	V
	M. xenopi	40–45	S	–	–	NA	NA	–

(continued)

Bacterial Diagnosis

Table 4.17 Differential characteristics of select, slow-growing *Mycobacterium* species *(continued)*

Descriptive term	Species	Optimal temp (°C)	Usual colony morphology[c]	Pigmentation[d]	Niacin	Growth on T2H (10 μg/ml)[b]	Susceptibility to Pyrazinamide	Nitrate reduction
Chromogens	*M. gordonae*	30–37	S/R	+	–	NA	NA	–
	M. kansasii	35–37	R	+	–	NA	NA	+
	M. marinum	30	S/R	+	V	NA	NA	–
	M. scrofulaceum	37	S	+	–	NA	NA	–
	M. simiae	37	S	+	V	NA	NA	+
	M. szulgai	37	S/R	+	–	NA	NA	+

NA, not applicable. The percentage of strains positive in each test is given in parentheses, and the test result is based on these percentages.

[a]R, rough; S, smooth; S/R, intermediate in roughness; Smt, smooth and transparent

[b]T2H, thiophere-2-carboxylic acid hydrazide.

[c]Requires hemin as growth factor.

Table 4.18 Differential characteristics of clinically relevant, rapidly growing, *Mycobacterium* species

Species	Pigment production	Unique phenotype	Unique *hsp65* PRA pattern	Unique (complete) 16S sequence
M. abscessus subsp. abscessus	-	-	+	+
M. chelonae	-	+	+	+
M. fortuitum	-	+	+	+
M. abscessus subsp. bolletii	-	-	-	-
M. abscessus subsp. massiliense	-	-	-	-
M. bacteremicum	+	-	+	+
M. boenickei	-	-	+	+
M. canariasense	-	-	+	+
M. cosmeticum	+	+	+	+
M. goodii	+	-	+	+
M. houstonense	-	-	-	-
M. immunogenum	-	-	+	+
M. mageritense	-	+	+	+
M. mucogenicum	-	+	+	+
M. neoaurum	+	-	+	+
M. peregrinum	-	-	+	-
M. porcinum	-	-	+	+
M. senegalense	-	-	+	+
M. smegmatis	+	-	+	+

Table 4.19 Differential characteristics of medically relevant *Neisseria* species

Species	Morphology	Growth on Selective Media[a]	Nitrate reductase	Acid from: Glucose	Maltose	Lactose	Sucrose	Fructose
N. animaloris	CR	NT	+	+	—	—	—	NT
N. bacilliformis	R	—	V	—	—	—	—	NT
N. cinerea	C	V	—	—	—	—	—	—
N. elongata[b]	C	—	V	V	—	—	—	—
N. flavescens	C	—	—	—	—	—	—	—
N. gonorrhoeae	C	+	—	+	—	—	—	—
N. lactamica	C	+	—	+	+	+	—	—
N. meningitidis	C	+	—	+	+	—	—	—
N. mucosa	C	—	+	+	+	—	+	+
N. polysaccharea	C	+	+	+	+	—	V	—
N. sicca	C	—	+	+	+	—	+	+
N. subflava[c]	C	V	—	+	+	—	V	V
N. weaver	R	NT	—	—	—	—	—	—
N. zoodegmatis	CR	NT	V	V	—	—	—	NT

[a] MTM, modified Thayer-Martin agar, ML, Martin-Lewis agar; NYC, New York City agar, CHOC, chocolate agar, BA, blood agar.

[b] Includes subspecies *elongata*, *glycolytica*, and *nitroreducens*

[c] Includes subspecies *flava, perflava,* and *subflava*.

Table 4.20 Differential characteristics of selected members of the *Neisseriaceae* and of the *Cardiobacteriaceae*

Species	Oxidase	Catalase	Growth on MacConkey agar	Indole	Nitrate reductase	Arginine dihydrolase	Ornithine decarboxylase	Esculin hydrolysis	Alkaline phosphatase	Acid from:				
										Glucose	Sucrose	Maltose	Mannitol	
Cardiobacterium hominis	+	—	—	+	—	—	—	—	—	+	+	+	+	Pleomorphic rods
Cardiobacterium valvarum	+	—	—	V	—	NT	NT	—	NT	V	V	V	V	
Chromobacterium violaceum	V	+	+	V	+	+	—	—	+	+	V	—	—	Produces violacein
Kingella kingae	+	—	—	—	—	—	—	—	+	+	—	+	—	Weak beta-hemolysis
Kingella denitrificans	+	—	—	—	+	—	—	—	—	+	—	—	—	
Kingella oralis	+	—	—	—	—	—	—	—	+	+	—	—	—	
Kingella potus	+	—	—	—	—	—	—	—	—	—	—	—	—	Yellow pigment
Eikenella corrodens	+	—	—	—	+	—	+	—	—	—	—	—	—	Slender rods; may pit agar
Simonsiella muelleri	+	—	—	—	V	—	—	—	—	+	—	—	—	Cells arrange in caterpillar-like filaments
Suttonella indologenes	+	—	—	+	—	—	—	—	+	+	+	+	—	

V, variable; NT, not tested.

Bacterial Diagnosis

Table 4.21 Differential characteristics of selected *Aggregatibacter, Actinobacillus,* and *Pasteurella* species

Species	Oxidase	Catalase	Growth on MacConkey agar	Urease	Esculin hydrolysis	Indole	ONPG	Gas from glucose	Acid from:							Comments
									Lactose	Maltose	Mannitol	Melibiose	Sucrose	Trehalose	Xylose	
Aggregatibacter actinomycetemcomitans	–/W	+	—	—	—	NT	—	V	—	+	V	—	—	—	V	Coccobacillus; star-shaped, adherent colonies
A. aphrophilus	V	—	V	—	—	NT	+	+	(+)	+	—	NT	+	(+)	—	Adherent colonies
A. segnis	—	V	—	—	—	NT	—	—	—	+	—	—	+	—	+	
Actinobacillus spp.	+	V	V	+	V	NT	V	—	V	+	V	V	+	V	V	Urease can differentiate from *Aggregatibacter* and *Pasteurella*
P. multocida	+	+	—	—	NT	+	NT	—	+	—	—	NT	+	NT	V	Coccobacillus
P. canis	+	+	—	—	NT	+	NT	—	—	—	—	NT	+	NT	—	Coccobacillus

Table 4.22 Differential characteristics of selected *Capnocytophaga, Dysgonomonas,* and *Streptobacillus* species

Species	Oxidase	Catalase	Indole	Nitrate reductase	ONPG	Acid from: Glucose	Lactose	Sucrose	Xylose	Melibiose	Comments
Capnocytophaga[a] (human flora)	—	—	—	V	V	NT	V	+	—	NT	Fusiform; pleomorphic
Capnocytophaga[b] (dog and cat flora)	+	+	—	V	+	NT	+	V	—	NT	Fusiform; pleomorphic
D. capnocytophagoides	—	—	V	—	+	+	+	+	+	+	Strawberry-like odor
S. moniliformis	—	—	—	—	—	+	—	—	—	NT	Typically form very long filaments

[a]Includes *C. ochracea, C. sputigena, C. gingivalis, C. granulosa,* and *C. haemolytica*
[b]Includes *C.canimorsus* and *C. cynodegmi*

Bacterial Diagnosis

Table 4.23 Differential characteristics of *Haemophilus* species

Species	Growth requirement:		Hemolysis	Urease	Ornithine decarboxylase	Indole	Catalase	Acid from:			
	Hemin (factor X)	NAD (factor V)						Glucose	Sucrose	Lactose	Mannose
H. influenzae	+	+	—	V	V	V	+	+	—	—	—
H. haemolyticus	+	+	+	+	—	V	+	—	—	—	—
H. parahaemolyticus	—	+	+	+	—	—	+	+	—	—	—
H. parainfluenzae	—	+	—	V	V	V	V	+	+	—	+
H. paraphrohaemolyticus	—	+	+	+	—	—	V	+	+	—	—
H. pittmaniae	—	+	+	NT	NT	NT	+[w]	+	+	—	+
H. ducreyi	+	—	—[a]	—	—	—	—	V	—	—	—
H. sputorum	—	+	+[b]	+	—	+	NT	+	NT	—	—
H. aegyptius	+	+	—	+	—	—	+	—	—	—	—

[a]Delayed hemolysis occurs in some strains.

[b]Hemolysis produced on sheep and horse blood.

w, weak reaction.

Table 4.24 Differential characteristics of selected members of the *Enterobacteriaceae*

Species	Indole production	Yellow pigment	Methyl red	Voges-Proskauer	Citrate utilization	Urease	Phenylalanine deaminase	Lysine decarboxylase	Arginine dihydrolase	Ornithine decarboxylase	Motility	Acid from: Glucose	Lactose	Sucrose	Mannitol	Dulcitol	Adonitol	Maltose	Xylose
Citrobacter freundii	V	—	V	—	V	V	—	—	V	—	+	+	V	+	+	—	—	+	+
Citrobacter koseri	+	—	+	—	+	V	—	—	V	+	+	+	V	V	+	V	+	+	+
Cronobacter (*Enterobacter*) *sakazakii*	—	+	—	+	+	—	V	—	+	+	+	+	+	+	+	—	—	+	+
Edwardsiella tarda	+	—	+	—	—	—	—	+	—	+	+	+	—	—	—	—	—	+	—
Enterobacter aerogenes	—	—	—	+	+	—	—	+	—	+	+	+	+	+	+	—	+	+	+
Enterobacter cloacae complex	—	—	—	+	+	V	—	—	+	+	+	+	+	+	+	V	V	+	+
Escherichia coli	+	—	+	—	—	—	—	+	V	V	+	+	+	V	+	V	—	+	+
Escherichia fergusonii	+	—	+	—	—	—	—	+	—	+	+	+	—	—	+	—	+	+	+
Escherichia hermannii	+	+	+	—	—	—	—	—	—	+	+	+	V	V	+	—	—	+	+
Klebsiella oxytoca	+	—	V	+	+	+	—	+	—	—	—	+	+	+	+	V	+	+	+
Klebsiella pneumoniae[a]	—	—	V	+	+	+	—	+	—	—	—	+	+	+	+	V	+	+	+
Morganella morganii	+	—	+	—	—	+	+	—	—	+	+	+	—	—	—	—	—	—	—
Plesiomonas shigelloides	+	—	+	—	—	—	—	+	+	+	+	+	V	—	—	—	—	—	—

(continued)

Table 4.24 Differential characteristics of selected members of the *Enterobacteriaceae (continued)*

Species	Indole production	Yellow pigment	Methyl red	Voges-Proskauer	Citrate utilization	Urease	Phenylalanine deaminase	Lysine decarboxylase	Arginine dihydrolase	Ornithine decarboxylase	Motility	Acid from:							
												Glucose	Lactose	Sucrose	Mannitol	Dulcitol	Adonitol	Maltose	Xylose
Proteus mirabilis	—	—	+	V	V	+	+	—	—	+	+	+	—	V	—	—	—	—	+
Proteus vulgaris	+	—	+	—	V	+	+	—	—	—	+	+	—	+	—	—	—	+	+
Providencia rettgeri	+	—	+	—	+	+	+	—	—	—	+	+	—	V	+	—	+	—	—
Providencia stuartii	+	—	+	—	+	V	+	—	—	—	V	+	—	+	—	—	—	—	—
Salmonella species	—	—	+	—	+	—	—	+	V	+	+	+	—	—	+	+	—	+	+
Serratia liquefaciens	—	—	+	+	+	—	—	+	—	+	+	+	—	+	+	—	—	+	+
Serratia marcescens	—	—	+	+	+	V	—	+	—	+	+	+	—	+	+	—	V	+	—
Shigella sonnei	—	—	+	—	—	—	—	—	—	+	—	+	—	—	+	—	—	+	—
Yersinia enterocolitica	V	—	+	—	—	V	—	—	—	+	—	+	—	+	+	—	—	V	V
Yersinia pestis	—	—	V	—	—	—	—	—	—	—	—	+	—	—	+	—	—	V	+

Table 4.25 Differential characteristics of *Citrobacter* species[a]

Species	Indole	ODC[b]	Malonate	Acid from: Sucrose	Dulcitol	Melibiose	Adonitol
C. amalonaticus	+	+	—	—	—	—	—
C. braakii	V	+	—	—	V	V	—
C. farmeri	+	+	—	+	—	+	—
C. freundii (sensu stricto)	V	—	—	V	—	+	—
C. koseri	+	+	+	V	V	—	+
C. rodentium	—	+	+	—	—	—	—
C. sedlakii	V	+	+	—	+	+	—
C. werkmanii	—	—	+	—	—	—	—
C. youngae	V	—	—	V	+	—	—
C. gillenii	—	—	+	V	—	V	—
C. murliniae	+	—	—	V	+	V	—

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]ODC, ornithine decarboxylase.

Bacterial Diagnosis

Table 4.26 Differential characteristics of select *Enterobacter, Pluralibacter, Cronobacter, Kosakonia,* and *Pantoea* species[a]

Species	Lysine decarboxylase	Arginine dihydrolase	Ornithine decarboxylase	Voges-Proskauer	Acid from:							Yellow pigment
					Sucrose	Adonitol	Sorbitol	Rhamnose	α-Methyl glucoside	Esculin	Melibiose	
E. aerogenes	+	—	+	+	+	+	+	+	+	+	+	—
P. agglomerans	—	—	—	V	V	—	V	V	—	V	V	V
E. amnigenus biogroup 1	—	—	V	+	+	—	—	+	V	+	+	—-
E. asburiae	—	V	+	—	+	—	+	—	+	+	—	—
E. cancerogenus	—	+	+	+	—	—	—	+	—	+	—	—
E. cloacae subsp. *cloacae*	—	+	+	+	+	V	+	+	V	V	+	—
K. cowanii	—	—	—	+	+	—	+	+	—	+	+	V
Pluralibacter gergoviae	+	—	+	+	+	—	—	+	—	+	+	—
E. hormaechei subsp. *hormaechei*	—	V	+	+	+	—	—	+	V	—	—	—
E. kobei	—	+	+	—	+	—	+	+	+	V	+	—
C. sakazakii	—	+	+	+	+	—	—	+	+	+	+	+

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

Table 4.27 Differential characteristics of *Klebsiella* and *Raoultella* species[a]

Species	Indole	Ornithine decarboxylase	Voges-Proskauer	Malonate	ONPG	Growth at:	
						10°C	44°C
K. oxytoca	+	—	+	+	+	—	+
K. ozaenae	—	—	—	—	V	NT	NT
K. pneumoniae	—	—	+	+	+	—	+
K. variicola[b]	—	—	+	+	+	—	+
K. rhinoscleromatis	—	—	—	+	—	NT	NT
R. ornithinolytica	+	+	V	+	+	+	NT
R. planticola	V	—	+	+	+	+	—
R. terrigena	—	—	+	+	+	+	—

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]A negative adonitol reaction may be an indication that the strain is *K. variicola* but *rpoB* gene sequencing is required to confirm identification.

Bacterial Diagnosis

Table 4.28 Differential characteristics of *Proteus, Providencia,* and *Morganella* species[a]

Organism	Indole	H_2S	Urea	Ornithine decarboxylase	Acid from: Maltose	D-Adonitol	D-Arabftol	Trehalose	*myo*-Inositol
Proteus									
P. hauseri	+	V	+	—	+	—	—	+	—
P. mirabilis	—	+	+	+	—	—	—	+	—
P. penneri	—	V	+	—	+	—	—	V	—
P. vulgaris	+	V	+	—	+	—	—	—	—
Providencia									
P. alcalifaciens	+	—	—	—	—	+	—	—	—
P. heimbachae	—	—	—	—	V	+	+	—	V
P. rettgeri	+	—	+	—	—	+	+	—	+
P. rustigianii	+	—	—	—	—	—	—	—	—
P. stuartii	+	—	V	—	—	—	—	+	+
Morganella									
M. morganii subsp. *morganii*	+	—	+	+	—	—	—	—	—
M. morganii subsp. *sibonii*	V	—	+	+	—	—	—	+	—

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

Table 4.29 Differential characteristics of *Yersinia* species after incubation at 25°C for 48 h[a]

Species	Motility	Urease	Voges-Proskauer	Indole	Esculin	Citrate	Ornithine decarboxylase	Acid from:				
								Sucrose	Rhamnose	Cellobiose	Melibiose	Sorbose
Y. pestis	—	—-	—	—	+	—	—	—	—	—	—	—
Y. pseudotuberculosis	+	+	—	—	+	—	—	—	(+)	—	+	—
Y. enterocolitica	+	+	V	V	—	—	+	+	—	+	—	+
Y. frederiksenii	+	+	+	+	+	+	+	+	+	+	—	+
Y. kristensenii	+	+	—	+	—	—	+	—	—	+	—	+
Y. ruckeri	V	—	—	—	—	—	+	—	—	—	—	—
Y. mollaretii	+	+	—	—	(+)	+	+	+	—	+	—	+
Y. bercovieri	+	+	—	—	—	—	+	+	—	+	—	—
Y. rohdei	+	+	—	—	—	+	+	+	—	+	+	—
Y. aldovae	+	+	+	—	—	+	+	—	+	—	—	—
Y. intermedia	+	+	+	+	+	+	+	+	+	+	+	+
Y. aleksiciae	+	+	—	—	—	—	+	—	—	+	—	—
Y. pekkanenii	—	+	—	—	—	—	—	—	—	+	—	—
Y. similis	+	+	—	—	+	+	—	—	+	—	—	—
Y. entomophaga	+	+	NT	+	+	+	+	+	—	NT	—	NT
Y. massiliensis	+	+	—	+	+	+	+	(+)	—	+	—	+
Y. nurmii	+	—	+	—	—	+	+	+	—	+	—	—

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

NT, not tested; (+), weak positive; V, variable.

Table 4.30 Differential characteristics of *Aeromonas* species[a]

Complex	Species	Voges-Proskauer	Indole	Lipase (corn oil) test	Gas from glucose	Acid from:				Ampicillin resistance
						Rhamnose	Lactose	Sucrose	Cellobiose	
A. hydrophila Complex	*A. hydrophila*	+	+	+	+	V	V	+	—	+
	A. bestiarum	V	+	+	V	V	—	+	V	+
	A. salmonicida	V	+	+	V	—	+	+	V	+
A. caviae Complex	*A. caviae*	—	V	V	—	—	V	+	+	+
	A. media	—	+	V	—	—	V	+	+	V
	A. eucrenophila	—	+	+	V	V	—	V	V	+
A. veronii Complex	*A. veronii*	+	+	+	+	—	—	+	V	+
	A. jandaei	+	++	+	+	—	—	—	V	+
	A. schubertii	V	V	+	—	—	—	—	—	+
	A. trota	—	+	—	V	—	—	V	+	—

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

V, variable.

Table 4.31 Differential characteristics of *Vibrio* species[a]

Species	Growth in: Nutrient broth + 0% NaCl	Growth in: Nutrient broth + 6% NaCl	Oxidase	Indole	Motility	ONPG	*myo*-Inositol fermentation	Arginine dihydrolase	Lysine decarboxylase	Ornithine decarboxylase
V. cholerae	+	V	+	+	+	+	—	—	+	+
V. mimicus	+	V	+	+	+	+	—	—	+	+
V. melschnikovii	—	V	—	V	V	V	V	V	V	—
V. cincinnatiensis	—	+	+	—	+	+	+	—	V	—
Grimontia (*Vibrio*) *hollisae*	—	V	+	+	—	—	—	—	—	—
Photobacterium (*Vibrio*) *damsela*	—	+	+	—	V	—	—	+	V	—
V. fluvialis	—	+	+	—	V	V	—	+	—	—
V. furnissii	—	+	+	—	+	V	—	+	—	—
V. alginolyticus	—	+	+	V	+	—	—	—	+	V
V. parahaemolyticus	—	+	+	+	+	—	—	—	+	+
V. vulnificus	—	V	+	+	+	V	—	—	+	V
V. harveyi	—	+	+	+	—	—	—	—	+	—

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

V, variable.

Bacterial Diagnosis

Table 4.32 Differential characteristics of *Pseudomonas* species[a]

Species	Oxidase	Growth at 42°C	Nitrate reductase	Nitrate to gas	Arginine dihydrolase	Cetrimide growth	Hydrolysis of:				Acid from:			
							Acetamide	Esculin	Gelatin	Starch	Glucose	Maltose	Mannitol	Xylose
P. aeruginosa	+	+	+	+	+	+	+	—	V	—	+	—-	V	+
P. fluorescens	+	—	V	—	+	+	—	—	+	—	+	—	V	+
P. putida	+	—	—	—	+	V	—	—	—	—	+	V	V	+
P. veronii	+	—	+	+	+	NT	—	NT	—	NT	+	NT	NT	+
P. montellii	+	—	—	—	+	+	—	—	—	—	+	—	—	—
P. mosselii	+	—	—	—	+	+	NT	—	+	—	+	V	V	—
P. stutzeri	+	V	+	+	—	—	—	—	—	+	+	+	+	+
P. mendocina	+	+	+	+	+	V	—	—	—	—	+	—	—	V
P. pseudoalcaligenes	+	+	+	—	V	V	NT	—	—	—	—	—	—	V
P. alcaligenes	+	V	V	—	—	V	NT	—	—	—	—	—	—	—
P. luteola	—	+	V	—	+	—	NT	+	V	—	+	+	V	+
P. oryzihabitans	—	V	—	—	—	V	NT	—	V	—	+	+	+	+

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

V, variable; NT, not tested.

Table 4.33 Differential characteristics of *Acidovorax, Brevundimonas, Delftia, Comamonas,* and *Stenotrophomonas* species[a]

Species	Oxidase	Growth: MacConkey	Growth: 42°C	Nitrate reduction	Nitrate to gas	Arginine dihydrolase	Lysine decarboxylase	Hydrolysis of: Citrate	Hydrolysis of: Gelatin	Hydrolysis of: Urea	Acid from: Glucose	Acid from: Maltose	Acid from: Mannitol	Acid from: Xylose	Comments
A. delafieldii	+	+	V	+	—	+	—	+	—	+	+	—	V	+	Yellow soluble pigment
A. facillis	+	—	—	+	—	+	—	—	+	+	+	—	+	+	
A. temperans	+	+	+	+	+	—	—	—	—	V	+	—	V	—	Yellow, soluble pigment
B. diminuta	+	+	V	—	—	—	—	—	V	—	V	—	—	—	Brown-tan colonies
B. vesicularis	+	V	V	—	—	—	—	—	V	—	V	+	—	V	Colonies may be yellow-orange
D. acidovorans	+	+	V	+	—	—	—	+	—	—	—	—	+	—	Colonies may be yellow-tan
Comamonas spp.	+	+	V	+	—	—	—	V	—	—	—	—	—	—	
S. maltophilia	V	+	V	V	—	—	+	V	+	—	V	+	—	V	Lavendar-green colonies; ammonia odor

[a]Adapted from Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (ed.), *Manual of Clinical Microbiology,* 8th ed., ASM Press, Washington, D.C., 2003.

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Table 4.34 Differential characteristics of selected *Bordetella* species

Organism	Oxidase	Catalase	Motility	Pigment	Growth on:		
					RL Medium	Columbia agar	MacConkey agar
Bordetella pertussis	+	+	-	-	+ (3 days)	-	-
Bordetella parapertussis	-	+	-	Brown	+ (2 days)	V	-
Bordetella holmesii	-	+	-	-	NT	+	+
Bordetella bronchiseptica	+	+	+	-	+ (1 day)	+	+
Bordetella trematum	-	+	+	Yellow	NT	+	+
Bordetella ansorpii	+	+	V	-	NT	+	+

Table 4.35 Differential characteristics of selected oxidase-negative, oxidative, Gram-negative rods

Genus or species	Growth on MacConkey agar	Catalase	Motility	Arginine dihydrolase	Lysine decarboxylase	Urease	Indole production	Esculin hydrolysis	Nitrate reductase	Acid from: D-Glucose	OF-lactose	OF-sucrose	OF-mannitol	OF-maltose	OF-xylose
Acinetobacter	+	+	—	V	—	V	—	—	—	+	V	—	—	V	
Chryseomonas	+	+	+	+	—	V	—	+	V	+	V	V	+	+	+
Flavimonas	+	+	+	V	—	V	—	—	—	+	V	V	+	+	+
Roseomonas	+	+	V	—	—	+	—	—	—	V	—	—	V	—	V
Sphingomonas	V	+	V	—	—	—	—	+	—	+	+	+	—	+	+
Stenotrophomonas	+	+	+	—	+	V	—	V	V	+	V	V	—	+	V
Francisella	—	+	NT	—	NT	—	—	NT	—	+	NT	—	NT	—	—

Table 4.36 Differential characteristics of selected oxidase-positive, nonfermentative, Gram-negative rods

Organism	Growth on MacConkey agar	Motility	Flexirubin pigment	Gram stain morphology	Urease	Esculin	Nitrate reductase	Nitrite reductase	Alkaline phosphatase	H_2S (On KIA)	Indole	Colistin resistant	Acid from:			
													Glucose	Ethylene glycol	Mannitol	Xylose
Moraxella catarrhalis	-	-	NT	C	-	NT	+	+	-	NT	-	-	-	-	-	-
Moraxella nonliquefaciens	-	-	NT	CB	-	NT	+	-	-	NT	-	-	-	-	-	-
Moraxella osloensis	V	-	NT	CB	-	NT	V	-	V	NT	-	-	-	+	-	-
Moraxella lacunata	-	-	NT	CB	-	NT	+	-	V	NT	-	-	-	V	-	-
Oligella ureolytica	-	V	NT	CB	+	NT	+	+	-	NT	-	NT	-	+	-	-
Oligella urethralis	V	-	NT	CB	-	NT	-	+	-	NT	-	NT	-	+	-	-
Paracoccus yeei	+	-	NT	C	+	NT	+	-	-	NT	-	NT	+	+	V	+

Inquilinus limosus	+	V	-	B	V	-	-	-	-	-	-	+	+	-	+	+
Myroides odoratus	V	-	+	B	+	+	-	+	+	-	-	+	-	-	-	-
Ochrabactrum anthropi	+	+	-	B	+	-	+	+	-	-	-	-	+	+	V	+
Rhizobium radiobacter	+	+	-	B	+	-	+	V	-	-	-	V	+	+	+	+
Shewanella algae	+	+	-	B	-	+	+	+	+	+	-	V	-	+	-	-
Shewanella putrefaciens	+	+	-	B	-	+	+	+	+	+	-	-	V	+	-	-
Sphingomonas spp.	-	V	-	B	-	-	V	-	+	-	-	V	V	+	-	V
Balneatrix alpica	-	+	-	B	-	-	+	-	NT	NT	+	-	+	-	+	-
Bergeyella zoohelcum	-	-	-	B	+	-	-	-	NT	NT	+	+	-	-	-	-
Chryseobacterium indologenes	V	-	V	B	-	+	V	V	NT	NT	+	+	+	-	-	-
Elizabethkingia meningoseptica	V	-	-	B	-	+	-	V	NT	NT	+	+	+	+	+	-
Sphingobacterium mizutaii	-	-	-	B	-	+	-	V	NT	NT	+	+	+	-	-	+
Weeksella virosa	-	-	-	B	-	-	-	-	NT	NT	+	-	-	-	-	-

C, coccoid; CB, coccobacillus; B, bacillus; V, variable; NT, not tested.

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Table 4.37 Differential characteristics of selected oxidase-positive, nonoxidative, Gram-negative rods

Species	Growth on:		Catalase	Motility	Flagella			Urease	Indole production	Nitrate reductase	Nitrate to gas	H_2S on TSI[a]	Acid from:		
	MacConkey agar	SS agar			1–2 polar	>2 polar	Peiitrichous						Glucose	OF-mannitol	OF-xylose
Aflpia felts	V	—	V	+	+	—	—	+	—	+	—	—	—	—	+
Alcaligenes faecalis	+	+	+	+	—	—	+	—	—	—	—	—	—	—	—
Alcaligenes xylosoxidans	+	+	+	+	—	—	+	—	—	+	+	—	—	—	—
Bordetella pertussis	—	—	+	—	—	—	—	—	—	—	—	NT	—	NT	—
Bordetella bronchiseptica	+	+	+	+	—	—	+	+	—	+	—	—	—	—	—
Brucella species	V	—	+	—	—	—	—	+	—	+	V	—	+	—	+
Campylobacter species	V	—	+	+	+	—	—	—	—	+	—	—	—	—	—
Methylobacterium species	V	—	+	+	+	—	—	V	—	V	—	—	V	—	+
Moraxella atlantae	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
Moraxella catarrhalis	—	—	+	—	—	—	—	—	—	V	—	—	—	—	—

Moraxella osloensis	V	—	+	—	—	—	—	—	—	V	—	—	—	—	—
Moraxella lacunata	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
Moraxella phenylpyruvica	V	—	+	—	—	—	—	+	—	V	—	—	—	—	—
Neisseria flavescens	V	—	+	—	—	—	—	—	—	—	—	—	—	—	—
Neisseria mucosa	V	—	+	—	—	—	—	—	—	+	+	—	+	—	—
Neisseria sicca	V	—	V	—	—	—	—	—	—	—	—	—	+	—	—
Ochrobactrum anthropi	+	+	+	+	—	—	+	+	—	+	+	V	+	V	+
Oligella ureolytica	V	—	+	V	—	—	V	+	—	+	V	—	—	—	—
Oligella urethralis	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
Pseudomonas diminuta	+	—	+	+	+	—	—	V	—	—	—	—	V	—	—
Roseomonas species	+	—	+	+	+	—	—	+	—	V	—	—	V	V	V
Bartonella species	—	—	+	—	—	—	—	—	—	—	—	NT	—	—	—
Eikenella corrodens	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—
Kingella denitrificans	—	—	—	—	—	—	—	—	—	+	V	—	+	—	—-
Weeksella virosa	—	—	+	—	—	—	—	+	+	—	—	—	—	—	—
Weeksella zoohelcum	—	—	+	—	—	—	—	—	+	—	—	—	—	—	—

[a]TSI, triple sugar iron.

Table 4.38 Differential characteristics of selected *Campylobacter, Arcobacter,* and *Helicobacter* species

Species	Catalase	Nitrate reductase	Urease	Alkaline phosphatase	Hippurate hydrolysis	Indoxyl acetate hydrolysis	ϒ-Glutamyl transferase	Growth: 25°C	Growth: 42°C	Growth: 1% Glycine
C. jejuni subsp. *jejuni*	+	NT	—	NT	+	+	NT	—	+	+
C. jejuni subsp. *doylei*	V	NT	—	NT	V	+	NT	—	+	+
C. coli	+	NT	—	NT	—	+	NT	—	+	+
C. fetus subsp. *fetus*	+	NT	—	NT	—	—	NT	+	—	+
C. lari subsp. *lari*/ *C. lari* subsp. *concheus*	+	NT	V	NT	—	—	NT	—	NT	+
C. upsaliensis	—	NT	—	NT	—	+	NT	—	+	V
A. butzleri	V	NT	—	NT	—	+	NT	+	V	V
A. cryaerophilus	V	NT	—	NT	—	+	NT	+	—	V
H. pylori	+	—	+	+	—	—	+	NT	—	—
H. cinaedi	+	+	—	—	—	—	—	NT	—	+
H. fennelliae	+	—	—	+	—	+	—	NT	—	+
H. pullorum	+	+	—	—	—	—	NT	NT	+	—
H. heilmannii	NT	NT	+	NT	NT	NT	NT	NT	NT	NT

Table 4.39 Differential characteristics of select non-spore-forming, anaerobic, Gram-positive rods

Genus	Strict anaerobe	Catalase	Nitrate reductase	Indole production	Metabolic products (GLC)[a]
Actinomyces spp.	V	V	V	—	S, L, a
Actinomyces israelii	—	—	+	—	S, L, a
Actinomyces neuii subsp. *Neuii*	—	+	+	—	S, L, a
Actinomyces odontolyticus	—	—	+	—	S, L, a
Actinomyces turicensis	—	—	—	—	S, L, a
Actinobaculum spp.	V	—	—	—	A
Bifidobacterium	+	—	—	—	A, L
Eubacterium	+	—	V	—	(A), (B)
Eggerthella lenta	+	+	+	—	(A, L, S)
Eggerthella sinensis	+	+	—	—	(A, L, S)
Lactobacillus	V	—	—	—	L, (a), (s)
Mobiluncus	+	—	V	—	S, A, (L)
Propionibacterium acnes	—	+	+	+	A, P, iv, s, 1
Propionibacterium avidum	—	+	—	—	A, P, iv, s, 1
Propionibacterium acidifaciens	+	—	—	—	A, P, iv, s, 1
Propionibacterium propionicum	+	—	+	—	A, P, iv, s, 1
Propionibacterium granulosum	—	+	—	—	A, P, iv, s, 1

[a]A, acetic acid; P, propionic acid; IB, isobutyric acid; B, butyric acid; IV, isovaleric acid; V, valeric acid; IC, isocaproic acid; C, caproic acid; L, lactic acid; S, succinic acid. Capital letters indicate a major acid peak, lowercase letters indicate a minor peak, and letters in parentheses indicate that the acids are irregularly observed.

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Table 4.40 Differential characteristics of selected *Clostridium* species

Species	Egg yolk agar		Gelatinase	Milk digestión	Spore Location	Indole	Acid from:				Metabolic products (GLC)
	Lecithinase	Lipase					Glucose	Maltose	Lactose	Sucrose	
C. perfringens	+	—	+	—	ST	—	+	+	+	+	A, B, (P), (L)
C. baratii	+	—	—	—	ST	—	+	+	+	+	A, B, (L)
C. novyi A	+	+	+	—	ST	—	+	+	—	—	A, P, B, (V)
C. bifermentans	+	—	+	+	ST	+	+	+	—	—	A, IC, (P), (TB), (IV), PP
C. sordellii	+	—	+	+	ST	+	+	+	—	—	A, IC, (P), (IB), (IV)
C. botulinum, A, B, F	—	+	+	+	ST	—	+	+	—	—	A, IB, B, IV, (P), (V), (IC), PP
C. botulinum B, E, F	—	+	+	—	ST	—	+	+	—	+	A, B
C. botulinum C and D	—	+	+	V	T	—	+	V	—	—	A, P, B
C. sporogenes	—	+	+	+	ST	—	+	+	—	—	A, IB, B, IV, (P), (V), (IC), PP
C. septicum	—	—	+	—	ST	—	+	+	+	—	A, B
C. difficile	—	—	+	—	ST	—	+	—	—	—	A, IB, B, IV, V, IC, (P), PP
C. tetani	—	—	+	—	T	+	—	—	—	—	A, P, B, (PP)
C. histolyticum	—	—	+	+	ST	—	—	—	—	—	A, (PP)
C. sphenoides	—	—	—	—	ST	+	+	+	+	—	A, F
C. tertium	—	—	—	—	ST	—	+	+	+	+	A, B, (L), (PP)
C. butyricum	—	—	—	—	ST	—	+	+	+	+	A, B
C. ramosum	—	—	—	—	T	—	+	+	+	+	A, L, (PY)
C. subterminale	—	—	+	+	ST	—	—	—	—	—	A, B, IV, ib (p, ic, l, s)

Table 4.41 Differential characteristics of anaerobic Gram-negative bacteria

Species	Susceptibility to:				Motility	Nitrate reductase	Indole	Catalase	Pigment production	Cell morphology
	Kanamycin (1,000 μg)	Vancomycin (5 μg)	Colistin (10 μg)	Growth in 20% bile						
Bacteroides	R	R	R	+	—	—	V	—	—	Short
Alistipes	R	R	R	V	—	—	V	V	V	Short
Porphyromonas[a]	R	S	R	—	—	—	V	V	+	Variable
Prevotella[a]	R	R	V	—	—	—	V	—	V	Coccobacillary
Parabacteroides	R	R	R	+	—	—	—	V	—	Short
Bilophila	S	R	S	+	—	+	—	+	—	Straight
Fusobacterium nucleatum	S	R	S	—	—	—	+	—	—	Long, thin, pointed ends
Fusobacterium necrophorum	S	R	S	V	—	—	+	—	—	Pleomorphic
Leptotrichia	S	R	S	—	—	—	—	V	—	Long, thin, pointed ends
Dialister	S	R	V	—	—	—	—	—	—	Coccoid
Veilonella spp.	S	R	S	—	—	+	—	V	—	Cocci
Anaerospirillium	S	R	V	V	+	—	—	—	—	Long, spiral
Desulfovibrio	R	S	R	V	+	V	—	V	+	Curved

[a]*Porphyromonas* and some *Prevotella* spp. initially fluoresce red and then develop pigmented colonies.

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Table 4.42 Differential characteristics of the *Bacteroides fragilis* group

Species	Indole	Catalase	α-Fucosidase	Acid from:		
				Arabinose	Trehalose	Xylose
B. caccae	-	-	+	+	+	+
B. cellulosilyticus	NT	-	W	W	-	+
B. clarus	+	-	-	-	W	+
B. coprocola	-	-	+	-	-	+
B. coprophilus	-	NT	+	-	NT	-
B. dorei	-	NT	+	+	-	+
B. eggerthii	+	-	-	+	-	+
B. faecis	+	-	+	+	-	+
B. finegoldii	-	NT	-	+	-	+
B. fluxus	+	-	+	+	+	+
B. fragilis	-	+	+	-	-	+

B. intestinalis	+	NT	+	+	-	+
B. massiliensis	-	-	+	-	-	-
B. nordii	+	-	-	-	-	+
B. oleiciplenus	+	+	-	+	+	+
B. ovatus	+	+	+	+	+	+
B. plebeius	-	-	+	+	-	+
B. salyersiae	+	-	-	+	-	+
B. stercoris	+	V	V	-	-	+
B. thetaiotaomicron	+	+	+	+	+	+
B. uniformis	+	-	+	+	-	+
B. vulgatus	-	-	+	+	-	+
B. xylanisolvens	-	-	+	+	+	+

W, weak reaction; NT, not tested.

Bacterial Diagnosis

SECTION 5

Viral Diagnosis

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Historically, viral infections have been diagnosed by culture in primary or continuous cell lines and by detection of antibody responses to infection. However, these methods are insensitive and slow, such that results are rarely available in a clinically relevant time frame. Molecular methods or nucleic acid amplification testing (NAAT) have replaced culture due to their superior sensitivity and more rapid turnaround time. In addition, antigen capture immunoassays, which allow for the direct detection of viruses in clinical specimens, have also been widely adopted. Despite these technological advances, serology remains an important diagnostic tool for many viral infections. Initially, many serological tests were conducted with the labor-intensive complement fixation and neutralization tests. However, those methods have largely been replaced by commercially prepared enzyme immunoassays. This technical change allows many smaller laboratories to perform tests that were previously available only in reference laboratories. This section summarizes the tests currently available for the laboratory diagnosis of the most common viral infections. For additional information, the reader is referred to the ASM *Manual of Clinical Microbiology* and *Clinical Virology Manual* by Specter et al. (see the Bibliography).

Table 5.1 Detection methods for viruses[a]

Virus[b]	Usefulness of detection by: Cell culture	Microscopy	Antigen detection methods	Antibody detection methods	Molecular diagnostics
RNA viruses					
Alphavirus	C	D	C	A	B
Arenavirus	C	C	C	B	C
Astrovirus, calicivirus, rotavirus	C	C	A	C	A
Bunyaviruses	D	D	D	A	C
Coronavirus	C	D	D	B	A
Coxsackievirus types A and B	B	D	D	A	A
Enterovirus	A	D	D	D	A
Filoviruses	D	D	C	A	A
Flavivirus	C	D	C	A	A
Hantavirus	D	D	D	A	B
Hepatitis A virus	C	D	D	A	B
Hepatitis C virus	D	D	D	A	A
Hepatitis E virus	D	C	D	A	B
HTLV-I and -II	C	D	D	A	B
HIV-I and -II	C	D	A	A	A
Influenzavirus types A to C	A	D	A	B	A
Measles virus	B	B	C	A	B
Mumps virus	A	C	C	A	B
Parainfluenzavirus types 1 to 4	B	C	C	C	A

(continued)

Viral Diagnosis

Table 5.1 Detection methods for viruses[a] *(continued)*

	Usefulness of detection by:				
Virus[b]	**Cell culture**	**Microscopy**	**Antigen detection methods**	**Antibody detection methods**	**Molecular diagnostics**
Poliovirus	A	D	D	D	A
Rabies virus	C	A	D	B	B
Respiratory syncytial virus	B	C	A	C	A
Rhinovirus	B	C	C	C	A
Rubella virus	A	D	D	A	B
DNA viruses					
Adenovirus	A	C	B	C	A
Cytomegalovirus	A	B	A	A	B
Epstein-Barr vims	C	B	D	A	B
Hepatitis B virus	D	C	C	A	A

Herpes simplex virus types I and II	A	C	B	B	A
Human herpesvirus 6	B	C	C	B	B
Orthopoxvirus	B	B	D	A	C
Papillomavirus	D	C	D	B	A
Parvovirus (B19)	C	D	D	A	A
Polyomavirus (BK virus, JC virus)	D	C	D	D	A
Varicella-zoster vims	A	A	D	B	A
Transmissible spongiform encephalopathy agents					
Bovine encephalopathy agent	D	C	D	D	D
Creutzfeldt-Jakob agent	D	C	D	D	D
Kuru agent	D	C	D	D	D

[a]A, test is generally useful; B, test is useful under certain circumstances; C, test is seldom used for general diagnosis but may be available in reference laboratories; D, test is generally not used for laboratory diagnosis.

[b]HTLV, human T-cell leukemia virus; HIV, human immunodeficiency virus.

Viral Diagnosis

Table 5.2 Cells used for viral isolation[a]

Type of cell	Tissue of origin	Viruses isolated[b]
Primary cell lines		
African green monkey	Kidney	HSV, VZV, mumps virus, rubella virus
CBMC, PBMC[c]	Human	HIV-1, HIV-2, HTLV-1, HTLV-2, HHV-6
Neonatal, human	Kidney	HSV, VZV, adenoviruses, mumps virus
Rabbit	Kidney	HSV
Rhesus or cynomolgus monkey	Kidney	Enteroviruses, influenza viruses, parainfluenza viruses, RSV, mumps vims, measles virus
Low-passage/finite cell lines		
Foreskin fibroblasts	Human	HSV, CMV
Lung fibroblasts	Human embryo	HSV, CMV, VZV, rhinoviruses, coronavirus
Kidney fibroblasts	Human fetus	Coronavirus, HSV, rhinovirus
WI-38, MRC-5	Human fetal lung	HSV, VZV, CMV, adenoviruses, enteroviruses, RSV, rhinoviruses
Continuous cell lines		
293	Human kidney	Adenoviruses (types 5, 40, and 41)
A549	Human lung	Adenoviruses (except types 40 and 41), HSV
HeLa	Human cervix	Poxviruses, RSV, rhinoviruses, enteroviruses
HEp-2	Human larynx	Adenoviruses, RSV, measles virus
MDCK	Canine kidney	Influenza viruses, parainfluenza viruses
Mink lung	Mink lung	HSV
RD	Human rhabdomyosarcoma	Enteroviruses (coxsackievirus type A), coronavirus, poliovirus
RKI_{13}	Rabbit kidney	Rubella virus, poxviruses
BGMK, Vero, CV-1	African green monkey kidney	HSV, VZV, enteroviruses, measles virus, poxviruses, rubella virus, RSV, parainfluenza viruses

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]CMV, cytomegalovirus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; HTLV, human T-cell leukemia virus; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.

[c]CBMC, cord blood mononuclear cells; PBMC, peripheral blood mononuclear cells.

RNA Viruses

Alphavirus (Eastern Equine Encephalitis Virus, Western Equine Encephalitis Virus, Venezuelan Equine Encephalitis Virus, Chikungunya Virus). Viruses grow in a variety of cell lines including Vero, A549, and MRC-5 cells. Virus can be found in blood at the time of clinical onset but is typically cleared when neurological symptoms develop. Additionally, antigen detection assays and reverse transcriptase PCR (RT-PCR) tests have been developed for some members of this group (e.g., Venezuelan equine encephalitis virus, Chikungunya). Of note, PCR assays quickly become negative shortly after symptoms develop; thus, negative results for patients who have been symptomatic for >7 days do not rule out disease. The most sensitive serological assays detect virus-specific immunoglobulin M (IgM) antibodies by capture enzyme-linked immunosorbent assay (ELISA). Some serological cross-reactivity has been observed between Chikungunya and the closely related Dengue virus. IgM antibodies are detected in serum and cerebrospinal fluid (CSF) within the first 7 to 10 days of clinical illness. Because IgM antibodies may persist for months, seroconversion should be demonstrated. Virus-specific assays are available for Chikungunya, Western, and Venezuelan equine encephalitis virus. However, due to a high degree of cross-reactivity, positive results should be confirmed with neutralization assays.

Arenavirus (Lymphocytic Choriomeningitis Virus, Lassa Virus, Junin Virus, Machupo Virus). Arenaviruses are a family of 43 named viruses divided into two groups: Old World complex (e.g., lymphocytic choriomeningitis [LCM] virus, Lassa virus) and New World or Tacaribe complex (e.g., Junin virus, Machupo virus). Indirect fluorescent-antibody (IFA) tests have been used with peripheral blood and urine sediment for detection of Junin virus. Antigen capture ELISAs have also been developed for detection of Lassa virus antigens in blood as well as for the identification of virus grown in cell cultures. The RT-PCR assay is a useful test for the rapid, definitive diagnosis of LCM and Lassa virus infections. The test sensitivity is approximately 80% for Lassa virus; this reflects the genetic variation among Lassa virus strains. Cell culture is a relatively sensitive diagnostic method for Lassa and related viruses; however, PCR has now replaced it as the most sensitive method. The viruses grow in Vero cells (and other cell lines), with viral antigens detected by immunofluorescent-antibody (IFA) staining of inoculated cell cultures or ELISA. LCM virus also grows in cell culture, but intracranial inoculation of weanling mice is a more sensitive diagnostic procedure. ELISA can be combined with serologic testing (ELISA) for IgM and IgG for a sensitive and rapid detection of Lassa virus infection. The diagnosis of infection can be confirmed for most patients at the time of clinical onset. ELISAs have also been

developed for detecting antibody responses to other arenaviruses and have replaced neutralization and IFA serologic tests. RT-PCR assays followed by genome analysis are replacing the above-mentioned diagnostic methods and are advantageous because it inactivates the virus, which would otherwise require BSL4 laboratory facilities.

Astrovirus, Calicivirus, and Rotavirus. Eight serotypes of astroviruses have been identified, with serotype 1 being the most common human pathogens, but all eight have been reported to cause human infection. Caliciviruses are subdivided into five genera with *Norovirus* and *Sapovirus* being responsible for the majority of human infections. Rotaviruses have been subdivided into seven antigenic groups A to G, with most human infections caused by group A. All of these viruses can be detected in stool specimens by electron microscopy. Only rotaviruses can be isolated in cell culture; however, growth is slow and culture is not generally performed. RT-PCR is a sensitive method for detection of these viruses, although inhibitors in fecal specimens can cause false-negative reactions. A wide range of ELISAs and latex agglutination tests have been developed for the detection of rotaviruses in fecal specimens, and an ELISA is commercially available for astroviruses. Some standalone PCR assays for the detection of *Norovirus* are now commercially available. In addition, some multiplex-PCR assays include *Norovirus, Sapovirus,* and *Rotavirus.*

Bunyavirus (Bunyamwera Virus, California Encephalitis Virus, La Crosse Virus, Hartland Virus, Rift Valley fever, Crimean Congo hemorrhagic fever). Virus can grow in Vero and BHK-21 cell lines; however, attempts to isolate virus from clinical specimens are generally unsuccessful. Serologic testing (e.g., neutralization, hemagglutination inhibition [HI], complement fixation [CF], and ELISA) is primarily used to establish infection. Most patients are seropositive by IgM ELISA at the time of onset of illness. Neutralizing antibodies are detected at the end of the first week of illness and persist for life. In contrast, HI antibodies are detected at the end of the first week, and CF antibodies develop a few weeks later; both antibodies disappear within 1 year.

Coronavirus. Although coronaviruses are recognized as a common source of upper respiratory disease, interest in this group of viruses has been stimulated by the onset of severe acute respiratory syndrome (SARS) and more recently, Middle Eastern respiratory syndrome (MERS). The viruses are difficult to grow in culture, so diagnosis has primarily depended on RT-PCR and serologic testing. Historically, RT-PCR has only been available through public health laboratories, tertiary-care centers, and commercial laboratories, but coronaviruses have now been included in most multiplex respiratory pathogen RT-PCR panels. Notably, these assays do not detect

SARS or MERS; thus, positive RT-PCR results do not suggest SARS or MERS, and similarly, negative results do not rule out infection with SARS or MERS. If SARS or MERS are suspected, testing should be coordinated with public health laboratories.

Enterovirus (Coxsackie A and B Viruses, Echovirus, Parechovirus, Enterovirus, Poliovirus). Isolation in culture is the method of choice for some enteroviruses and for specimens from which RT-PCR cannot be performed. Some serotypes of coxsackie A virus fail to grow in culture. These serotypes can be recovered by inoculation of suckling mice, but the procedure is not usually performed in clinical laboratories. In addition, parechoviruses require a combination of human and primate cell lines. Human rhabdomyosarcoma cells, WI-38, and human embryonic lung cells are best for coxsackie A virus and monkey (e.g., Buffalo green, rhesus, and cynomolgus) kidney or HeLa cells are best for coxsackie B virus. Isolation in culture is the method of choice for enterovirus and poliovirus, with growth being observed in a wide range of cell lines. RT-PCR is also a useful assay, particularly for CSF samples, for which this assay is as sensitive as culture. Serological testing is restricted primarily to research laboratories.

Filovirus (Ebola Virus, Marburg Virus). Filoviruses are biosafety level 4 (BSL-4) pathogens, and so all work with the viruses is restricted to BSL-4 facilities. Virus can be cultured from serum at the time of clinical onset. Vero cells are permissive. Antigen capture ELISA has been used to detect viral antigens in serum. Filovirus-specific IgM capture and IgG ELISA are used to assess the serological response to infection. IgM and IgG appear 8 to 10 days after onset of disease. IgM antibody levels decrease over the first few months of infection, but IgG antibodies will persist for 2 years or more. For patients who have been symptomatic for a short time (<3 days), Ebola virus nucleic acid may not be detectable in serum. Patients without an alternative diagnosis should be retested at a later time to rule out Ebola infection. Ebola virus nucleic acid may be detected in nonblood specimens, and public health laboratories should be consulted to determine whether testing is appropriate.

Flavivirus (Yellow Fever Virus, Dengue Virus, St. Louis Encephalitis Virus, West Nile Virus). Yellow fever virus antigen can be detected by antigen capture assays or RT-PCR; however, these assays are not commercially available. Most infections are diagnosed by IgM capture ELISA, with a presumptive diagnosis being based on the presence of IgM antibodies and the diagnosis being confirmed by the demonstration of a significant rise in antibody levels. Dengue virus infections are diagnosed on the basis of clinical presentation and detection of viral RNA by RT-PCR. Serological is of greatest value in patients who have been symptomatic for

longer than 5 days. Therefore, negative IgM test results should be confirmed by testing a convalescent specimen. Although antigen capture assays and RT-PCR tests have been developed for St. Louis encephalitis virus, serologic testing is the most sensitive diagnostic test. Cross-reactivity with West Nile virus and Japanese encephalitis virus occurs, and so neutralization assays must be performed to demonstrate which virus is responsible for the infection. Likewise, West Nile virus infections are diagnosed primarily by serologic testing, except in immunocompromised patients, for whom RT-PCR assay may remain positive. Positive serologic tests must be confirmed by neutralization assays, and a fourfold change in antibody levels must be demonstrated because IgM and IgG antibodies can persist for months to years.

Flavivirus (Hepacivirus [Hepatitis C Virus]). Diagnosis of infections caused by hepatitis C virus (HCV) is by either serologic testing or nucleic acid amplification testing (NAAT). Current serological assays used for screening blood donors and patients are directed against a variety of antigens, including core, NS3, NS4, and NS5 antigens. Seroconversion is detected by 10 weeks after exposure. False-positive reactions occur at a low rate. To improve the test specificity, a strip immunoassay (recombinant immunoblot assay [RIBA]) was developed. However, the performance of the RIBA was similar to the initial screening assays and is no longer offered in lieu of more definitive NAATs. In the setting of acute hepatitis, qualitative and quantitative NAATs have been developed for detecting viral nucleic acids in serum or plasma. However, patients are often asymptomatic in acute infection, and thus most patients are not diagnosed until the chronic infection has been established. In the setting of chronic hepatitis, EIAs are highly sensitive and specific, and confirmation by NAA tests is not necessary. For patients presenting with acute hepatitis, qualitative NAA tests are used to confirm active infection. Quantitative NAA tests can be used to monitor the response to therapy or the progression of disease.

Hantavirus (Hantaan Virus). Hantaviruses are difficult to grow in culture. Diagnosis is most commonly made by serologic testing, and nearly all patients who develop severe symptoms from Hantaan virus infection will have high IgM titers at or near the time of symptom development. IgG antibody is also commonly detectable during the acute phase of disease. RT-PCR assays have also been developed and are increasingly being used to diagnose Hantaan virus infection. Viral RNA can be detected from blood and plasma, as well as from lung and kidney tissue.

Hepatitis A Virus. Hepatitis A virus (HAV) is difficult to culture, and so this is done only in research laboratories. Commercially available assays for anti-HAV IgM are the methods of choice for diagnosis of acute type hepatitis, with solid-phase antibody capture

immunoassay being the most commonly used method. Antibodies are detected at the time of onset of symptoms and have disappeared by 6 months following infection. EIAs are used to measure total anti-HAV antibody levels (IgM, IgG, and IgA), which increase during acute infections and then persist indefinitely. Detectable anti-HAV antibodies in the absence of IgM antibodies are indicative of past infection and immunity. RT-PCR can be used to detect viremic patients in the early stages of disease, but these assays are not widely available and are not commonly used.

Hepatitis E Virus. Although hepatitis E virus (HEV) has been grown in culture, this is inefficient and is performed only in research laboratories. The method of choice for diagnosis of acute HEV infections is detection of IgM antibodies, which are detectable at the time of onset of symptoms and disappear within several weeks after symptoms resolve. IgG antibodies are also short-lived, typically becoming undetectable within several months of resolution of symptoms. Acute HEV infections may also be diagnosed by detecting HEV RNA in serum or plasma. This NAA assay remains positive for 2 to 7 weeks after onset, although viral RNA may be detected in some individuals for a more prolonged period.

Human Immunodeficiency Virus Types 1 and 2. Human immunodeficiency virus (HIV) infections can be diagnosed by culture, antigen or antibody detection, and NAA methods. Most infections are made initially by screening with a combined approach that detects HIV-specific antibodies and the p24 antigen which are produced within a few weeks after infection. It is currently recommended that positive screening assays be confirmed with an HIV 1 and 2 differentiation assay. Rapid immunoassays and tests designed for home diagnosis are also available. Quantitative NAA methods are available for monitoring the viral load, which has prognostic implications. The complexity of the available diagnostic tests precludes a detailed discussion here; the user of this Pocket Guide is referred to the ASM *Manual of Clinical Microbiology.*

Human T-Cell Lymphotropic Virus Types 1 and 2. Amplification of proviral DNA is the preferred method of diagnosing HTLV infection. NAATs can also be used to distinguish between the four HTVL groups. EIA measuring the serologic response to human T-cell lymphotropic virus type 1 (HTLV-1) and HTLV-2 infection is the primary diagnostic test, and a WB assay is used to confirm the diagnosis. It is recommended that a test giving an initial positive EIA result be repeated. If both tests are positive, the band profile observed in the WB assay is used to distinguish between HTLV-1 and HTLV-2.

Influenza Virus (Types A to C). Influenza infection is typically diagnosed in one of two ways. Rapid antigen detection is a commonly used method, but these assays suffer from poor performance

and are discourage by most microbiologists. With the advancement of rapid PCR assays that can be performed with minimal hands-on-time, and yield high sensitivity and specificity, rapid antigen tests are less commonly used. RT-PCR detection of influenza viruses can be done in several formats. Stand-alone assays typically include detection and differentiation of influenza A and B without subtyping. Multiplex PCR assays will also detect influenza A and B, but also subtype influenza A. The Madin-Darby canine kidney (MDCK) cell line is most commonly used for isolation of influenza viruses, although growth is observed in a variety of cell lines (e.g., Vero, MRC-5, and baby hamster kidney cells). Cytopathic effect (CPE) is typically observed within 2 to 3 days, but negative cultures should be tested by hemadsorption. Immunologic staining of infected cells at 1 and 2 days (shell vial assay) is more common than traditional tube culture. DFA can be performed with nasopharyngeal washes, although this test has a sensitivity of only 80 to 90% compared with culture. Specific EIAs for either influenza A virus or influenza A plus B viruses are available but have limited utility in the diagnosis of active infection. Serologic tests are used primarily for epidemiological surveys.

Measles Virus. Measles virus can be isolated from the conjunctiva, nasopharynx, and blood during the late prodromal period and early stage of rash development. Viremia clears within 2 to 3 days of the rash, but virus can be detected in urine for up to 7 days. B95-8 (B-lymphoblastoid) cells are used for isolation of virus. However, few clinical laboratories attempt to culture the virus. Virus-infected cells can be detected by cytologic examination (detection of intracytoplasmic and intranuclear inclusions and giant cells), and RT-PCR is offered by reference and public health laboratories. The recommended laboratory method for confirmation of infection is a serum-based IgM EIA. Commercial assays are available. Serum can be collected at the time of rash onset or up to 4 weeks later. IgG assays are also available and, in combination with IgM assays, can be used to assess immunity as well as primary disease.

Mumps Virus. Mumps is diagnosed by viral isolation, NAA, or serologic testing. Mumps virus is cultured most commonly in primary rhesus monkey kidney cells and human neonatal kidney cells. Cells are examined for CPE for 14 days, and negative cell cultures are tested by hemadsorption with guinea pig erythrocytes. Rapid antigen tests are infrequently used because mumps infections are uncommon in vaccinated populations. Mumps virus can be detected by RT-PCR, although this technique is not widely used as there are no commercially available assays. Serologic testing can be used to define an acute infection or immunity. A single positive IgG test is sufficient to identify an immune patient; seroconversion is necessary to identify a primary infection. EIAs are available for

measuring IgM and IgG antibodies with whole virus, sonicated virus, or purified viral antigens (e.g., HN or nucleocapsid [NP]) used in the assays. Serologic testing is of limited utility in diagnosing possible infection in vaccinated individuals as IgM is only weakly produced in a secondary immune response.

Parainfluenza Virus. RT-PCR is the preferred method of diagnosing parainfluenza virus infection. Most multiplex respiratory pathogen PCR panels include parainfluenza 1-4 and perform with high sensitivity and specificity. Primary human embryonic kidney and primary monkey kidney cells are the most sensitive cell line for culture of parainfluenza virus (PIV). Other cell lines can support the growth of PIV but are not recommended for primary isolation. Cultures are examined for CPE for 10 to 14 days, with 50% of positive cultures being detected at 5 days. Positive cultures can also be detected as early as 48 h after inoculation if cultures have been stained with virus-specific fluorescent antibodies. In most clinical laboratories, culture has been replaced with the shell vial assay, which has comparable sensitivity and is more rapid. Direct and indirect immunofluorescent antibody tests are commonly used to examine respiratory specimens for virus-infected cells. Specimens are typically examined with pooled reagents (for influenza A and B viruses, PIV-1 to PIV-3, respiratory syncytial virus [RSV], and adenoviruses), and those giving positive reactions are tested with virus-specific reagents. A variety of serologic assays (CF, HI, IFA, neutralization, and EIA) have been developed. Cross-reactions with mumps virus limit the utility of these tests.

Rabies Virus. The preferred method for the diagnosis of rabies in animals is the DFA test for rabies virus antigen in brain tissue. Fluorescein isothiocyanate-labeled antirabies antibodies can be prepared against whole rabies viruses or purified RNA-nucleoprotein complex or nucleoproteins (N proteins). For the diagnosis of rabies in humans, the following specimens are collected: saliva (collected with an eye dropper and placed in a sterile container with no preservatives), neck biopsy specimen (collected from the hair line and of sufficient depth to include cutaneous nerves and placed in a sterile container with no preservatives), 0.5 ml of serum (not whole blood) or CSF, and brain biopsy specimen (only if the specimen was collected for other diagnostic procedures). The following tests are recommended: saliva, RT-PCR and culture; neck biopsy specimen, RT-PCR, and IFA; serum and CSF, serologic testing; brain biopsy specimen, RT-PCR, and IFA. Serologic testing can be used to assess the response to vaccination. The neutralization test is most commonly used, although a rabies surface glycoprotein (G)-specific ELISA is available in Europe.

Respiratory Syncytial Virus. NAAT are now the preferred diagnostic method for RSV infection. These assays exist as stand-alone

assays or as part of multiplex panels. Both forms of NAAT have high sensitivity and specificity. Rapid antigen tests are available and are commonly found in smaller laboratory settings or physician offices. Like the influenza rapid antigen tests, RSV rapid antigen tests suffer from poor performance. Viral infectivity is rapidly lost at room temperature, and so specimens for culture should be processed promptly. The most sensitive cell lines for culture are HEp-2 and HeLa; less sensitive cells include primary monkey kidney and human fibroblast cell lines. CPE is observed on average at 4 to 5 days. The shell vial assay is slightly more sensitive, and positive cultures are detected at 1 to 2 days. Direct antigen detection tests (IFA and EIA) have a sensitivity equivalent to that of culture, are more rapid, and are not adversely affected by specimen transportation problems. Serologic testing is useful for epidemiological surveys but is not as sensitive as culture or antigen tests.

Rhinovirus. Multiplex respiratory pathogen PCR panels commonly include Rhinovirus detection. These assays perform with high sensitivity but are typically unable to differentiate Rhinovirus from Enterovirus. The cell lines used most commonly for growth of rhinoviruses are WI-38 and MRC-5. Cultures should be incubated at 33°C, with CPE seen as early as 1 to 2 days after inoculation. Negative cultures should be held for 7 days or more. EIAs are insensitive because there are a large number of serotypes of rhinoviruses and no common antigen exists. The large number of serotypes also makes serologic testing impractical.

Rubella Virus. Throat swabs and nasopharyngeal specimens are reliable sources of rubella virus, with positive cultures detected a few days before the rash develops to up to 4 days after onset. The virus grows in a variety of cell lines (e.g., Vero, BHK21, AGMK, and RK-13). Cultures are maintained for 1 week and then passaged. Viral growth is detected by IFA or RT-PCR. RT-PCR assays have also been used for primary detection of virus but are restricted primarily to research laboratories and are not used routinely for clinical diagnosis. Detection of rubella virus-specific IgM is the fastest and most efficient method to diagnose recent postnatal infection. However, only 50% of infected newborns are IgM positive on the day of symptom onset. By 8 days after the onset of rash, the infant should be positive for both IgM detectable by IgM capture ELISA and IgG detectable by indirect ELISA. False-positive tests can occur; care must be exercised in interpreting the test results.

DNA Viruses

Adenovirus. Syndromic multiplex NAATs are now the preferred method of detection for adenovirus-caused upper respiratory tract infection and gastroenteritis. In addition, quantitative and qualitative Adenovirus PCR assays are commonly used for the diagnosis

and monitoring of disseminated infection, typically in immunocompromised patients. All adenoviruses except types 40 and 41 replicate and produce CPE in cell cultures (e.g., HeLa, KB, A549, HEp-2, and HEK cells). CPE usually appears in 2 to 7 days, but passage of cell cultures for up to 1 month is recommended for negative cultures. Shell vial assays are as sensitive as traditional culture and more rapid (taking 2 to 5 days). Clinical specimens can also be examined by IFA, but this is significantly less sensitive than culture. Commercial EIAs are also available and are particularly useful for detecting types 40 and 41 in patients with gastroenteritis. Serologic tests are used primarily for epidemiological purposes. A seroconversion must be demonstrated to confirm a current infection, because seroreactivity to adenovirus is common.

Cytomegalovirus. NAATs are the preferred method of diagnosing and monitoring disseminated infection in immunocompromised patients. NAATs can also be done from urine or saliva in cases of suspected congenital infection. In addition, culture is a sensitive method for detecting cytomegalovirus (CMV) in respiratory specimens, urine, and anticoagulated whole blood (leukocytes). Intermittent shedding in urine is possible, and so multiple specimens should be processed. Recovery of CMV from leukocytes is a better indicator of symptomatic infection. Human fibroblast cell lines (e.g., WI-38, MRC-5, and IMR-90) are best, but growth is typically slow and may require serial passage of the cells and prolonged incubation (for up to 6 weeks). Results of this test compare favorably with the quantitative detection of CMV DNA in leukocytes or plasma by NAA methods (PCR). A variety of serologic tests are available (e.g., EIA, IFA, and passive latex agglutination), including IgM- and IgG-specific tests. IgM results must be interpreted with caution because IgM antibody is found in both primary and reactivated infections and can persist for months. Demonstration of IgG seroconversion is diagnostic of primary infection. Serologic testing is important in assessing organ donors and recipients but is not useful in diagnosing infections in immunocompromised patients.

Epstein-Barr Virus. Epstein-Barr virus (EBV) can be cultured in human cord blood lymphocytes, but this is rarely done for diagnostic purposes. Indirect, direct, and anticomplement immunofluorescence are the main methods used for the detection of EBV antigens in tissues and cell cultures. Quantitative PCR assays are commonly performed from whole blood and plasma, but these assays are not intended to be used for the diagnosis of EBV infection. Rather, these assays are used to monitor the development of post-transplant lymphoproliferative disease in transplant patients. EBV-positive CSF is significantly associated with primary lymphoma in HIV-positive individuals and with encephalitis in immunocompetent individuals. The diagnosis of acute EBV infection

(infectious mononucleosis) is established through the detection of heterophile antibodies (nonspecific antibodies) or detection of EBV-specific serological markers (preferred method). For interpretation of serologic test results, refer to Table 5.3.

Erythrovirus (B19 Virus). B19 virus, a member of the *Parvoviridae*, is difficult to grow *in vitro*. Viral particles or DNA can be detected in blood about 6 days after infection, with peak viremia occurring 2 to 3 days later. Viral titers decrease, but B19 DNA can be detected by PCR for up to 2 months. Serologic tests to detect antibodies are commercially available and are the most commonly used methods for diagnosis of acute infections and immune status. In immunocompetent individuals, IgM antibodies develop 2 weeks after infection and persist for up to 30 weeks. In patients with aplastic crisis, antibodies appear several days after onset of clinical symptoms. In patients with fetal hydrops, detection of IgM antibodies at the time of clinical onset is more variable. In immunocompetent patients, IgG antibodies appear several days after IgM antibodies and persist for years. The presence of IgG antibodies is consistent with immunity. In immunocompromised patients, IgG and IgM antibody responses are unpredictable, and so serologic testing is not used for these patients. NAA and PCR are the most common methods used to detect B19 DNA. Serologic diagnosis of recent infection is generally performed by IgM capture EIAs. If IgM assays are negative for immunocompromised patients, DNA detection methods should be used. B19 IgG antibodies detected by EIA in the absence of seroconversion are indicative of past infection. See Table 5.5 for a description of diagnostic methods for the various presentations of parvovirus infection.

Hepatitis B Virus. Diagnosis of hepatitis B virus (HBV) infections is based primarily on the detection of virus-specific antigens and antibodies. A variety of assays have been developed to detect early and late antigens and the antibody response to each. Refer to Table 5.4 for interpretation of these assays in specific clinical presentations. NAATs are used for the quantitation of HBV DNA, which can be used for the initial evaluation of infection as well as monitoring the progression of chronic infection during treatment.

Herpes Simplex Virus Types 1 and 2. Culture is a sensitive method for detecting virus in mucocutaneous, genital, and ocular lesions. Viral growth, as indicated by a CPE, is rapid in most cell lines (95% of specimens are positive by 5 days). Some cell lines (e.g., mink lung cells) are better than others (e.g., MRC-5 and Vero cells). Culture is insensitive for CSF infections, for which PCR is the recommended test. DFA and IFA tests are available and provide a rapid result if positive, but they are relatively insensitive compared with culture and PCR. DFA and IFA can be used to distinguish between herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) when the

Table 5.3 EBV serologic profiles under different conditions[a,b]

Condition	Presence of antibodies to antigen and Ig isotope[c]:						
	VCA, IgG	**VCA, IgM**	**VCA, IgA**	**EA/D, IgG**	**EA/R, IgG**	**EBNA, Ig**	**EBNAI, IgG**
Seronegative	—	—	—	—	—	—	—
Ongoing primary infection	+++	++++	++	++		—	—
Recent primary infection	+++	++	++	++	++	—	—
Past primary infection	++	—	—	—	—	++	++
Chronic active EBV infection	++++	++	+++	+++	++		

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]The most frequently employed antigens and Ig isotypes are included. The characteristic reactivity is given. For all conditions apart from seronegativity, there are exceptions to the rule. Clinical data including other laboratory parameters supporting the likelihood of a diagnosis must always be kept in mind.

[c]–, antibodies completely absent; ±, antibodies absent or present in low titers; +, antibodies present in low titers; ++, antibodies present in medium liters; +++, antibodies present in elevated titers; ++++, antibodies present in strongly elevated titers. VCA, viral capsid antigen; EA/D, early antigen—diffuse component; EA/R, 3 early antigen—restricted component; EBNA, Epstein-Barr nuclear antigen; EBNAI, Epstein-Barr nuclear antigen 1.

Viral Diagnosis

Table 5.4 Hepatitis B virus markers in different stages of infection and convalescence[a,b,c]

Stage of infection	HBV DNA	HBsAg	HBeAg	Anti-HBc		Anti-HBe	Anti-HBs
				Total	IgM		
Early incubation	+	—	—	—	—	—	—
Late incubation	+	+	+ or —	—	—	—	—
Acute infection	+	+	+	+	+	—	—
HBsAg-negative acute infection	+ or —	—	—	—	+	+	—	—
Chronic infection	+	+	+	+++	+ or —	—	—
Healthy HBsAg carrier	—	+	—	+++	+ or —	+	—
Recent infection	+ or —	—	—	++	+	+	+ or ++
Remote infection	—	—	—	+	—	—	+ or —
Vaccination response	—	—	—	—	—	—	+

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis Be antigen; HBc, hepatitis B core antigen.

[c]—, antibodies absent; +, antibodies present; ++, antibodies present in moderately high titers; +++, antibodies present in very high titers.

Table 5.5 Clinical diseases of parvovirus B19 and method of diagnosis[a]

Host(s)	Disease presentation	IgM	IgG	PCR
Healthy children	Fifth disease	Positive	Positive	Positive
Healthy adults	Polyarthropathy syndrome	Positive within 3 month of onset	Positive	Positive
Healthy children	Petechial or pupruic rash	Negative/positive	Negative/positive	Positive
Patients with increased erythropoiesis	TAC	Negative/positive	Negative/positive	Positive
Immunodeficient or immunocompetent patients	Persistent anemia/pure red cell aplasia	Negative/weakly positive	Negative/weakly positive	Positive
Fetus (<20 weeks)	Hydrops fetalis/congenital anemia	Negative/positive	Positive	Positive amniotic fluid or tissue

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

Viral Diagnosis

viruses are isolated in culture. An EIA has also been developed but is less sensitive than culture, particularly for specimens from asymptomatic patients. PCR assays can detect all strains of HSV and can distinguish between HSV-1 and HSV-2. Rapid PCR assays are now commercially available for the testing of lesions from skin and mucocutaneous specimens. Type-specific IgG assays are also available commercially.

Human Herpesvirus 6. Human herpesvirus 6 (HHV-6) causes roseola infantum (exanthem subitum) in children and opportunistic infections in immunocompromised patients. HHV-6 can be isolated from peripheral blood mononuclear cells by cocultivation with human cord blood lymphocytes; however, this test is not commonly performed. Quantitative PCR assays have been developed and have proved useful for monitoring viral concentrations in peripheral blood mononuclear cells. A variety of serologic assays (e.g., neutralization, immunoblot, IFA, and ELISA) are available for measuring IgG antibodies to HHV-6. Some cross-reactivity with HHV-7 and CMV occurs. Seroconversion can be used to define a primary infection.

Orthopoxvirus (Vaccinia Virus, Smallpox Virus, Monkeypox Virus). Orthopoxviruses can grow in a variety of established cell lines (BSC-1, CV-1, and LLCMK-2 cells, monkey kidney cells, human embryonic lung fibroblasts, HeLa cells, chicken embryo fibroblasts, and MRC-5 cells). Growth is rapid, with most cultures being positive within 48 h. PCR-based NAA tests are also available for detecting virus in serum and vesicular lesions. NAA tests are offered by the CDC and WHOCC. Virus- and family-specific assays have been developed. Neutralizing antibodies can be detected as early as 6 days after infection or vaccination. The absence of antibodies does not define susceptibility to infection, because the level of antibodies required for protective immunity is not known.

Papillomavirus. Human papillomavirus (HPV) cannot be grown in culture. Late-structure antigens have been detected in tissue biopsy specimens by using virus-specific polyclonal antibodies. This assay is specific but insensitive and is rarely used for diagnostic purposes. HPV DNA can be detected by using type specific DNA or RNA probes in a variety of hybridization techniques. The use of target amplification to increase the sensitivity of this method has made this the diagnostic test of choice. In addition, molecular assays now offer the ability to subtype HPV into high- and low-risk genotypes. Serologic tests are used for epidemiological studies because type-specific antibodies can be detectable for many years after exposure.

Polyomavirus (JC Virus, BK Virus). Viral culture is not routinely used for clinical diagnosis because JC virus and BK virus have long growth cycles and a limited range of host cells. Serologic

testing has been used primarily for epidemiological studies. Most individuals are infected at a young age. IgM antibodies develop initially, as measured by EIAs. The role of this antibody response in recurrent infections is not well characterized. NAA tests are the primary tests used to document infection with JC virus and BK virus. JC virus DNA is detected in patients with progressive multifocal leukoencephalopathy, and BK virus DNA is detected in the blood and urine of renal transplant recipients.

Varicella-Zoster Virus. Diploid human cell lines (e.g., human fetal diploid kidney [HFDK] and human fetal diploid lung [HFDL] cells) are the most sensitive cells for isolation of varicella-zoster virus (VZV). Other cell lines can support the growth of VZV but are much less sensitive. CPE is generally observed in the first week of incubation, but prolonged incubation may be required. DFA tests are available and are much more sensitive than culture (98 and 50%, respectively) because the virus is highly labile. Cellular material from the base of a vesicular lesion must be collected; vesicle fluid alone is unsatisfactory. PCR is also more sensitive than culture. The value of serologic testing (many tests are commercially available) is limited because increases in the titer of heterotypic antibody to VZV may occur in HSV-infected patients who have had a prior infection with VZV. Serologic testing is used primarily to assess immunity in unvaccinated health care workers exposed to patients with documented VZV infections.

Transmissible Spongiform Encephalopathies

Bovine Encephalopathy. Diagnosis is based on clinical history and histopathologic examination of brain tissues. Early diagnosis has also been made on the basis of examination of tonsillar and appendix biopsy specimens.

Creutzfeldt-Jakob Disease. Diagnosis is based on clinical history and histopathologic examination of brain tissues.

Kuru. Diagnosis is based on clinical history and histopathologic examination of brain tissues.

SECTION 6

Fungal Diagnosis

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Mycology: Specimen Collection and Transport Guidelines

General Guidelines

1. Although the recovery of some fungi in clinical specimens is always considered significant (e.g., dermatophytes, dimorphic fungi, *Cryptococcus neoformans*), most fungi are part of the patient's normal flora (e.g., *Candida* spp.) or found in the environment (e.g., most dematiaceous and moniliaceous fungi). Specimens must be carefully collected to avoid contamination with indigenous or exogenous fungi.

2. Bacteria can rapidly overgrow fungi, so care must be used in cleaning the site where the specimen will be collected (e.g., skin surface, nail beds). Transport conditions must be selected to minimize the risk of bacterial overgrowth.

3. Microscopic examination of specimens is important for the rapid detection of a fungal infection and for assessing the significance of an isolate.

Dematiaceous Fungi

Specimens submitted for microscopy and culture include aspirates, scrapings, and tissues. Cotton-wrapped swabs should not be used because an inadequate quantity of material is recovered and desiccation occurs. Flocked swabs, although not officially approved for use in collection of specimens for fungal culture, may sufficiently capture fungal elements so as to be acceptable for specimen collection. Unpublished data suggest that this is the case, but more evidence is needed to confirm that these specimens can be recommended for use.

Transport medium is unnecessary if the specimen is processed immediately.

Serologic testing for some of the thermally dimorphic fungi is available, but cross-reactivity may exist between *Blastomyces* and *Histoplasma*. In addition, direct detection of fungal antigens is available and commonly used for several fungi. Urine antigen testing is commonly performed for the diagnosis of *Blastomyces* and *Histoplasma* infection, but like serologic testing, there is a high degree of cross-reactivity between the two pathogens. Cryptococcal antigen testing is commonly used to diagnose disseminated infection and can be performed from both serum and cerebral spinal fluid.

Dermatophytes (*Epidermophyton, Microsporum,* and *Trichophyton* spp.)

Collect infected hairs with sterile forceps (guided by the use of a Wood's lamp if the suspected dermatophyte is fluorescent). Endo-

thrix fungi may require the use of a sterile scalpel to collect the hair root. Sample skin lesions at the active border of the lesion with a sterile scalpel to collect the sample. Disinfect nails with alcohol before collecting the sample by clipping or scraping. Do not place hair, skin, or nail samples in closed tubes. The high humidity fosters overgrowth of contaminating bacteria. If possible, directly inoculate the sample to appropriate media.

Dimorphic Fungi (*Blastomyces, Coccidioides, Histoplasma, Paracoccidioides,* and *Sporothrix* spp.)

Process specimens (e.g., respiratory specimens and wound aspirates) promptly to avoid overgrowth of contaminating bacteria. Do not use swabs, because these organisms are susceptible to desiccation. *Histoplasma capsulatum* can be recovered in fungal blood cultures, particularly from patients with AIDS and other immunosuppressive diseases. The lysis-centrifugation system (Isolator [Wampole]) and the MycoF/Lytic blood culture bottle (Becton Dickinson) are useful for the isolation of dimorphic molds from blood.

Eumycotic Mycetoma Agents

Examine pus, exudate, or biopsy material for the presence of granules (sclerotia) consisting of the eumycotic agents and matrix material. Wash the granules with saline containing antibiotics (e.g., penicillin and streptomycin), and then culture them. Organisms can be visualized by examining crushed granules microscopically.

Moniliaceous Fungi

Process specimens (e.g., biopsy specimens, lower respiratory secretions, nails, eye specimens) promptly to avoid overgrowth of contaminating bacteria. Do not transport specimens on swabs, because organisms are susceptible to desiccation. Specimens should be examined microscopically and cultured. *Fusarium* spp. are among the few filamentous fungi that can be reliably recovered in blood cultures and will occasionally grow in routine bacterial blood cultures. Collect blood specimens in the lysis-centrifugation system (Isolator) and/or the MycoF/Lytic blood culture bottle. Serologic tests are available for some of these fungi.

Pneumocystis (carinii) jiroveci

Respiratory specimens should be limited to induced sputa or bronchoscopy specimens. Patients can only rarely expectorate sputum, and throat washings are insensitive. Collect first morning specimens when possible. A 24-h collection is unacceptable. The presence of oral contamination, signified by squamous epithelial cells, does not invalidate the specimen.

Yeast

Yeasts are relatively easy to isolate from clinical specimens, although overgrowth of contaminating bacteria should be avoided. Yeasts are isolated commonly from blood specimens. Lysis centrifugation, biphasic systems, and the MycoF/Lytic blood culture bottle are reliable methods for isolating yeasts from blood. Automated continuous-monitoring systems are reliable for common yeasts but less reliable for *Cryptococcus neoformans.* Direct detection includes microscopy (Gram stain, KOH, India ink, Calcofluor white) and antigen tests. Serologic testing is available for antibodies to *Candida* spp. but is not commonly used.

Table 6.1 Methods for the identification of fungi

Organism	Applicability of[a]:				
	Direct microscopy	**Culture**	**Antigen detection**	**Antibody detection**	**Nucleic acid detection[b]**
Candida spp.	A	A	C	D	B
Cryptococcus spp.	A	A	A	C	B
Malassezia spp.	A	B	D	D	D
Trichosporon spp.	A	A	D	D	D
Blastomyces dermatitidis[c]	A	A	A	B	D
Coccidioides spp.[c]	A	A	B	A	D
Histoplasma capsulatum[c]	A	A	B	B	D
Paracoccidioides brasiliensis	A	A	A	A	D
Talaromyces marneffei[c]	A	A	C	C	D
Sporothrix schenckii	A	A	C	C	D
Aspergillus spp.	A	A	A	B	D
Moniliaceous fungi (not *Aspergillus* spp.)	A	A	D	D	D
Dematiaceous fungi	A	A	D	D	D
Dermatophytes	A	A	D	D	D
Zygomycetes	A	A	D	D	D
Eumycotic mycetoma agents	A	A	D	D	D
Pneumocystis spp.	A	D	D	D	C

[a]A, test is generally useful for diagnosis; B, test is useful under certain circumstances; C, test is seldom useful for general diagnostic purposes but may be available in reference laboratories; D, test is generally not used for laboratory diagnosis of infection.

[b]Nucleic acid techniques will probably be used in the future as primary tools for the diagnosis of fungal infections. At present, further technical developments and clinical studies are needed.

[c]For laboratory safety purposes, please notify the laboratory if this agent is suspected. The conidia of the mycelial form of these dimorphic fungi are highly infectious and easily transmissible by aerosolization.

Fungal Diagnosis

Acridine Orange Stain

Acridine orange stains fungi red-orange, but the background material stains green-yellow. For stain details, see section 4.

Calcofluor White Stain

Calcofluor white is a nonspecific fluorochrome that binds to cellulose and chitin in the cell walls of fungi. The dye can be mixed with 10% potassium hydroxide so that mammalian cells can be dissolved, thus facilitating visualization of fungal elements. Fungi (including *Pneumocystis jiroveci*) appear green or blue against a dark background when the stained slide is examined under UV illumination. Care must be used to distinguish specific staining from stained debris. Optimal detection of fluorescence requires the use of a 400- to 500-nm excitation filter and 500- to 520-nm barrier filter.

Fluorescent-Antibody Stain

Direct and indirect fluorescein-conjugated monoclonal anti-Pneumocystis antibodies are used for immunofluorescence assays and target a family of surface glycoproteins that contain both common and distinct epitopes within and among *Pneumocystis* species. Depending on the monoclonal antibody supplied with the kit, staining may target only the cyst form or may target all forms of the organism. The typical fluorophore that is conjugated to the antibody or used in an indirect assay is fluorescein isothiocynate, which produces a brilliant apple green color. The staining reaction shows a diffuse pattern distributed over the surface of the entire cluster of organisms and often over the matrix in which the organisms are embedded. Single cysts usually appear with a distinctive rim of fluorescence and a duller interior fluorescence.

Giemsa Stain

The Giemsa stain combines methylene blue and eosin. It is useful for the detection of *Histoplasma capsulatum* in bone marrow, peripheral smears, and touch preparations, as well as intracystic bodies and trophozoites of *Pneumocystis jiroveci* in induced sputum, bronchoscopy specimens, and lung tissue. *H. capsulatum* appears as tiny blue-purple budding yeast cells. The cyst wall of *P. jiroveci* appears as a clear ring around spores or intracystic bodies. The nuclei stain red-purple, and the cytoplasm generally stains light to dark blue.

Gram Stain

The Gram stain detects most fungi if present. Most yeast appear Gram positive; however, *Cryptococcus* and *Malassezia* spp.

stain weakly and in some instances exhibit only stippling. The hyphae of molds generally appear Gram-negative. *Pneumocystis* produces a negative (pink) reaction with poorly defined organism morphology.

India Ink Stain (Nigrosin)

The use of India ink is not technically a staining method. Detection of encapsulated fungi (i.e., *Cryptococcus neoformans*) is made possible by exclusion of the ink particles by the polysaccharide capsule of the organism. Care in interpretation is required because artifacts (e.g., leukocytes, erythrocytes, powder, and bubbles) may be confused with yeast cells. The morphologic characteristics of the yeast cells must be recognized before the preparation can be interpreted. Although a rapid detection method for encapsulated yeasts, the India ink procedure is an insensitive method for the detection of *Cryptococcus neoformans*; cryptococcal antigen testing (latex or enzyme immunoassay) is recommended.

Kinyoun Stain

Some ascomycetous fungi produce ascospores when grown on a medium that promotes their formation. Ascospores are acid-fast and stain red, whereas the ascomycete cell wall and cytoplasm appear blue. (For stain details, see section 4.)

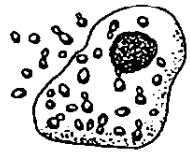
Potassium Hydroxide (KOH)

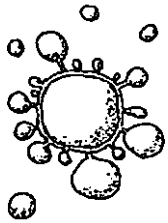
A 10 to 15% solution of potassium hydroxide can be used to dissolve cellular and organic debris and facilitate the detection of fungal elements, which are not affected by strong alkali solutions (although fungal elements dissolve after exposure for a few days). The hyphae of dematiaceous fungi can be distinguished from those of hyaline molds by their brown melanin pigment on these direct preparations. Ink (e.g., permanent blue-black Parker Super Quick Ink) can be added as a contrasting agent to aid the detection of fungi. Lactophenol cotton blue (i.e., Poirrier's blue) can also be added to the KOH. The aniline blue stains the outer cell wall of fungi, and the lactic acid is a clearing agent.

Toluidine Blue-0 Stain

Toluidine blue-0 stain is used primarily for the detection of *Pneumocystis jiroveci* in respiratory specimens. *P. jiroveci* cysts stain reddish blue to dark purple against a light blue background. Trophozoites do not stain by this method. This staining method is rapid and inexpensive, but some skill is required to recognize *P. jiroveci* cysts (usually present in clumps). Many laboratories prefer the direct fluorescent-antibody test for the detection of *P. jiroveci*, even though the stain is more expensive.

Table 6.2 Characteristic fungal elements seen by direct examination of clinical specimens

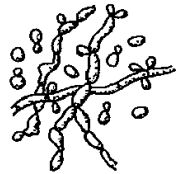
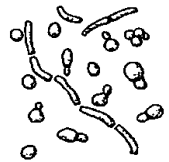
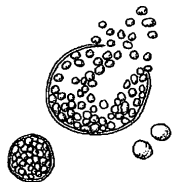
Morphologic fungal structure found	Organism(s)	Diam range (μm)	Characteristic features	Illustration[a]
Yeast forms	*Histoplasma capsulatum*	2–5	Oval to round budding cells; often found clustered within histiocytes	
	Sporothrix schenckii	2–6	Oval to round to cigar-shaped, single or multiple buds present; yeast uncommonly seen in clinical specimens	
	Cryptococcus neoformans	2–15	Cells vary in size; usually round to oval; buds usually single and "pinched off"; capsule may or may not be evident; pseudohyphal forms rare	

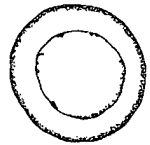
Talaromyces marneffei	3	Fission yeast, do not bud; round to oval with a central septum	
Blastomyces dermatitidis	8–15	Cells usually large and spherical, doubly refractile; buds usually single but may remain connected to parent cells by broad base	
Paracoccidioides brasiliensis	5–60	Cells usually large and surrounded by smaller buds around periphery (mariner's wheel appearance); small cells (2–5 μm) that resemble *H. capsulatum* may be present	

(continued)

Fungal Diagnosis

Table 6.2 Characteristic fungal elements seen by direct examination of clinical specimens *(continued)*

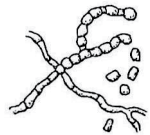
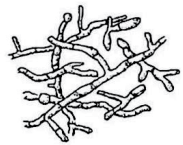
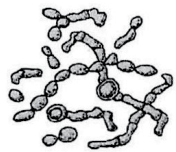
Morphologic fungal structure found	Organism(s)	Diam range (μm)	Characteristic features	Illustration[a]
Yeast forms and pseudohyphae or true hyphae	*Candida* spp.	3–4 (yeast forms), 5–10 (pseudohyphae)	Cells usually exhibit single budding; pseudohyphae, when present, are constricted at ends and remain attached; true hyphae, when present, have parallel walls	
	Malassezia spp.	3–4 (yeast forms), 2.5–4 (pseudohyphae)	Short, curved hyphal elements may be present along with round-oval yeast cells that are round at one end and have a flat collarette at the opposite end	
Spherules	*Coccidioides* spp.	10–200	Spherules vary in size; some contain endospores; hyphae may be found in cavitary lesions	

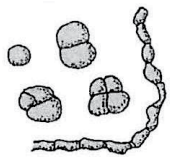
Sporangium	*Rhinosporidium seeberi*	6–300	Large, thick-walled sporangia containing sporangiospores; mature sporangia are larger than *Coccidioides* spherules	
Adiaconidia	*Emmonsia crescens*	20–140	Large, round, thick walled, no budding; interior of adiacondium usually appears empty	
Wide nonseptate or Rarely septate hyphae	Zygomycetes, *Pythium* spp.	10–30	Large, ribbonlike hyphae, often fractured or twisted, branching usually at right angles	

(continued)

Fungal Diagnosis

Table 6.2 Characteristic fungal elements seen by direct examination of clinical specimens *(continued)*

Morphologic fungal structure found	Organism(s)	Diam range (μm)	Characteristic features	Illustration[a]
Hyaline septate hyphae	Dermatophytes	3–15	Branched, septate hyphae; chains of arthroconidia may be seen	
	Other hyaline moulds; *Aspergillus, Fusarium*	3–12	Hyphae are septate and may exhibit 45° (dichotomous) and 90° angle branching	
Dematiaceous septate hyphae	Organisms causing phaeohyphomycosis	2–6	Brown-pigmented septate hyphae, dark budding yeastlike forms may also occur	

Dematiaceous sclerotic bodies	Organisms causing chromoblastomycosis	5–12	Sclerotic bodies are brown pigmented and thick walled and have horizontal and vertical septations	
Cysts and trophozoites	*Pneumocystis (carinii) jiroveci*	3–5	Nonbudding, round, ovoid, or collapsed crescent forms appear in small clusters against a foamy background	

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.

Fungal Diagnosis

Birdseed Agar

Birdseed (also called niger seed) agar is used for the selective isolation and identification of *Cryptococcus neoformans*. The agar medium contains an extract of *Guizotia abyssinica* seed, caffeic acid. *C. neoformans* produces phenol oxidase, and dark brown colonies develop in the presence of caffeic acid. The medium contains chloramphenicol to suppress the growth of bacteria.

Brain Heart Infusion Agar (BHI)

Brain heart infusion agar is a nutritionally enriched medium that can be used for the isolation of a variety of fastidious bacteria, yeast, and molds. It is prepared with infusions of calf brains and beef hearts, peptones, glucose, sodium chloride, and disodium phosphate. Supplementation with 5 to 10% sheep blood can enrich the medium, and the addition of antibiotics (e.g., gentamicin, chloramphenicol, and penicillin) can make this medium selective for fungi.

CHROMagar Candida

CHROMagar *Candida* is a selective, differential agar medium for the isolation and presumptive identification of *Candida albicans*, *C. krusei*, and *C. tropicalis.* The medium consists of peptones, glucose, chloramphenicol, and "chromogenic mix." The antibiotic inhibits the growth of most bacteria. *C. albicans* forms green colonies, *C. krusei* forms pink colonies, and *C. tropicalis* forms purple colonies.

Dermatophyte Test Medium (DTM)

Dermatophyte test medium is a selective agar medium used for the isolation and identification of dermatophytes. It consists of digests of soybean meal supplemented with glucose, cycloheximide, chlortetracycline, gentamicin, and phenol red. The antibiotics suppress the growth of bacteria, saprophytic yeasts, and molds. Dermatophytes growing on this medium produce alkaline by-products that change the phenol red indicator from yellow to red. This color change may be obscured when grossly contaminated specimens (e.g., nails) are processed on this medium. The pigment produced by dermatophytes, which is used for their identification, is obscured by the intense red color produced on this medium.

Inhibitory Mold Agar (IMA)

Inhibitory mound agar is an enriched, selective medium that is used for the isolation of pathogenic fungi other than dermatophytes. It consists of digests of animal tissue and casein, yeast extract,

dextrin, starch, glucose, salts, and chloramphenicol. Contaminating bacteria are inhibited by chloramphenicol.

Mycosel (Mycobiotic) Agar

Mycosel (Mycobiotic) agar is a selective medium used for the isolation of pathogenic fungi from contaminated specimens. Mycosel and Mycobiotic (BD Diagnostic) agars consist of digests of soybean meal supplemented with glucose, cycloheximide, and chloramphenicol. Cycloheximide-susceptible fungi, including *Cryptococcus neoformans*, *Pseudallescheria boydii*, the zygomycetes, many species of *Candida* and *Aspergillus*, *Trichosporon* spp., and most saprophytic or opportunistic fungi, do not grow on this medium.

Sabouraud Agar–Brain Heart Infusion (SABHI)

Sabouraud agar–brain heart infusion (SABRI), an enriched agar medium, is a variation of Sabouraud dextrose agar (described below). The medium consists of infusions of beef heart and calf brains, peptones, salts, glucose, blood, and chloromycetin (chloramphenicol). It is used for the cultivation of dermatophytes and other pathogenic and nonpathogenic fungi.

Sabouraud Dextrose Agar (SDA)

Sabouraud dextrose agar is an enriched agar medium used for the isolation of saprophytic and pathogenic fungi. The original formulation of SDA consists of digests of casein and animal tissue supplemented with 4% glucose and adjusted to pH 5.6. The Emmons modification is preferred by many mycologists. It contains a reduced concentration of glucose (2%) and is buffered to neutrality (pH 6.9). Yeast, dermatophytes, and other filamentous fungi grow on these media. The original formulation of SDA was acidic to suppress the growth of bacteria. This problem can be circumvented by the addition of antibiotics (e.g., cycloheximide and chloramphenicol) to the media. However, cycloheximide-susceptible fungi (refer to Mycosel agar above) do not grow on this medium.

Yeast Extract-Phosphate Agar

Yeast extract-phosphate agar is a selective medium used for the isolation of pathogenic fungi such as *Histoplasma* and *Blastomyces* spp. It consists of yeast extract and phosphate buffer supplemented with chloramphenicol to suppress the growth of bacteria. The pH is adjusted to 6.0.

Table 6.3 Mycology plating guide

Source	Direct exam[a] (wet mount, Calcofluor white, KOH)	MAF for *Nocardia*[b]	Enriched SABHI, SDA, BHI	Selective	Comments
Blood	N		× (or automated systems media)		Lysis and centrifugation; automated systems include BacT/Alert (bioMerieux), BACTEC (Becton Dickinson), and ESP (Trek); if *Malassezia* suspected, add olive oil to plates.
Body fluids	O		×	IMA, Mycosel, yeast extract, phosphate	>2 ml, filtration or centrifugation at 2,000 x *g* for 10 min to concentrate fungal organisms; for quantification of urines, uncentrifuged urine can be streaked with a calibrated loop onto a plate of noninhibitory media.
Bone marrow	N		×		
Eye					
Corneal Scrapings	O		×		Inoculate media directly using C or X marks.
Eyelid, conjunctiva	O		×	IMA, Mycosel	
Vitreous fluid	O		×		>2 ml, filtration or centrifugation at 2,000 x *g* for 10 min to concentrate fungal organisms.
Exudates, pus, drainage	O	×	×	IMA, Mycosel, yeast extract	If granules present, wash, centrifuge, examine, and crush granules.

Mouth			R or Gram stain	CHROMagar *Candida,* I MA	Candidiasis is usually diagnosed on the basis of clinical symptoms and direct microscopic examination.
Nails, hair, skin scrapings	R			IMA, Mycosel, DTM	Cut into small pieces and embed directly into agar; if *Malassezia* suspected, add olive oil to plates.
Respiratory secretions	R, O (TCP stain)	×	×	CHROMagar *Candida,* IMA, Mycosel, yeast extract, phosphate, birdseed	Liquefication with a mucolytic agent; necessary with centrifugation for induced sputum specimens for the detection of *Pneumocystis* spp.
Sinus	R		×	CHROMagar *Candida,* IMA	
Tissue	R, O (PCP stain, lung)	×	×	IMA, Mycosel, yeast extract, phosphate, birdseed	Mincing (zygomycetes), grinding (*Histoplasma*), or use of stomacher
Vagina	R, Gram stain			CHROMagar *Candida,* IMA	Vaginal candidiasis is usually diagnosed on the basis of clinical symptoms and direct microscopic examination.

[a]R, staining should be routinely performed; O, staining is optional and should be performed if requested; N, staining should not be performed unless the request is discussed with the physician.

[b]Although *Nocardia* is a bacterium, stains and cultures are commonly pursued through the mycology laboratory. For appropriate bacterial culture media, please see Primary Plating Media in section 4 of this handbook.

Fungal Diagnosis

Abbreviation Guide. CF, complement fixation; CIE, counterimmunoelectrophoresis; EIA, enzyme immunoassay; ID, immunodiffusion; IFA, indirect fluorescent-antibody test; LA, latex agglutination; RIA, radioimmunoassay; TA, tube agglutination; TP, tube precipitin.

***Aspergillus* Species.** Microscopy and culture are sensitive detection methods for *Aspergillus* species. Molecular methods are being developed for both direct specimen and *Aspergillus* spp. identification. EIA and RIA for *Aspergillus* antigens and CF, CIE, and ID tests for antibodies have been developed. Antigen tests are used primarily to diagnose invasive aspergillosis. Commercial EIAs detect galactomannan (GM) in serum and respiratory specimens. The tests have a sensitivity between 71 and 95% (higher when monoclonal antibodies are used) and good specificity. Antibody tests are most sensitive for immunocompetent patients with allergic bronchopulmonary aspergillosis (ABPA), pulmonary aspergilloma, and invasive aspergillosis (IA). The sensitivities of the ID and CIE tests are comparable, while ID is more specific. The CF test is more specific but less sensitive than ID. The sensitivity of ID is improved by the use of multiple antigens from *A. fumigatus, A. flavus, A. niger,* and *A. terreus*. Precipitins are present in more than 90% of patients with aspergillomas, 70% of patients with ABPA, and fewer patients with IA. A fourfold concentration of serum and retesting is recommended for patients with suspected IA and a negative ID result. The ID is highly specific, with false-positive precipitins developing only against C-reactive protein. A complement fixation antibody titer of 1:8 is considered positive. Skin test reactivity to *Aspergillus* antigen extracts is useful for patients with suspected allergic bronchopulmonary aspergillosis, atopic dermatitis, or allergic asthma sensitized to aspergilli.

***Blastomyces dermatitidis* (Blastomycosis).** Demonstration of broad-based budding yeast cells and culture are the most reliable detection methods for *B. dermatitidis.* This organism can be identified by using microscopic and macroscopic morphology as well as molecular probe and exoantigen methods. Due to the lack of sensitivity and specificity of available serologic tests (ID, EIA, CF, and RIA), these tests are generally not helpful for diagnosing blastomycosis. The use of the A antigen, obtained from yeast culture filtrates, has improved the specificity of these tests. The commercially available EIA is more sensitive but less specific than ID. An EIA titer of 1:32 or greater is considered diagnostic for blastomycosis. Titers of 1:8 to 1:16 should be confirmed with ID or culture because cross-reactivity with *Histoplasma* antibodies can occur at this level. ID has a sensitivity of approximately 80% and a speci-

ficity approaching 100%. Antibodies are detected within 1 month of onset, and the level declines with successful treatment. RIA has a sensitivity and specificity similar to ID but is rarely used. A CF antibody titer of ≥1:8 is considered positive. This test is relatively insensitive and nonspecific for blastomycosis and has generally been replaced by EIA and ID. *Blastomyces* urine antigen testing can be useful in diagnosing infection, but there is near complete cross-reactivity in cases of *Histoplasma* infection, and thus positive results do not confirm *Blastomyces* infection.

***Candida* Species (Candidiasis).** *Candida* species can be detected by direct examination with Gram stain, KOH, or Calcofluor white preparations. *Candida* species grow well on most culture media. The identification of *Candida* species can be pursued through use of color production on differential agars, germ tube production, sugar assimilation and/or fermentation, temperature tolerance, cycloheximide tolerance, and urea and nitrate testing. Currently, yeast identification using nucleic acids is not widely practiced. LA, ID, and CIE tests for antibodies and EIAs for antigens have been developed for the diagnosis of candidiasis. In general, the sensitivity and specificity of available tests are low.

***Coccidioides* Species (Coccidioidomycosis).** Microscopy and culture are reliable detection methods for *Coccidioides* species. There are two species of *Coccidioides*: *C. immitis* and *C. posadasii*. *C. immitis* can be identified using microscopic and macroscopic morphology as well as Accuprobe and exoantigen methods, while molecular methods are required to definitively identify *C. posadasii*. CF, TP, ID, and EIA have been developed for detection of antibodies against C. *immitis*. The test antigens, called coccidioidin, are prepared from filtrates of mycelial cultures. Two primary antigens are used: a heat-stable 120-kDa glycoprotein (factor 2 antigen) located in the walls of arthroconidia and spherules, and a heat-labile 110-kDa chitinase enzyme (F antigen). The former protein is detected in the TP test, and the latter is detected in the CF test. Both antigens can be detected in the ID test and EIAs. Another antigen, spherulin, is prepared from spherules of *C. immitis* and has been used in CF tests. Factor 2 antigen is not specific for *C. immitis* and is also found in morphologically similar saprophytic fungi. The TP test is used to detect early disease (80% positive at 2 to 3 weeks, disappearing by 6 months), and the CF test detects persistent antibodies. TP remains positive in patients with disseminated disease. The combination of CF and TP tests is positive in more than 90% of infected patients. ID is comparable to CF and TP. The commercially prepared EIA measures both immunoglobulin M (IgM) and IgG antibodies. Both tests must be performed for maximum sensitivity. A positive EIA result must be confirmed by ID. CF antibody titers of 1:2 to 1:4 usually indicate early, residual,

or meningeal disease. Antibody titers of 1:16 indicate disseminated disease. Negative titers do not exclude the disease. Coccidioidin skin tests are of limited usefulness, although failure to develop a positive skin test has been associated with poor response to therapy.

***Cryptococcus neoformans* (Cryptococcosis).** Microscopy and culture are useful detection methods for *C. neoformans. Cryptococcus* grows readily in culture but is inhibited by cycloheximide. Cryptococcal antigens can be measured by LA and EIA. EIA is more sensitive for capsular glucuronoxylomannan polysaccharide, and the method is suitable for testing of multiple specimens. Titers are generally determined using the LA method. A titer of 1:8 or greater in serum or cerebrospinal fluid (CSF) is considered diagnostic. Titers of 1:4 or less may be indicative of early disease or nonspecific reactions (prozone, patients with rheumatoid arthritis, syneresis fluid, platinum wire loops, *Capnocytophaga* [DF-1], *Trichosporon beigelii*, disinfectants, and soaps). These nonspecific reactions have been documented by LA only. Titers in CSF can be helpful in monitoring therapy when the titers are tested over appropriate intervals (at least 2 weeks). Interpretation of follow-up titers is sometimes difficult because the antigen is harbored within the body; therefore, the definitive decision often depends on the results of culture. More than 99% of patients with culture-confirmed cryptococcosis have positive antigen tests. IFA, EIA, and LA have been developed for measuring cryptococcal antibodies. These tests are not useful for diagnosis because capsular polysaccharide may inhibit antibody synthesis or mask the presence of antibody. Antibody testing may have prognostic value during the recovery of non-AIDS patients.

***Histoplasma capsulatum* (Histoplasmosis).** Definitive diagnosis of histoplasmosis requires growth of the fungus. Mycelial forms mature within 20 days and display diagnostic tuberculate macroconidia. Identification is performed by mound-yeast conversion, Accuprobe, and exoantigen methods. CF, EIA, LA, and ID have been developed to measure antibodies to *H. capsulatum*. RIA and EIA have been developed to detect *Histoplasma* antigens in urine and serum. The CF test is sensitive (more than 90% of culture-confirmed patients have antibodies), but cross-reactions can occur in patients with blastomycosis, coccidiodomycosis, other mycoses, and leishmaniasis. Two antigens are used in the CF test: yeast phase antigen and mycelial phase antigen (histoplasmin). CF antibodies develop within 4 weeks after exposure in patients with pulmonary infections, with antibodies against the yeast phase being detected first and those against histoplasmin developing later. Patients with chronic histoplasmosis generally have higher titers to histoplasmin. Antibody titers between 1:8 and 1:32 are considered presumptive

evidence of histoplasmosis; however, high titers can be observed in patients with other diseases, so serology should be confirmed by culture. Antigen EIA and RIA provide rapid diagnosis, but cross-reactivity occurs with other mycoses. Cross-reactivity does not detract from the value of these tests because, depending of the severity of the clinical picture, antifungal therapy is essentially the same. If these tests are used, the results must be confirmed by ID tests. The ID test can detect as many as six precipitin bands when histoplasmin is used as the test antigen. Two bands, H and M, have diagnostic value. The M band generally appears first and is an indicator of early disease. The presence of both the M and H bands is indicative of active disease, past disease, or recent skin testing. The presence of both M and H bands is consistent with active disease. The LA test is used to detect acute histoplasmosis, with reactivity occurring 2 to 3 weeks after exposure. Positive reactivity should be confirmed with the ID test. A heat-stable polysaccharide antigen can also be detected in serum, urine, and CSF specimens in patients with disseminated histoplasmosis (90% sensitivity), as well as localized pulmonary disease (<50% sensitivity). The urine test is the most sensitive for disseminated disease, but false-positive reactions have been reported with other diseases (e.g., Coccidioidomycosis paracoccidioidomycosis, penicilliosis, and blastomycosis). Positive reactions should be confirmed with culture.

***Malassezia* Species.** Direct examination and culture are the methods of choice for the detection of *Malassezia* species. All species are lipophilic (except *M. pachydermatitis*) and require the addition of long-chain fatty acids (e.g., sterile olive oil) to culture media for growth. *Malassezia* exists both as a skin commensal and as an etiological agent of cutaneous and systemic disease. Differentiation of lipophilic species is not generally performed.

Paracoccidioides brasiliensis. Diagnosis is established when direct examination demonstrates the organism's characteristic "ship's wheel." Mycelial forms mature within 21 days, but their presence is not diagnostic. Mold-yeast conversion or exoantigen testing is necessary for definitive identification. CF, ID, EIA, and CIE have been developed to measure antibodies to *P. brasiliensis.* The CF test detects antibodies (titer of 1:32 or greater) in at least 80 to 95% of patients with paracoccidioidomycosis, while positive serologic test results are reported for 98% of patients when both the CF and ID tests are used. Cross-reactivity with *H. capsulatum* can occur in the CF test. Declining CF titers are consistent with a response to therapy, and the presence of persistently high titers indicates a bad prognosis. One to three unique precipitin bands are observed in the ID test. Antigen 1 has been characterized as a 43-kDa glycoprotein. This antigen has also been used in EIA. Both the CF and ID tests are available through the CDC.

***Talaromyces* (*Penicillium*) *marneffei*.** The diagnostic test of choice is demonstration of fission yeast cells in direct examinations and the recovery of *T. marneffei* in clinical specimens. These infections are usually disseminated, with multiple-organ involvement including lymphadenitis, subcutaneous abscesses, bone lesions, arthritis, splenomegaly, and lesions in the lungs, liver, or bowel.

***Pneumocystis jiroveci*.** Demonstration of the organism in clinical specimen by microscopy is diagnostic. *P. jiroveci* grows poorly in cell culture, and reliable antigen and antibody tests have not been developed. The presence of the PCR product has not been strictly correlated with disease. Toluidine blue 0, Calcofluor white, and methenamine silver stain the cyst wall; Gram Weigert and Papanicoalou stain the intracystic bodies and faintly stain trophozoite forms; Giemsa and fluorescein-labeled antibodies (IFA, DFA) stain both cysts and trophozoites.

***Sporothrix schenckii* (Sporotrichosis).** Isolation and mold-yeast conversion are required for the diagnosis of sporotrichosis. Although antigen and antibody tests are available, they are not widely used. EIA, LA, and TA can be used reliably to detect antibodies to *S. schenckii*, while the CF and ID tests are less reliable and are not recommended. Antibodies to at least two cell wall antigens (40- and 70-kDa antigens) are detected. EIA titers of at least l:16 in serum and l:8 in CSF are considered diagnostic. Elevated titers can be observed, which decline with successful therapy. LA titers of 1:4 or greater are consistent with disease, although nonspecific reactions can occur at titers of 1:8. Antibody titers in the LA test do not change predictably with therapy, so they cannot be used for prognostic purposes.

***Zygomycetes* (Zygomycosis, Mucormycosis).** EIA and ID have been developed to detect antibodies in patients with active zygomycosis. The tests have a sensitivity of approximately 70% and a specificity of greater than 90%. They are rarely used because the etiologic agents of zygomycosis grow rapidly.

Biomarkers of Invasive Fungal Infection

Galactomannan. This is a diagnostic test that detects circulating galactomannan in serum (a component of *Aspergillus* cell wall) as a marker of invasive aspergillosis (IA). The diagnosis of IA is often very difficult due to the low yield of culture, nonspecific symptoms, and the fact that those patients at greatest risk for IA are of too unstable to undergo diagnostic biopsy procedures. The performance of galactomannan detection in serum yields relatively high sensitivity and specificity, although false positive results have been noted in patients receiving piperacillin/tazobactam. In addition to

testing the serum, there is evidence that testing of respiratory specimens may be useful in the diagnosis of pulmonary aspergillosis.

1, 3 Beta-D-Glucan. Detection of circulating beta-D-glucan (BDG) is used as a marker of invasive fungal infection. BDG is a nonspecific component of the cell wall of many fungal species. Importantly, *Cryptococcus* and the Zygomycetes do not possess this element of their cell wall and therefore negative BDG results do not rule out infection with these organisms. In addition, BDG is controversial due to its overall poor performance. In particular, false positive results (poor specificity) are a significant problem with this test, as a wide range of predisposing factors can lead to falsely positive results. Notably, patients exposed to gauze or medications that have been filtered through a cellulose-containing membrane are among the most common causes of false positive results.

The following tables summarize organisms described in the eighth edition of the *Manual of Clinical Microbiology.* They are organized in parallel with the discussions of the organisms within the manual. Due to the dependence on phenotypic growth characteristics for the identification of molds, the information summarized includes colony morphology, line drawings, and key differential characteristics. All line drawings are used with the author's permission and come from the book *Medically Important Fungi, a Guide to Identification,* fourth edition (2002), by D. H. Larone. For further organism information, please refer to the eighth edition of the *Manual of Clinical Microbiology* and the fourth edition of *Medically Important Fungi, a Guide to Identification*.

Table 6.4 Cultural and biochemical characteristics of yeasts frequently isolated from clinical specimens[ab]

Species	Growth at 37°C	Pellicle in Broth	Pseudo—or true hyphae	Chlamydospores	Germ tubes	Capsule India Ink	Assimilation of:												Fermentation of:						Urease	KNO_3 utilization	Phenol oxidase
							Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose			
Candida albicans	+	−	+	+	+	−	+	+	+*	—	+	—	—	—	+	—	+	—	F	F	—	—	F	F	—	—	—
C. catenulata	+*	−	+	−	—	−	+	+	—	—	+	—	—	—	+	—	—	—	F*	—	—	—	—	—	—	—	—
C dubliniensis	+	−	+	+	+	−	+	+	+	—	+	—	—	—	+*	—	+	—	F	F	—	—	F	F	—	—	—
C famata	+	−	—	−	—	−	+	+	+	+*	+	+	+	—	+	+	+	+*	W	—	W	—	—	W	—	—	—
C. glabrata	+	−	—	−	—	−	+	+	—	—	—	—	—	—	—	—	+	—	F	—	—	—	—	F	—	—	—
C. guilliermondii	+	−	+	−	—	−	+	+	+	—	+	+	+	—	+	+	+	+	F	—	F	—	F*	F	—	—	—
C. kefyr	+	−	+	−	—	−	+	—	+	+	+	—	+*	—	+*	+	—	F	—	F	F*	F	—	—	—	—	
C. krusei[c]	+	+	+	−	—	−	+	—	—	—	—	—	—	—	+*	—	—	—	F	—	—	—	—	—	+*	—	—
C lambica	+*	+	+	−	—	−	+	—	—	—	—	—	—	—	+	—	—	—	F	—	—	—	—	—	—	—	—
C. lipolytica[c]	+	+	+	−	—	−	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
C. lusitaniae[d]	+	−	+	−	—	−	+	+	+	—	+	—	+	—	+	—	+	—	F	—	F	—	F	F	—	—	—
C parapsilosis[e]	+	−	+	−	—	−	+	+	+	—	+	—	—	—	+	—	+	—	F	—	—	—	—	—	—	—	—
C. pintolopepsii		−		−		−																					
C. rugosa	+	−	+	−	+	−	—	—	—	+	—	—	—	+*	—	—	—	—	—	—	—	—	—	—			
C. tropicalis[d,e]	+	+	+	−	—	−	+	+	+	—	+	—	+	—	+	—	+	—	F	F	F	—	F*	F*	—	—	—

C. zeylanoides	—	−*	+	−	—	−	+	—	—	—	—*	—	—*	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—
Cryptococcus neoformans	+	−	R	−	—	+	+	+	+	—	+	—	+	+	+	+*	+	+	—	—	—	—	—	—	—	+	—
C. albidus	—*	−	—	−	—	+	+	+	+	+*	+*	+	+	+	+	+	+	+*	+	+	—	—	—	—	+	—	+
C. laurentii	+*	−	—	−	—	+	+	+	+	+	+	+*	+	+	+	+*	+	+	—	—	—	—	—	—	+	—	—
C. luteolus	—	−	—	−	—	+	+	+	+	—	+	+	+	+	+	+	+	+	—	—	—	—	—	—	+	—	—
C. terreus	—*	−	—	−	—	+	+	+*	—	+*	+*	—		+	+	+	+	—*	—	—	—	—	—	—	+	+	—
C. uniguttulatus	—	−	—	−	—	+	+	+	—	—*	—	—*	+	+	+*	—*	—	—	—	—	—	—	—	—	+	+	—
R. glutinus	+	−	—	−	—	−*	+	+	+	—	+*	—	+	—	+	+	+	—	—	—	—	—	—	—	+	+	—
R. rubra	+	−	—	−	—	−*	+	+	+	—	+	—	+*	—	+	+	+	—	—	—	—	—	—	—	+	—	—
S. cerevisiae	+	−	—	−	—	−	+	+	+	—	+	—	—	—	—	+	+*	—	F	F	F	—	F	F*	—	—	—
C. peliculosa		−		−		−																					
G. candidum	—*	+	+	−	—	−	+	—	—	—	+	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
B. capitalus	+	+	+	−	—	−	+	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Prototheca wickerhamii	+	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Sporobolomyces salmonicolor	+*	+	+*	−	−	−	+	+	+	−	+*	−	+*	−	+	−	W	−	−	−	−	−	−	−	+	+	−
Trichsoposon asahii	+	NT	+	−	−	−	+	+	+*	+	+	−	+	+*	+*	−	+*	−	−	−	−	−	−	+	+	−	−
Trichosporon mucoides	+	NT	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−	−
Trichosporon ovoides	+*	NT	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−

[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

[b]Symbols: +, growth greater than that of the negative control;—, negative reaction; *, some isolates may give the opposite reaction; R, rare; F, the sugar is fermented (i.e., gas is produced); W, weak fermentation.

[c]*C. lipolytica* assimilates erythritol; C. *krusei* does not. Maximum growth temperatures are 43 to 45°C for C. *krusei* and 33 to 37°C for C. *lipolytica.*

[d]*C. lusitaniae* assimilates rhamnose; C. *tropicalis* usually does not.

[e]*C. parapsilosis* assimilates L—arabinose; C. *tropicalis* usually does not.

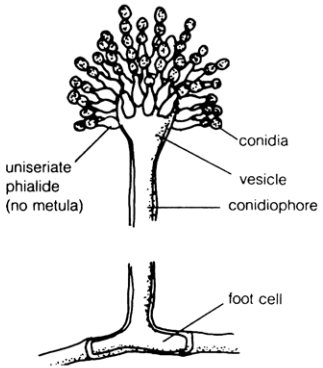
Fungal Diagnosis

Table 6.5 Characteristics of selected *Trichosporon* species[a]

Characteristic	*T. asahii*	*T. asteroides*	*T. cutaneum*	*T. inkin*	*T. loubieri*	*T. mucoides*	*T. mycotoxinivorans*	*T. ovoides*
Assimilation of:								
L–Rhamnose	+	—	+	—	V	+	+	+
Melibiose	—	—	+	—	+	+	+	—
Raffinose	—	—	+	—	+	+	+	V
Ribitol	V	V	+	—	NT	+	NT	—
Xylitol	V	+	+	—	V	+	W	V
L–Arabinitol	+	+	+	—	V	+	W	—
Galactitol	—	—	—	—	-	+	+	—
Sorbitol	–	V	+	–	V	+	+	–
Growth at 37°C	+	V	—	+	+	+	+	+
Growth at 42˚C	–	–	–	V	+	–	–	–
Urease	+	+	+	+	+	+	+	+
0.01% Cycloheximide	+	V	—	V	+	+	+	+
0.1% Cycloheximide	—	V	—	—	+	+	+	—
Appresoria	—	—	—	+	–	—	–	+
Fusiform giant cells	–	–	–	–	+	–	+	–

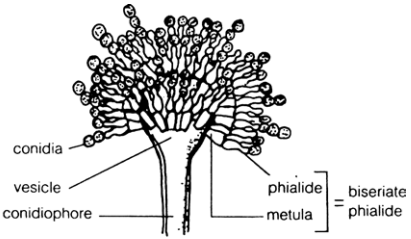
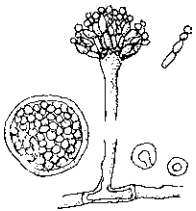
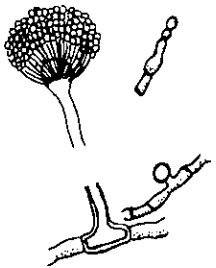
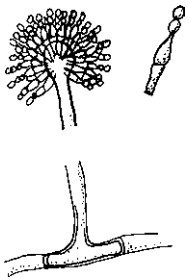
[a]Adapted from Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

Table 6.6 Characteristics of *Aspergillus* species[a]

Illustration	Diagnostic characteristics	*A. fumigatus*	*A. flavus*	*A. niger*
	Colony morphology	Velvety-powdery, blue-green to gray, reverse white-tan	Velvety-powdery, yellow-dark yellowish green, reverse gold to red-brown	Velvety-powdery, yellow-black, reverse buff
	Seriation Conidiophore Microscopic morphology	Unseriate Smooth	Biseriate Rough	Biseriate Smooth, long, straight

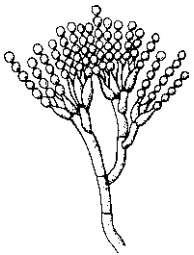
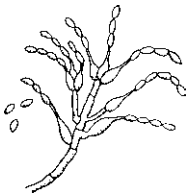
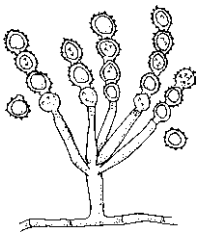
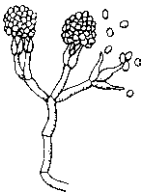
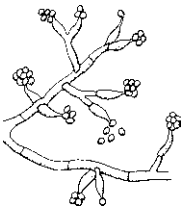
(continued)

Table 6.6 Characteristics of *Aspergillus* species[a] *(continued)*

Illustration	Diagnostic characteristics	*A. nidulans*	*A. terreus*	*A. versicolor*
	Colony morphology	Velvety, dark green to purplish-brown, reverse buff to deep red	Velvety, tan to cinnamon brown, reverse yellow to tan	Green to gray-green or tan with patches of pink or yellow, reverse variable
	Seriation	Biseriate	Biseriate	Biseriate
	Conidiophore	Smooth, short, brown	Smooth	Smooth
	Microscopic morphology			

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.

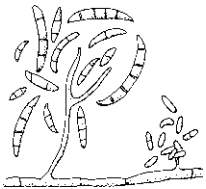
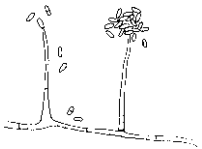
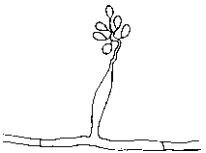
Table 6.7 Opportunistic moniliaceous fungi[a]

Diagnostic characteristics	***Talaromyces***	***Paecilomyces***	***Scopulariopsis***	***Gliocladium***	***Trichoderma***
Colony morphology	Velvet, green, reverse white to cream; if red diffusing pigment, rule out *P. marneffei*	Velvety, yellowish brown or mauve, never bright green or blue-green; off-white, pinkish, yellow or pale brown reverse	Powdery, cream-cinnamon or dark gray to brown-black; reverse usually tan, occasionally darker	Fluffy dark green, reverse white	Fluffy green, reversecolorlessoryellow-orange
Microscopic morphology					

(continued)

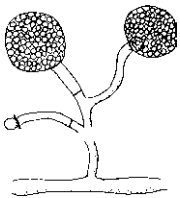
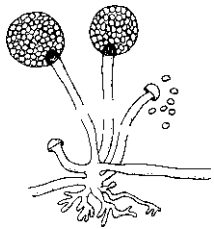
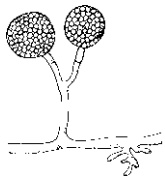
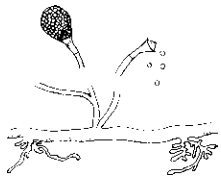
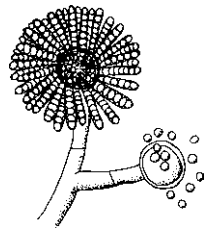
Fungal Diagnosis

Table 6.7 Opportunistic moniliaceous fungi[a] *(continued)*

Diagnostic characteristics	***Talaromyces***	***Acremonium***	***Phialemonium***	***Lecythophora***	***Beauveria***
Colony morphology	Cottony surface variable in color (white, cream, violet, or pink), reverse variable (white to dark pink); also consider *Cylindrocarpon*	Glabrous-feltlike, white, tan, light gray, or pale rose; reverse colorless, pale yellow, or pinkish; some species are *dematiaceous*	Flat, spready white-cream, yellow or green; reverse light with pale wine-buff or brown; some have green diffusing pigment	Moist-slimy, pink to salmon-orange; reverse pink or tan	Fluffy cream to pink; reverse white; also consider *Engyodontium*
Microscopic morphology					

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002. The *Paecilomyces* illustration is from the third edition.

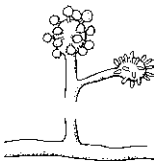
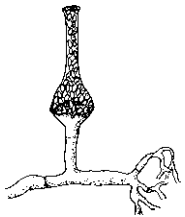
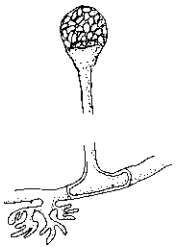
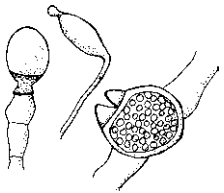
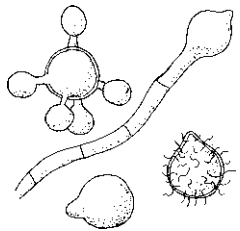
Table 6.8 Zygomycetes[a]

Diagnostic characteristics	***Mucor***	***Rhizopus***	***Rhizomucor***	***Lichtheimia***	***Syncephalastrum***
Colony morphology	Fluffy gray to gray-brown, reverse white	Fluffy gray-brown, reverse white	Fluffy gray to dark brown, reverse white	Fluffy gray, reverse white	Fluffy dark gray, reverse white
Microscopic morphology					
Maximum growth temp	37°C	45–50°C	54–58°C	45–50°C	40°C

(continued)

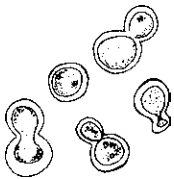
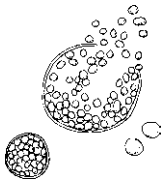
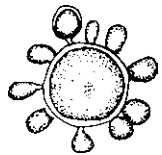
Fungal Diagnosis

Table 6.8 Zygomycetes[a] *(continued)*

Diagnostic characteristics	***Cunninghamella***	***Saksenaea***	***Apophysomyces***	***Basidiobolus***	***Conidiobolus***
Colony morphology	Fluffy gray, reverse white	Fluffy white, reverse white; use special media to enhance sporulation	Cream, yellow, or gray-brown; reverse white to pale-yellow; use special media to enhance sporulation	Flat, waxy, buff to gray-brown; satellite colonies formed by ejected conidia	Glabrous flat cream becoming covered with a white powdery mycelium, reverse white
Microscopic morphology					
Maximum growth temp	42°C	<37°C	42°C	37°C	35°C

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., Fungal Diagnosis, 2002

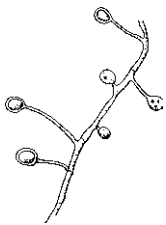
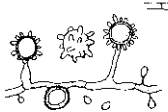
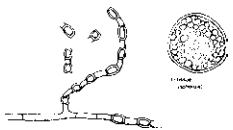
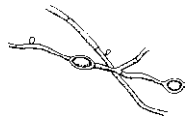
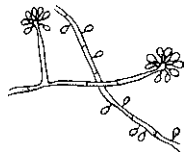
Table 6.9 Dimorphic molds[a]

Diagnostic characteristics	***Blastomyces dermatitidis***	***Histoplasma capsulatum***	***Coccidioides* spp.**	***Talaromyces marneffei***	***Paracoccidioides brasiliensis***	***Sporothrix schenckii***
Direct exam						
Colony morphology at 25°C	Glabrous to velvety, white-gray, becoming tan; reverse cream-brown	Glabrous to velvety, white, becoming tan; reverse cream-brown	Glabrous to velvety, white-gray, becoming tan; reverse cream-brown	Green with gray-orange or purple-orange periphery; reverse buff, red diffusible pigment	Glabrous brown to wrinkled floccose, beige or white	Moist yeast-like texture, becoming wrinkled; surface reverse cream-brown

(continued)

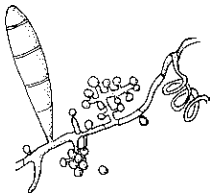
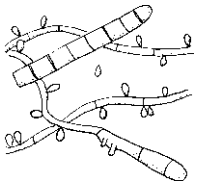
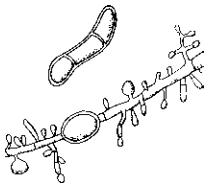
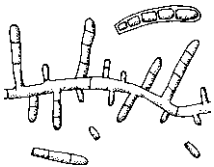
Fungal Diagnosis

Table 6.9 Dimorphic molds[a] *(continued)*

Diagnostic characteristics	***Blastomyces dermatitidis***	***Histoplasma capsulatum***	***Coccidioides* spp.**	***Talaromyces marneffei***	***Paracoccidioides brasiliensis***	***Sporothrix schenckii***
Microscopic exam						
Rule out	*Emmonsia, Chrysosporium, Scedosporium apiospermum*	*Emmonsia, Sepedonium*	*Arthrographis, Geotrichum, Malbranchea, Trichosporon*	Other *Penicillium* spp. producing red diffusible pigment	*Emmonsia;* on direct exam, *Blastomyces dermatitidis*	*Acrodontium* spp.

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.

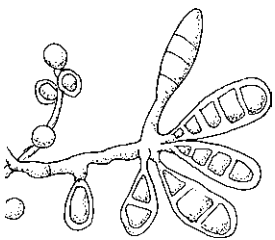
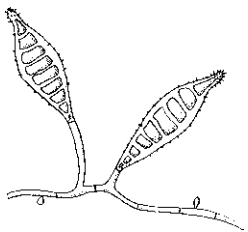
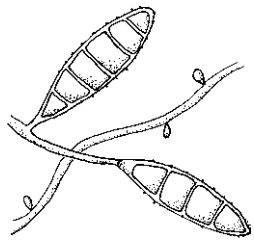
Table 6.10 Characteristics of common *Trichophyton* species[a]

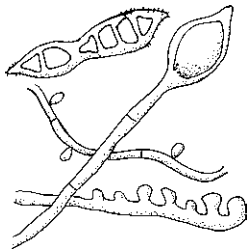
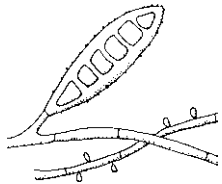
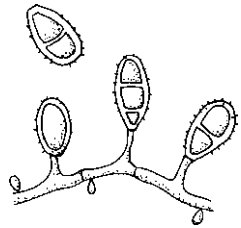
Diagnostic characteristics	*T. mentagrophytes*	*T. rubrum*	*T. tonsurans*	*T. terrestre*	*T. verrucosum*
Colony morphology	Variable cottony, velvety or granular, generally white; reverse white to pale yellow	Variable, cottony white; reverse deep red-brown	White to creamy yellow powdery or velvety surface, reverse lemon-yellow or red-brown	White to cream powdery to velvety surface, reverse pale, slightly yellow-gray	Very slow growing white to cream heaped colonies, reverse white to yellow-brown
Microscopic morphology					
Urease	+	—	+	+	
Trichophyton agars 1/4[b]	4+/4+	4+/4+	–/+	+/+—	–/V
Growth at 37°C	+	+	+	–	+

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.
[b]—, no growth; +, restricted growth; 4+, maximum growth; V, variable.

Fungal Diagnosis

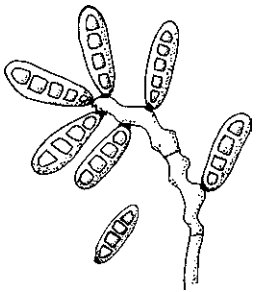
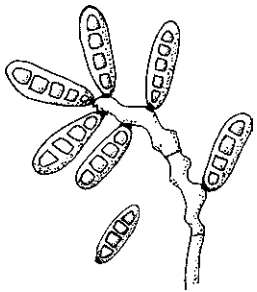
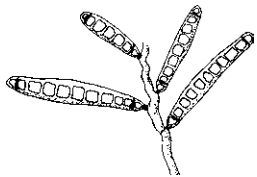
Table 6.11 *Epidermophyton floccosum* and common *Microsporum* species[a]

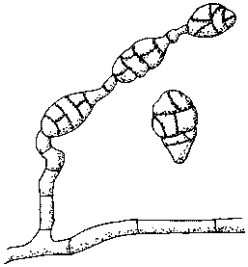
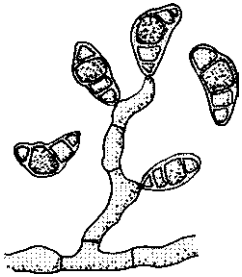
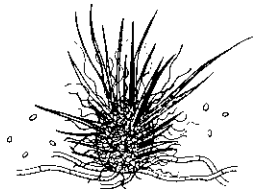
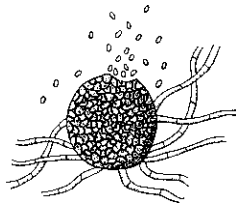
Diagnostic characteristics	***Epidermophyton floccosum***	***Microsporum canis* var. *canis***	***Microsporum gypseum* complex**
Colony morphology	Flat, slightly granular, sandy to olive-brown; reverse pale to yellow	Flat to velvety, pale to yellow; reverse yellow; also consider *M. praecox*	Granular, sandy color; reverse usually pale to brown
Microscopic morphology			

Diagnostic characteristics	*Microsporum audouinii*	*Microsporum cookei*	*Microsporum nanum*
Colony morphology	Flat to velvety; reverse pale salmon to pale brownish	Granular to velvety; reverse wine red	Powdery, sandy color, reverse reddish-brown
Microscopic morphology			

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.

Table 6.12 Dematiaceous fungi with macroconidia or other structures[a]

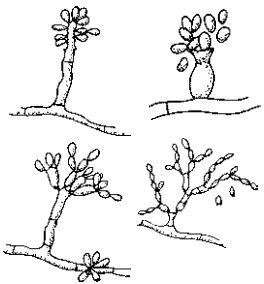
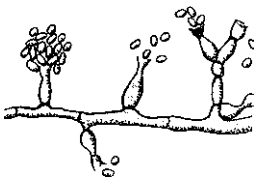
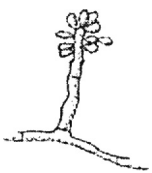
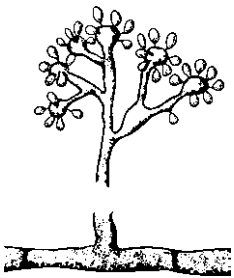
Diagnostic characteristics[b]	***Bipolaris***[c]	***Drechslera***	***Exserohilum***	***Helminthosporium***
Microscopic morphology				

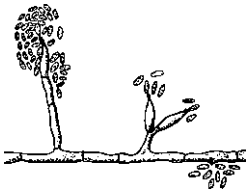
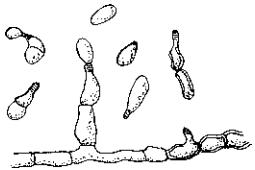
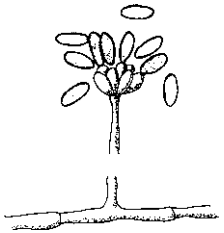
Diagnostic characteristics[b]	*Alternaria*	*Curvularia*	*Chaetomium*	*Phoma*
Microscopic morphology				

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.
[b]Colonies are woolly, rapidly growing, and shades of green and gray to black. Reverse is dark.
[c]In the past, most isolates of *Bipolaris* were mistakenly called *Drechslera*; a germ tube test is needed for differentiation.

Fungal Diagnosis

Table 6.13 Dematiaceous fungi with small conidia[a]

Diagnostic characteristics[b]	***Fonsecaea pedrosoi***	***Phialophora verrucosa***	***Rhinocladiella***	***Botrytis***
Microscopic morphology			 Also consider *Ramichloridium*	

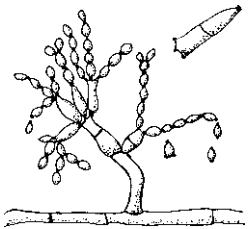
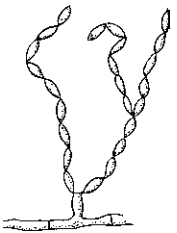
Diagnostic characteristics[b]	*Exophiala* spp.	*Exophiala dermatitidis*	*Hortaea werneckii*	*Stachybotrys*
Microscopic morphology				
Colony morphology and differential test	Nitrate positive; growth at 40°C variable	Nitrate negative; growth at 40°C positive	Nitrate positive; growth at 40°C variable	

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.

[b]Colonies are woolly, rapidly growing, and shades of green and gray to black. Reverse is dark. *Exophiala*, *Wangiella*, and *Phaeoannellomyces* are usually yeast-like when young.

Fungal Diagnosis

Table 6.14 Differentiation of *Cladosporium* and *Cladophialophora* species[a]

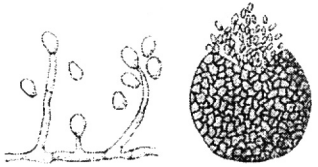
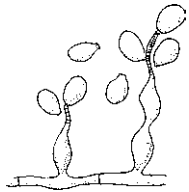
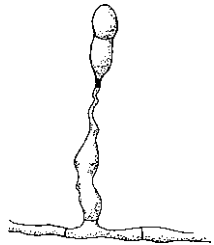
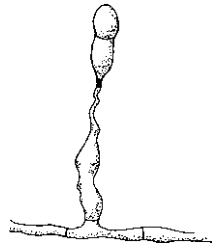
Diagnostic characteristics[b]	***Cladosporium* spp.**	***Cladophialophora carrionii***	***Cladophialophora bantiana***[c]
Microscopic morphology			
Gelatin hydrolysis	+	—	—
15% salt tolerance	+	—	—
Growth at 37°C	—	+	+
Growth at 42°C	—	—	Variable

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.

[b]Colonies rapidly growing, velvety or cottony, olive-gray to olive-brown or black. Reverse is black.

[c]*C. bantiana* now includes isolates previously classified as *Xylophypha ernmonsii.* Isolates of *C. bantiana* from cerebral lesions exhibit growth at 40°C; some isolates (those previously classified as *X. ernmonsii*) do not grow above 37°C.

Table 6.15 *Scedosporium* and *Dactylaria* species[a]

Diagnostic characteristics	***Scedosporium* spp. complex**	***Scedosporium prolificans***	***Ochroconis gallopava***	***Dactylaria constricta* var. *constricta* (not covered in MIF)**
Colony morphology	Cottony white to gray or brown; reverse white, becoming gray or black	Cottony, light gray to black; reverse gray to black	Woolly and dark, olive-gray, reddish brown, or gray-black; reverse dark with a red to brown diffusible pigment	Woolly and dark, olive-gray, reddish brown, or gray-black; reverse dark with a red to brown diffusible pigment
Microscopic morphology	Graphium may be present  **Asexual** **Sexual**			

(continued)

Fungal Diagnosis

Table 6.15 *Scedosporium* and *Dactylaria* species[a] *(continued)*

Diagnostic characteristics	***Scedosporium* spp. complex**	***Scedosporium prolificans***	***Ochroconis gallopava***	***Dactylaria constricta* var. *constricta* (not covered in MIF)**
Gelatin hydrolysis in <7 days	NA	NA	+	—
Cycloheximide tolerance	+ (the sexual stage may be inhibited by cycloheximide)	—	—	+
Growth at 37–45°C	37°C	45°C		

[a]Illustrations from D. H. Larone, *Medically Important Fungi, a Guide to Identification,* 4th ed., ASM Press, Washington, D.C., 2002.

SECTION 7

Parasitic Diagnosis

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Diagnosis of most parasitic infections has traditionally been made by the microscopic examination of clinical material, necessitating that highly trained technologists spend a significant amount of time examining individual specimens. In most developed countries, the prevalence of parasitic infection is very low, and as a result, many laboratories send their parasitic testing to large referral laboratories where expertise is centralized and competency can more easily be maintained. For the detection of more common parasites, immunoassays have been developed (e.g., *Entamoeba histolytica, Giardia duodenalis,* and *Cryptosporidium parvum*). However, these tests are adjuncts to the microscopic examination of specimens for ova and parasites and can rarely replace microscopy. Likewise, a number of tests have been developed to detect parasite-specific nucleic acids, and recently, these assays have been FDA cleared for direct detection from stool.

This section summarizes the tests currently available for the laboratory diagnosis of the most common parasitic infections. For additional information, the reader is referred to the *Manual of Clinical Microbiology*, 11th ed., 2015, and Garcia's *Diagnostic Medical Parasitology*, 4th ed., 2001.

Table 7.1 Detection methods for parasites[a]

Parasite	Culture	Microscopy	Antigen detection	Antibody detection	Molecular diagnostics
Free-living amebae					
Acanthamoeba	A	A	D	D	C
Naegleria	D	A	D	D	C
Intestinal and urogenital protozoa					
Balantidium coli	D	A	D	D	D
Blastocystis hominis	B	A	D	D	D
Cryptosporidium parvum	D	A	A	D	B
Cyclospora cayetanensis	D	A	D	D	B
Dientamoeba fragilis	D	A	C	D	D
Entamoeba histolytica/dispar	D	A	A	B	B
Giardia duodenalis	D	A	A	D	B
Isospora belli	D	A	D	D	C
Trichomonas vaginalis	A	A	A	D	A
Blood and tissue protozoa					
Babesia	D	A	D	B	C
Leishmania	B	A	D	C	C
Plasmodium	C	A	B	C	C
Toxoplasma gondii	C	B	D	A	B
Trypanosoma	C	A	C	A	C

(continued)

Parasitic Diagnosis

Table 7.1 Detection methods for parasites[a] *(continued)*

Parasite	Culture	Microscopy	Antigen detection	Antibody detection	Molecular diagnostics
Microsporidia					
Many genera	D	A	D	D	C
Helminths—nematodes					
Ancylostoma duodenale	D	A	D	D	D
Ascaris lumbricoides	D	A	D	D	D
Brugia spp.	D	A	C	C	C
Capillaria philippinensis	D	A	D	D	D
Dracunculus medinensis	D	A	D	D	D
Enterobius vermicularis	D	A	D	D	D
Loa loa	D	A	C	C	C
Mansonella perstans	D	A	C	C	C
Necator americanus	D	A	D	D	D
Onchocerca volvulus	D	A	D	D	D
Strongyloides stercoralis	D	A	C	C	C
Toxocara canis	D	D	D	A	D
Trichinella spiralis	D	A	D	A	C

Trichuris trichiura	D	A	D	D	D
Wuchereria bancrofti	D	A	C	C	C
Helminths—trematodes					
Clonorchis sinensis	D	A	D	D	D
Fasciola hepatica	D	A	D	D	D
Fasciolopis buski	D	A	D	D	D
Paragonimus westermani	D	A	D	C	D
Schistosoma spp.	D	A	C	C	C
Helminths—cestodes					
Diphyllobothrium latum	D	A	D	D	D
Dipylidium canium	D	D	D	D	D
Echinococcus granulosus	D	D	D	A	D
Echinococcus multilocularis	D	D	D	A	D
Hymenolepis diminuta	D	A	D	D	D
Hymenolepis nana	D	A	D	D	D
Taenia saginata	D	A	D	D	D
Taenia solium	D	A	D	A	D

[a]A, test is generally useful; B, test is useful under certain circumstances; C, test is seldom used for general diagnosis but may be available in reference laboratories; D, test is generally not used for laboratory diagnosis

Parasitic Diagnosis

Acid-Fast Trichrome Chromotrope Stain

The acid-fast trichrome chromotrope stain is used to detected microsporidia, *Cryptosporidium, Cyclospora*, and *Isospora*. Specimens are stained with carbol fuchsin followed by Didier's trichrome solution (Chromotrope 2R, aniline blue, and phosphotungstic acid in acetic acid) and then washed with acid-alcohol followed by 95% ethanol. *Cryptosporidium*, *Cyclospora*, and *Isospora* stain bright pink or violet, and microsporidia appear pink.

Calcofluor White Stain

Calcofluor white binds to cellulose and chitin; it fluoresces best when exposed to long-wavelength UV light. Free-living amebae (i.e., *Acanthamoeba*, *Balamuthia*, and *Naegleria*) and larvae of *Dirofilaria* fluoresce.

Delafield's Hematoxylin Stain

Delafield's hematoxylin stain is used for thin and thick blood films for the detection of microfilaria. Structural detail (e.g., nuclei and sheaths) may show greater detail than with Giemsa or Wright's stains. This stain is not commercially available and so is typically used only in specialty laboratories.

Direct Fluorescent-Antibody Stain

A variety of organisms (e.g., *Cryptosporidium parvum* and *Giardia duodenalis*) are detected directly in clinical specimens by using specific fluorescein-labeled antibodies. The labeled antibodies bind to the organisms and fluoresce green under UV light. The sensitivity and specificity of the stain are determined by the quality of the antibodies used in the reagents. Optimal detection of fluorescence requires the use of either a 420- to 490-nm (wide band) or 470- to 490-nm (narrow band) excitation filter and a 510- to 530-nm barrier filter.

Giemsa Stain

Giemsa stain and Wright's stain are modifications of Romanowsky stain, which combines methylene blue and eosin. Both stains are used for the detection of blood parasites (e.g., *Plasmodium*, *Babesia*, and *Leishmania*). A protozoan trophozoite has a red nucleus and gray-blue cytoplasm.

Iron Hematoxylin Stain

Iron hematoxylin stain is used for the detection and identification of fecal protozoa. Helminth eggs and larvae generally retain too much stain and are more easily identified with wet-mount preparations. Iron hematoxylin stain can be applied to either fresh stool specimens or ones preserved with polyvinyl alcohol or a similar preservative. Formalin-fixed specimens cannot be used.

Lugol's Iodine Stain

Iodine is added to "wet" preparations of parasitology specimens to enhance the contrast of the internal structures (e.g., nuclei and glycogen vacuoles). One disadvantage of this method is that protozoa are killed by the iodine and hence motility cannot be observed.

Modified Acid-Fast Stain

Acid-fast stains are used for detecting *Cryptosporidium*, *Cyclospora*, and *Isospora*. Because the protozoa can be readily decolorized, a weak acid-alcohol solution is used for removing the basic carbol fuchsin from non-acid-fast organisms. Organisms that retain this modified stain are referred to as being partially acid-fast.

Modified Acid-Fast Stains (Weber Green, Ryan Blue)

The trichrome stain has been modified specifically for the detection of microsporidia. A higher concentration of dye and longer staining time are used to facilitate the staining of microsporidia. Weber Green stains the organisms pink with a green background, whereas the Ryan Blue also stains the organisms pink but with a blue background.

Trichrome Stain

The trichrome stain, like the iron hematoxylin stain, is a permanent stain that is used for the detection and identification of protozoa. The stain consists of a solution of three dyes (Chromotrope 2R, light green SF, and fast green FCF) in phosphotungstic acid and acetic acid. When staining is done properly, the specimen background is green and the protozoa have a blue-green to purple cytoplasm with red or purple-red nuclei, chromatoid bodies, erythrocytes, and bacteria. Parasite eggs and larvae usually stain red.

Wright's Stain

Wright's stain is a polychromatic stain that contains a mixture of methylene blue, azure B (from the oxidation of methylene blue), and eosin Y dissolved in methanol. The eosin ions are negatively charged and stain the basic components of cells orange to pink, whereas the other dyes stain the acidic cell structures various shades of blue to purple.

Specific Diagnostic Tests

Free-Living Amebae

Acanthamoeba. Chronic granulomatous amebic encephalitis, caused by several species of *Acanthamoeba*, can be diagnosed by microscopic examination of Giemsa- or trichrome-stained brain tissue and, rarely, cerebrospinal fluid (CSF). *Acanthamoeba* keratitis is diagnosed by direct microscopic examination of corneal scrapings or by culture of the specimen. Nucleic acid amplification (NAA) tests and serologic testing have been used only in research laboratories.

Naegleria. Primary meningoencephalitis, caused by *Naegleria fowleri*, is diagnosed by microscopic examination of Giemsa- or trichrome-stained brain tissue or detection of mobile trophozoites in CSF. Giemsa or trichrome staining can be performed on CSF, but Gram stains are unreliable (giving false-positive and false-negative results). NAA tests and serologic testing have been used only in research laboratories.

Intestinal and Urogenital Protozoa

Balantidium coli. *B. coli* is best detected by wet mount examination of stool specimens. The organism tends to overstain with trichrome stains and may be misidentified. *B. coli* ciliated and the trophozoites have a rapid rotary motion, which makes them easy to miss as they move across a field of view.

Blastocystis hominis. The role of *B. hominis* in human disease remains controversial. The protozoa can be detected by microscopic examination (iodine wet mount, trichrome, or direct fluorescent antibody [DFA] assay) or antigen tests (enzyme immunoassay [EIA]) of fecal specimens collected from symptomatic and asymptomatic individuals. Serologic testing is not useful because prolonged exposure is required before an antibody response is detected.

Cryptosporidium parvum. *C. parvum* infections are diagnosed by examining fecal specimens. This protozoon does not stain adequately with iodine or with permanent stains (trichrome or iron hematoxylin). It can be recognized by using a wet mount; however, modified acid-fast stains or the DFA test is more sensitive and specific. EIAs and lateral flow assays now commercially available for the rapid detection of *C. parvum* have sensitivities and specificities approaching 100%. Multiplex, PCR-based, syndromic panels now include the detection of *C. parvum*. Some laboratories are adopting syndromic gastrointestinal panels to replace routine stool culture. This practice is somewhat controversial but may have the benefit of detecting more *C. parvum* disease, which has historically been difficult to detect without the order of a specific diagnostic test. Serologic testing has not been used for diagnostic purposes.

Cyclospora cayetanensis. *C. cayetanensis* is detected by the microscopic examination of fecal specimens. The protozoon does not stain well with iodine, Giemsa, trichrome, or chromotrope stains. It is most commonly detected by using a modified acid-fast stain, although even the experienced parasitologist can find it difficult to identify this organism with the acid-fast stain. *Cyclospora* will also autofluoresce under UV epifluorescence (green with 450- to 490-nm excitation filter; blue with 365-nm excitation filter), which can greatly enhance one's ability to detect the organism. PCR-based NAA tests are now commercially available but thus far only exist on large multiplex pan-

els designed to be tested from stool specimens. Serologic testing is not used for diagnostic purposes.

Dientamoeba fragilis. Microscopic examination of a concentrated, permanently stained specimen is the method of choice to diagnose *D. fragilis* infections. Stool antigen detection assays have been developed but are not commonly used. This may be due to the historical controversy over whether *D. fragilis* is a true pathogen.

Entamoeba histolytica/dispar. Microscopy cannot reliably differentiate between *E. histolytica* (pathogenic) and *E. dispar* (nonpathogenic) unless erythrocytes are detected in the cytoplasm of *E. histolytica* trophozoites (an uncommon finding). These two protozoa are detected by examining clinical specimens (e.g., feces, tissue biopsy specimens, and abscess aspirates) using wet mount or permanent stains. A number of antigen detection tests (EIAs) can be used to identify *E. histolytica.* These tests are now more sensitive and specific than microscopy. PCR-based NAA tests are also available for the detection and identification of *E. histolytica.* Thus far, commercially available NAA tests are unable to differentiate between *E. histolytica* and *E. dispar.* Tests that include these organisms are typically multiplexed to varying degrees. *E. histolytica/dispar* can be found on both large gastrointestinal panels, which include bacteria, viruses, and parasites, or on smaller panels which include on parasites. Serologic testing is valuable for the diagnosis of extraintestinal infections because cysts or trophozoites may not be detected in stool specimens. Indirect hemagglutination (IHA) is the reference test. The Centers for Disease Control and Prevention (CDC) recommend the use of 1:256 as a criterion for a positive IHA serologic result. This level identifies 95% of patients with extraintestinal infections, 70% of patients with active disease localized to the intestines, and 10% of asymptomatic intestinal carriers. Positive titers may persist for years after successful therapy. EIA is a sensitive assay, which identifies significantly more patients with hepatic disease than does IHA. No cross-reactions with other amoebas are observed. Detection of immunoglobulin M (IgM) antibodies is insensitive, even for patients with active invasive disease (positive in only 65% of these patients).

Giardia duodenalis. Microscopic examination of fecal specimens (wet mount, permanent stain, or DFA test [cyst specific]) for *G. duodenalis* trophozoites and cysts is used to establish infection. Antigen detection by EIA and lateral flow are used extensively and are more sensitive and specific than microscopic methods. Antigen detection assays have the benefit of detecting both cysts and trophozoites (preferred test). PCR-based NAA methods have been developed. *Giardia* can be found on both large gastrointestinal PCR panels, which include bacteria, viruses, and parasites, or on smaller panels which include parasites.

Cystisospora belli. As with *Cryptosporidium* and *Cyclospora*, the most common method used to detect *C. belli* in fecal specimens is

the modified acid-fast stain. NAA tests are restricted to research laboratories, and serologic testing is not useful for diagnosis.

Trichomonas vaginalis. *Trichomonas vaginalis* is now recognized as one of the most common sexually transmitted diseases. Historically, *T. vaginalis* infections have most commonly been diagnosed by microscopic examination (wet mount, DFA) of vaginal and urethral discharges, prostatic secretions, and urine sediments. The sensitivity of a microscopic examination is between 50 and 70%. If microscopy is negative, culture can be performed to enhance sensitivity (>80%) and is considered the "gold standard." A limitation of microscopic and culture-based diagnostic methods is that rapid transport is required to maintain organism viability. Antigen detection with lateral flow assays is an alternative diagnostic method that obviates the need for rapid specimen transport because organism viability is not required. More recently, NAA tests have been developed and are being adopted due to the superior sensitivity and rapid turnaround times. Serologic testing is not useful.

Blood and Tissue Protozoa

***Babesia* spp.** *Babesia* infections are most commonly diagnosed by detecting parasitized erythrocytes in Giemsa-stained thin films of peripheral blood. For patients with low-grade parasitemia or inconclusive peripheral smears, serologic testing can be helpful, although in practice this is rarely performed. Antibody titers in the immunofluorescent-antibody (IFA) test rise rapidly during the first weeks of disease to 1:1,024 or higher and then gradually decline over the next 6 months. Low but detectable titers may persist for 1 year or more. Elevated antibody titers may be present in healthy individuals living in areas of endemic infection. Therefore, a positive serologic test result should be confirmed by detection of the parasite in blood smears. Cross-reactivity among *Babesia* species is variable; therefore, regional differences in serologic reactivity may be observed.

***Leishmania* spp.** Leishmaniasis is diagnosed by detection of amastigotes in clinical specimens or promastigotes in culture. Specimens should be collected from the margin of the lesion by aspiration, scraping, or punch biopsy. Tissue is used to make touch preparations and is submitted for histopathologic examination. Amastigotes are found in macrophages in Giemsa-stained preparations. PCR-based NAA tests have been developed to identify specific *Leishmania* species in tissue biopsy specimens. Specimens can also be cultured in Schneider's *Drosophila* medium supplemented with 30% fetal bovine serum. Although this is a sensitive procedure, cultures must be held for 4 weeks or longer. Serologic tests, including the IFA test, enzyme-linked immunosorbent assay (ELISA), and immunoblot (IB) test, have been developed for diagnosis but are available only in reference laboratories and at the CDC.

***Plasmodium* spp**. Malaria is most commonly diagnosed by detecting parasitized erythrocytes in Giemsa-stained thick and thin films of peripheral blood. If blood is collected with anticoagulant, EDTA but not heparin should be used. Examination of thick films is the most sensitive microscopic method, but identification of the *Plasmodium* species requires examination of thin films. Acridine orange has also been used to stain blood films. This method is sensitive, but species identification is difficult. PCR-based NAA tests have been developed and can identify *Plasmodium* at the species level. These NAA tests are at least as sensitive as examination of thick films but are currently restricted to reference laboratories. Antigen detection tests specific for *P. falciparum* histidine-rich protein 2 (HRP-2) and parasite lactate dehydrogenase (LDH) specific for *P. falciparum* and non-*P. falciparum* plasmodia are commercially available. These assays have poor sensitivity for low-parasitemia infections such as those typically seen with non-falciparum infection. However, despite limitations in sensitivity, antigen testing can offer a rapid preliminary result in settings where experienced parasitologists are not available. Although species-specific serologic tests have been developed, there is extensive cross-reactivity among *Plasmodium* species, and these tests have not been used for diagnostic purposes.

***Toxoplasma gondii*.** Microscopic examination of tissues and fluids is generally unrevealing, although tachyzoites and cysts may be observed in Giemsa-stained specimens. Additionally, parasites can be recovered by inoculating mice or cell cultures, but this also has a low yield. EIA antigen tests are insensitive and are not recommended. A PCR test has been developed and is useful for confirmation of congenital infections and for testing the CSF of patients with characteristic ring-enhancing lesions seen on imaging. This test is less useful for other forms of toxoplasmosis and is typically only available through reference laboratories. Serologic testing is the method of choice for the diagnosis of toxoplasmosis. A variety of commercial tests are available (IFA test, EIA, and agglutination) for measuring the IgM and IgG response to toxoplasma. Care must be used when tests using different assay methods are compared. For the diagnosis of an acute acquired infection, an IgG IFA test or EIA should be performed. If the test is negative in an immunocompetent person, the diagnosis is excluded. Detection of IgM antibodies or a fourfold or greater increase in the level of IgG antibodies (rarely observed) is consistent with an acute infection. The following describes serological testing for Toxoplasma in several important clinical scenarios.

Pregnancy: Immunocompetent women who have detectable IgG antibody before becoming pregnant are essentially immune, and there is low risk of transmitting the organism to the fetus. Seronegative women are at risk for transmitting the organism to the fetus and in some countries are testing monthly for the development of IgG antibody. If a woman is tested for the first time after becoming pregnant

and has IgG antibody, she should be tested for IgG and IgM avidity to determine whether the acute infection occurred during pregnancy.

Newborns: An attempt to isolate the organism from the placenta should be made as ~95% of placentas of untreated, congenitally infected newborns. The child's serum should be tested for total antibody as well as IgG, IgM, and IgA specific antibodies. CSF should also be analyzed with serologies as well as direct examination for *T. gondii* tachyzoites. Persistent increasing IgG titers in the infant as compared to the mother are diagnostic for congenital infection. Detection of parasite-specific DNA by PCR in amniotic fluid is also definitive evidence of disease.

Ocular infections: Ocular infections can be diagnosed by demonstrating local production of antibody or detection of parasite DNA.

Immunocompromised patients: Most infections in immunocompromised patients represent reactivation disease. IgM antibody is usually not detected, and IgG antibody titers are consistent with chronic infections. Diagnosis is typically confirmed by detection of parasites or Toxoplasma DNA in tissue biopsy specimens or aspirated fluids.

Trypanosoma brucei. African trypanosomiasis is diagnosed by detection of trypanomastigotes in blood, lymph node aspirates, sternum bone marrow, or CSF. Parasites are present in the blood during febrile periods but are found in only small numbers when the patient is afebrile. Thick and thin films, as well as buffy coat cells, should be examined using the Giemsa stain. CSF should be concentrated before examination. ELISA has been used to detect parasitic antigens in serum and CSF. PCR-based NAA tests have also been developed in reference laboratories. Serologic tests (IFA, ELISA, IHA, and agglutination) are used for epidemiologic studies but not for diagnosis.

Trypanosoma cruzi. American trypanosomiasis (Chagas disease), caused by *T. cruzi*, is diagnosed during the acute phase of illness by detection of trypanomastigotes in Giemsa-stained peripheral blood (thick film, thin film, or buffy coat cells). Blood smears are less reliable for detection of congenital infections and chronic disease. Immunoassays for parasitic antigens in sera and urine have been used for these infections. PCR-based NAA tests have been developed but are used primarily in research laboratories. Aspirates, blood, and tissues can be cultured with samples incubated for 4 weeks or longer. Serologic tests are available in reference laboratories and at the CDC. These tests include complement fixation (CF), IFA test, IHA, and ELISA. Most tests use an epimastigote antigen, and cross-reactions occur with other trypanosomes, *Leishmania*, and *Toxoplasma*. An elevated titer cannot be used to discriminate between active and past disease.

Microsporidia

As many as 140 genera have been described for the phylum Microsporidia, with at least 7 being implicated in human disease. Diagnosis is most commonly made by examination of fecal specimens or by cytologic or histopathologic testing. Fecal smears are prepared on glass slides (concentration of specimens results in a loss of organisms) and then stained with chromotrope-based stains or chemofluorescent agents (Calcofluor white). Immunofluorescent stains have been developed but are not widely used. Microsporidial spores have been detected by cytologic examination of concentrated fluids such as bronchoalveolar lavage fluid, biliary aspirates, duodenal aspirates, and CSF. Histologic examination of biopsy specimens has also been useful. NAA tests have been developed but are restricted to research laboratories. Serologic testing is not useful for the diagnosis of human infections.

Helminths: Nematodes

Ancylostoma duodenale. Hookworm infections are diagnosed by microscopic examination of fecal specimens with a direct smear for characteristic eggs. Heavy infections (e.g., >25 eggs per coverslip) are associated with anemia. Delays in examining the specimen should be avoided because eggs can hatch in unpreserved specimens and release larval forms that can be misidentified as *Strongyloides*. Infection with other species of *Ancylostoma* (and other hookworms and *Strongyloides* species) can cause cutaneous larva migrans, where filariform larvae migrate through the skin layers and stimulate an inflammatory response. This disease is diagnosed on the basis of clinical presentation.

Ascaris lumbricoides. Roundworm infections are diagnosed by microscopic examination of fecal specimens for characteristic eggs (fertilized, decorticated, and unfertilized eggs). Fertilized eggs can be detected in a direct fecal smear or in concentrated specimens. Unfertilized eggs are not concentrated in floatation concentration methods. Adult worms may also be passed in feces or regurgitated.

***Brugia* spp.** Infections are detected by examining blood for the presence of microfilaria. Most infections consist of relatively few microfilaria in the blood, so that a large volume must be examined by either thick films or, more appropriately, concentration on a membrane filter (Knott technique). The worms are stained with Giemsa or hematoxylin. Identification of the specific microfilariae is based on their morphology (size, nuclear arrangement in the tail, and presence or absence of sheath). Antigen, antibody, and PCR-based NAA tests have also been developed for the detection of microfilarial infections. These tests are generally available through the CDC and research laboratories. Microfilariae circulate in blood in well-defined periodic cycles corresponding to the biting habits of the insect vector.

***Capillaria philippinensis*.** Diagnosis is made on the basis of microscopic detection of characteristic eggs in fecal specimens. Larvae and adults are occasionally detected.

***Dracunculus medinensis*.** Infections with the "Guinea worm" are diagnosed by recovery of the adult female worm when it migrates from the subcutaneous tissues to the skin surface. Adult male worms are small and are only rarely detected.

***Enterobius vermicularis*.** Pinworm infections are diagnosed by microscopic examination of parasite eggs collected from the perianal folds. Eggs are collected with cellulose tape or a commercial paddle, transferred to a microscope slide, and examined directly or after exposure to one drop of toluene or xylene. Multiple specimens may have to be examined.

***Loa loa*.** Refer to *Brugia. L. loa* has a diurnal periodicity and specimens should be collected at midday. Adult worms may be detected when they migrate through the conjunctivae.

***Mansonella perstans*.** Refer to *Brugia. M. perstans* has no periodicity.

***Necator americanus*.** Refer to *Ancylostoma duodenale*.

***Onchocerca volvulus*.** Adult worms live in subcutaneous tissues and deposit microfilaria in the skin tissue. Diagnosis is made by detecting the microfilaria in skin snips suspended in saline solutions. Skin snips should be collected from the scapular region or the iliac crest. Care must be used to not contaminate the specimen with blood.

Strongyloides stercoralis. Strongyloidiasis is diagnosed on the basis of microscopic examination of fecal specimens for characteristic larval forms. Eggs are rarely observed, and larvae may be scarce even in concentrated specimens, particularly in those from patients with chronic infections. Techniques developed to detect light infections include the Baermann procedure (fecal material is placed in a funnel with water, the larvae are allowed to migrate into the water, and the specimen is examined microscopically) and the agar plate method (fecal material is placed on an agar plate and then examined after 1 to 3 days for tracks of larvae migrating from the fecal mass). Multiple specimens may be needed to make the diagnosis. Adult worms, eggs, and larvae may be observed by histopathologic testing. Serologic tests (EIA and IB analysis) are available through the CDC. EIAs have a reported sensitivity between 84 and 92%. Cross-reactions can occur in patients with other nematode infections. Titers may persist, so serologic testing therefore cannot be used reliably to differentiate between current and past infections. Polymicrobial blood cultures resembling stool flora in immunocompromised patients may be an indication that the patient has *Strongyloides* hyperinfection. In these cases, the larvae migrate through the intestinal wall into the blood stream, carrying with them intestinal flora.

***Toxocara canis*.** Human ingestion of *T. canis* eggs leads to visceral larva migrans, characterized by hypereosinophilia, hepatomegaly, fever, and pneumonitis. Diagnosis is based on clinical findings and serologic testing (EIA). The test sensitivity and specificity cannot be precisely assessed because alternative methods to demonstrate infection have not been developed. However, the test sensitivity is estimated to vary from 70 to 80%, and the specificity is estimated to be >90%.

Trichinella spiralis. Trichinosis is diagnosed by demonstration of encapsulated larvae in biopsy specimens of skeletal muscle, particularly deltoid and gastrocnemius muscles. Detection of larvae may be improved by digestion of muscle tissue with an acidic solution. Detectable antibodies do not develop until 3 to 5 weeks after infection (after the acute phase of disease); their levels peak in the second or third month and then decline slowly for several years. Antibodies are detected earlier by EIA than by other methods, but EIA is less specific. Positive EIA results can be confirmed by flocculation tests.

***Trichuris trichiura*.** Diagnosis in patients with heavy infections is made by microscopic examination of a direct wet mount preparation of a fecal specimen. Concentration methods may be required to detect eggs in light infections.

Wuchereria bancrofti. Refer to *Brugia*. *W. bancrofti* has a nocturnal periodicity.

Helminths: Trematodes

***Clonorchis sinensis*.** Infections with the Oriental liver fluke are diagnosed by microscopic examination of fecal specimens for characteristic eggs.

Fasciola hepatica. Infection with the intestinal fluke, *F. hepatica*, is diagnosed by microscopic examination of fecal specimens for characteristic eggs. Serologic tests (EIA and m assay) are available through the CDC. EIA uses the excretory-secretory antigens. Specific antibodies appear within 2 to 4 weeks after infection. Sensitivity is excellent (95%); however, cross-reactivity with Schistosoma may occur. This can be resolved by using IB assays. Antibody titers fall rapidly following treatment and can be used to predict the response to therapy.

***Fasciolopis buski*.** Infections with the liver fluke, *F. buski*, are diagnosed by microscopic examination of fecal specimens for characteristic eggs.

***Paragonimus* spp**. Infections with the lung fluke, *Paragonimus*, are diagnosed by microscopic examination of fecal specimens and, less commonly, sputum for characteristic eggs. Serologic tests (EIA and m assay) are available through the CDC. EIA has a high sensitivity

and specificity, and antibody titers can be monitored to assess the response to therapy.

***Schistosoma* spp.** The three most important blood flukes that infect humans are *S. mansoni, S. japonicum*, and *S. haematobium*. They produce morphologically characteristic eggs that can be detected in fecal specimens (*S. mansoni* and *S. japonicum*) or urine (*S. haematobium*). In chronic *S. mansoni* and *S. japonicum* infections, eggs accumulate in the walls of the intestine, rectum, and liver and may be scarce in fecal specimens. Biopsy of the rectum or cecum may be required to make a diagnosis. Likewise, biopsy of the bladder wall may be required to diagnose *S. haematobium* infection. Antigen (EIA) and antibody (EIA and IB assay) tests are available through the CDC. The tests have a high sensitivity for *S. mansoni* infections but a lower sensitivity for *S. japonicum* and *S. haematobium* infections. IB analysis is used to discriminate among the Schistosoma species.

Helminths: Cestodes

Diphyllobothrium latum. Fish tapeworm infections are diagnosed on the basis of detection of characteristic eggs or proglottids in fecal specimens.

Dipylidium caninum. Infections with the dog tapeworm, *D. caninum*, are diagnosed on the basis of detection of proglottids or egg packets in fecal specimens.

Echinococcus granulosus. Diagnosis of unilocular hydatid infection is difficult but is made by detecting cysts in tissues by using imaging techniques (e.g., X-ray analysis, ultrasonic scanning, and computed tomography). Aspiration of the cyst contents is not recommended. Serologic testing (IHA, IFA tests, and EIA) is also useful. The test sensitivity ranges from 60 to 90% and is improved when a combination of tests is used. Antibody reactivity in patients is influenced by the location and integrity of the cyst. Detectable antibodies are more common in patients with cysts in the bones and liver than in those with cysts in the lungs, brain, and spleen. Seroreactivity is always lower in patients with intact cysts. False-positive reactions may occur in persons with other helminthic infections, cancer, collagen vascular disease, and cirrhosis.

Echinococcus multilocularis. As with *E. granulosus*, infection with *E. multilocularis* (multilocular hydatid infection) is difficult. Definitive diagnosis is made by histologic examination of hepatic tissue. Serologic tests (EIA) have also been developed for diagnosis of infections with *E. multilocularis.* Purified antigens are used, which has improved the test sensitivity and specificity.

***Hymenolepis diminuta*.** *H. diminuta* (mouse tapeworm) infections are diagnosed by finding characteristic eggs in fecal specimens. Proglottids are rarely observed.

Hymenolepis nana. *H. nana* (rat tapeworm) infections are diagnosed by finding characteristic eggs in fecal specimens. Proglottids are rarely observed.

Taenia saginata. Beef tapeworm infections are diagnosed by finding characteristic eggs or proglottids in fecal specimens.

***Taenia solium*.** Infections with the pork tapeworm following ingestion of cysticerci are diagnosed by finding characteristic eggs or proglottids in fecal specimens. *T. solium* eggs are also infectious for humans. Ingestion of eggs leads to cysticercosis. Cysticerci can develop in any tissue, with diagnosis made on the basis of detection of the parasite in histologic preparations or on the basis of a serologic response (EIA and bentonite flocculation). Seropositivity is reported in 50 to 70% of patients with a single cyst, 80% of patients with multiple calcified lesions, and >90% of patients with multiple, noncalcified lesions. EIAs are less sensitive than the IB assay and cross-react with antibodies specific for other helminth infections. Current tests do not differentiate between active and inactive infections.

Table 7.2 Trophozoites of common intestinal amebae[a]

Organism	Size[b] (diam or length)	Motility	Nucleus (no. and visibility)	Appearance of stained:			
				Peripheral chromatin	Karyosome	Cytoplasm	Inclusions
Entamoeba histolytica	5–60 µm; usual range 15–20 µm; invasive forms may be >20 µm	Progressive, with hyaline fingerlike pseudopodia; may be rapid	1; difficult to see in unstained preparations	Fine granules, uniform in size and usually evenly distributed; may appear beaded	Small, usually compact; centrally located but may also be eccentric	Finely granular, "ground glass," clear differentiation of ectoplasm and endoplasm; if present, vacuoles are usually small	Noninvasive organism may contain bacteria; erythrocytes, if present, are diagnostic
Entamoeba hartmanni	5–12 µm; usual range 8–10 µm	Usually nonprogressive	1; usually not seen in unstained preparations	Nucleus may stain more darkly than that of *E. histolytica,* although morphology is similar, chromatin may appear as solid ring rather than beaded	Usually small and compact; may be centrally located or eccentric	Finely granular	May contain bacteria; no erythrocytes

Entamoeba coli	15–50 µm; usual range, 8–10	Sluggish, nondirectional, with blunt, granular pseudopodin	1; often visible in unstained preparations	May be clumped and unevenly arranged on membrane; may also appear as solid dark ring with no beads or clumps	Large, not compact; may or may not be eccentric; may be diffuse and darkly stained	Granular, with little differentiation into ectoplasm and endoplasm; usually vacuolated	Bacteria, yeast cells, other debris
Entamoeba polecki	10–12 µm	Usually nonprogressive, sluggish	1; occasionally seen on a wet preparation	Fine granules; may be interspersed with large granules; evenly arranged on membrane; chromatin may also be clumped at edge of membrane	Small, usually centrally located	Finely granular	May contain ingested bacteria
Endolimax nana	6–12 µrn; usual range, 8–10 µm	Sluggish, usually nonprogressive	1; occasionally visible in unstained preparations	Usually no peripheral chromatin; nuclear chromatin may be quite variable	Large, irregularly shaped; may appear "blotlike"; many nuclear variations are common; may mimic *E. hartmanni* or *Dientamoeba fragilis*	Granular, vacuolated	Bacteria

(continued)

Table 7.2 Trophozoites of common intestinal amebae[a] *(continued)*

Organism	Size[b] (diam or length)	Motility	Nucleus (no. and visibility)	Appearance of stained:			
				Peripheral chromatin	Karyosome	Cytoplasm	Inclusions
Iodamoeba butschlii	8–20 µm; usual range, 12–15 µm	Sluggish, usually nonprogressive	1; usually not visible in unstained preparations	Usually no peripheral chromatin	Large; may be surrounded by refractile granules that are difficult to see ("basket nucleus")	Coarsely granular; may be highly vacuolated	Bacteria, yeast cells, other debris
Dientamoeba fragilis[c]	5–15 µm	Nonprogressive, pseudopodia are angular	1 (40%) or 2 (60%)	No peripheral chromatin	Karyosome clusters of 4–8 granules	Finely granular, vacuoles may be present	

[a] Data from L. S. Garcia, *Diagnostic Medical Parasitology,* 4th ed., ASM Press, Washington, D.C., 2001.

[b] Wet-preparation measurements (in permanent stains, organisms usually measure 1 to 2 um less).

[c] Now considered a flagellate, but historically considered an amoeba, thus its inclusion in this table. Also included in Table 7.4.

Table 7.3 Cysts of common intestinal amebae[a]

Organism	Size[b] (diam or length)	Shape	Nucleus (no. and visibility)	Appearance of stained: Peripheral chromatin	Karyosome	Cytoplasm chromatoidal bodies	Glycogen[c]
Entamoeba histolytica	10–20 µm; usual range, 12–15 µm	Usually spherical	Mature cyst, 4; immature, 1 or 2; characteristics difficult to see on wet preparation	Fine, uniform granules, evenly distributed; nuclear characteristics may not be as clearly visible as trophozoite	Small, compact, usually centrally located but occasionally eccentric	May be present; bodies usually elongate with blunt, rounded, smooth edges; may be round or oval	May be diffuse or absent in mature cyst; clumped chromatin mass may be present in early cysts
Entamoeba hartmanni	5–10 µm; usual range, 6–8 µm	Usually spherical	Mature cyst, 4; immature, 1 or 2; 2 nucleated cysts very common	Fine granules evenly distributed on membrane; nuclear characteristics may be difficult to see	Small, compact, usually centrally located	Usually present; bodies usually elongate with blunt, rounded, smooth edges; may be round or oval	May or may not be present, as in *E. histolytica*

(continued)

Parasitic Diagnosis

Table 7.3 Cysts of common intestinal amebae[a] *(continued)*

Organism	Size[b] (diam or length)	Shape	Nucleus (no. and visibility)	Appearance of stained: Peripheral chromatin	Karyosome	Cytoplasm chromatoidal bodies	Glycogen[c]
Entamoeba coli	10–35 µm; usual range, 15–25 µm	Usually spherical; may be oval, triangular, or other; may be distorted on permanent stained slide owing to inadequate fixative penetration	Mature cyst, 8; occasionally ≥16; immature cysts with ≥2 nuclei occasionally seen	Coarsely granular; may be clumped and unevenly arranged on membrane; nuclear characteristics not as clearly defined as in trophozoite; may resemble *E. histolytica*	Large, may or may not be compact and/or eccentric; occasionally centrally located	May be present (less frequently than in *E. histolytica*); *splinter* shaped with rough, pointed ends	May be diffuse or absent in mature cyst; clumped mass occasionally seen in mature cysts
Entamoeba polecki	5–11 µm	Usually spherical	Mature cyst, 1; may be visible in wet preparations; rarely 2 or 4 nuclei	Similar to trophozoite	Similar to trophozoite	Abundant, angular pointed ends; threadlike chromatoidal bodies may be present	May or may not be present

Endolimax nana	5–10 μm; usual range, 6–8 μm	Usually oval, may be round	Mature cyst, 4; immature cysts, 2, very rarely seen and may resemble cysts of *Enteromonas hominis*	Rarely present; small granules or inclusions are occasionally seen; fine linear chromatoidal bodies may be faintly visible on well-stained smears	Smaller than karyosome seen in trophozoites but generally larger than those of genus *Entamoeba*	No peripheral chromatin	Usually diffuse if present
Iodamoeba butschlii	5–20 μm; usual range, 10–12 μm	May vary from oval to round; cyst may collapse owing to large glycogen vacuole space	Mature cyst, 1	No peripheral chromatin	Larger, usually eccentric refractile granules may be on one side of karyosome ("basket nucleus")	None; small granules are occasionally present	Large, compact, well-defined mass
Blastocystis hominis	6–40 μm	Usually spherical	Multiple nuclei surrounding a large central body	Not observed	Not observed	Large central body dominates internal structure	Not present

[a]Data from L. S. Garcia, *Diagnostic Medical Parasitology,* 4th ed., ASM Press, Washington, D.C., 2001.
[b]Wet-preparation measurements (in permanent stains, organisms usually measure 1 to 2 pm less).
[c]Stains reddish-brown with iodine.

Parasitic Diagnosis

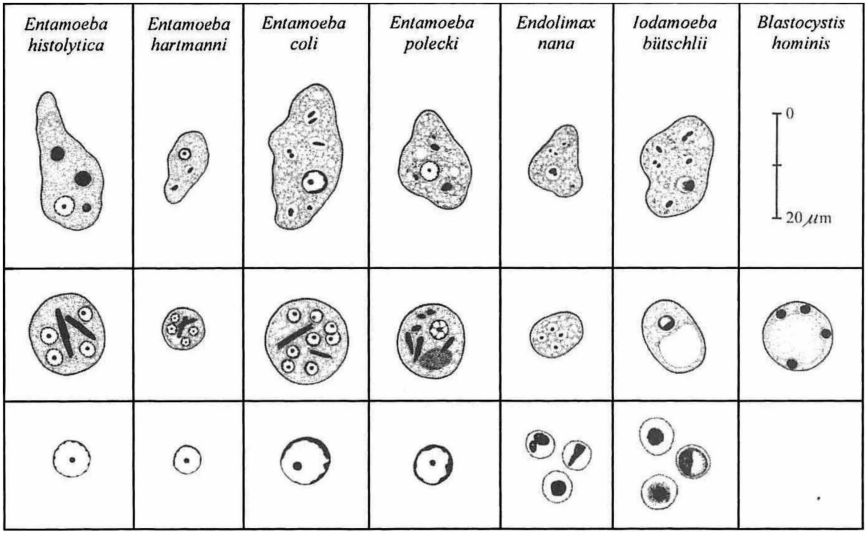

Figure 7.1 Intestinal amebae of humans. (Top row) Trophozoites. (Middle row) Cysts. (Bottom row) Trophozoite nuclei, shown in relative proportion. From Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

Table 7.4 Trophozoites of flagellates[a]

Organism	Shape and size	Motility	Nucleus (no. and visibility)	No. of flagella[b]	Other features
Dientamoeba fragilis	Shaped like amebae; 5–15 µm (usual range, 9–12 µm)	Usually nonprogressive; pseudopodia are angular, serrated, or broad lobed and almost transparent	Percentage may vary, but 40% of organisms have 1 nucleus and 60% have 2 nuclei; not visible in unstained preparations; no peripheral chromatin; karyosome is cluster of 4–8 granules	No visible flagella	Cytoplasm finely granular and may be vacuolated with ingested bacteria, yeasts, and other debris; may be great variation in size and shape on single smear
Giardia duodenalis	Pear shaped; 10–20 µm long; 5–15 µm wide	Falling-leaf motility may be difficult to see if organism is in mucus	2; not visible in unstained mounts	4 lateral, 2 ventral, 2 caudal	Sucking disk occupying 1/2–3/4 of ventral surface; pear shaped from front, spoon shaped from side
Chilomastix mesnil	Pear shaped; 6–24 µm long (usual range, 10–15 µm long), 4–8 µm wide	Stiff, rotary	1; not visible in unstained mounts	3 anterior, 1 in cytostome	Prominent cytostome extending 1/3–1/2 length of body; spiral groove across ventral surface

(continued)

Parasitic Diagnosis

Table 7.4 Trophozoites of flagellates[a] *(continued)*

Organism	Shape and size	Motility	Nucleus (no. and visibility)	No. of flagella[b]	Other features
Trichomonas hominis	Pear shaped; 5–15 µm long (usual range, 7–9 µm long), 7–10 µm wide	Jerky, rapid	1; not visible in unstained mounts	3–5 anterior, 1 posterior	Undulating membrane extends length of body; posterior flagellum extends free beyond end of body
Trichomonas vaginalis	Pear shaped; 7–23 µm long (usual range, 13 µm) 5–15 µm wide	Jerky, rapid	1; not visible in unstained mounts	3–5 anterior; 1 posterior	Undulating membrane extends 1/2 length of body; no free posterior flagellum; axostyle easily seen
Enteromonas hominis	Oval, 4–10 µm long (usual range, 8–9 µm long), 5–6 µm wide	Jerky	1; not visible in unstained mounts	3 anterior, 1 posterior	One side of body flattened; posterior flagellum extends free posteriorly or laterally
Retortamonas intestinalis	Pear shaped or oval; 4–9 µm long (usual range, 6–7 µm long), 3–4 µm wide	Jerky	1; not visible in unstained mounts	1 anterior, 1 posterior	Prominent cytostome extends approximately 1/2 length of body

[a]Data from L. S. Garcia, *Diagnostic Medical Parasitology,* 4th ed., ASM Press, Washington, D.C., 2001.
[b]Usually difficult to see.

Table 7.5 Cysts of flagellates[a]

Species	Size	Shape	Nuclei (no. and visibility)	Other features
Dientamoeba fragilis,	4–7 μm in diameter	Round	2; often fragmented into distinct granules, referred to as chromatin packets	Rarely encountered in clinical samples
Trichomonas hominis	No cyst stage	NA[b]	NA	NA
Giardia duodenalis	8–19 μm long (usual range, 11–14 μm long), 7–10 μm wide	Oval, ellipsoidal, or round	4; not distinct in unstained preparations; usually located at one end	Longitudinal fibers in cysts may be visible in unstained preparations; deeply staining median bodies usually lie across longitudinal fibers; there is often shrinkage, and cytoplasm pulls away from cyst wall; may also be "halo" effect around outside of cyst wall due to shrinkage caused by dehydrating reagents
Chilomastix mesnili	6–10 μm long (usual range, 7–9 μm long), 4–6 μm wide	Lemon shaped with anterior hyaline knob	1; not distinct in unstained preparations	Cytostome with supporting fibrils, usually visible in stained preparation; curved fibril alongside of cytostome usually referred to as "shepherd's crook"
Enteromonas hominis	4–10 μm long (usual range, 6–8 μm long), 4–6 μm wide	Elongate or oval	1–4; usually 2 lying at opposite ends of cyst; not visible in unstained mounts	Resembles *Endolimax nana* cyst; fibrils or flagella usually not seen
Retortamonas intestinalis	4–9 μm long (usual range, 4–7 μm long), 5 μm wide	Pear shaped or slightly lemon shaped	1; not visible in unstained mounts	Resembles *Chilomastix* cyst; shadow outline of cytostome with supporting fibrils extends above nucleus; bird beak fibril arrangement

[a]Data from L. S. Garcia, *Diagnostic Medical Parasitology,* 4th ed., ASM Press, Washington, D.C., 2001.

[b]NA, not applicable.

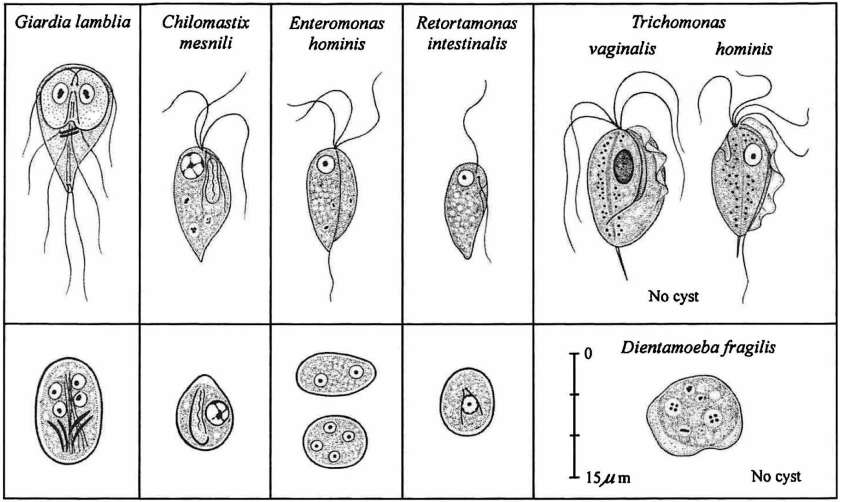

Figure 7.2 Intestinal and urogenital flagellates of humans. (Top row) Trophozoites. (Bottom row) Cysts. *D. fragilis* trophozoite is shown; a cyst stage for *D. fragilis* has recently been found but is not shown here. From Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (ed.), *Manual of Clinical Microbiology,* 11th ed., ASM Press, Washington, D.C., 2015.

Table 7.6 Morphological characteristics of ciliates, coccidia, microsporidia, and tissue protozoa[a]

Species	Shape and size	Other features
Balantidium coli	Trophozoite: ovoid with tapering anterior; 50–100 µm long; 40–70 µm wide (usual width range, 40–50 µm) Cyst: spherical or oval; 50–70 µm in diam (usual range, 50–55 µm)	Trophozoite: 1 large, kidney-shaped macronucleus; 1 small, round micronucleus, which is difficult to see even in stained smears; macronucleus may be visible in unstained preparations; body is covered with cilia, which tend to be longer near cytostome; cytoplasm may be vacuolated. Cyst: 1 large macronucleus visible in unstained preparations; micronucleus difficult to see; macronucleus and contractile vacuoles are visible in young cysts; in older cysts, internal structure appears granular; cilia difficult to see within cyst wall.
Cryptosporidium parvum	Oocyst generally round, 4–5 µm in diam; each mature oocyst contains sporozoites, which may or may not be visible	Oocyst is the usual diagnostic stage in stool. Various other stages in life cycle can be seen in biopsy specimens taken from GI tract (brush borders of epithelial cells in intestinal tract) and other tissues (respiratory tract, biliary tract).
Cyclospora cayetanensis	Organisms generally round, 8–9 µm in diam; acid-fast like *Cryptosporidium* spp., but larger	Resemble nonrefractile spheres in wet-preparation smears; autofluoresce with epifluorescence; stain variably with acid-fast stains; appear clear, round, and somewhat wrinkled in trichrome stains.

(continued)

Parasitic Diagnosis

Table 7.6 Morphological characteristics of ciliates, coccidia, microsporidia, and tissue protozoa[a] *(continued)*

Species	Shape and size	Other features
Cystisospora belli	Ellipsoidal oocyst; usual size, 20–30 µm long, 10–19 µm wide; sporocysts rarely seen out of oocysts but measure 9–11 µm	Mature oocyst contains 2 sporocysts with 4 sporozoites each; immature oocysts are usually seen in fecal specimens.
Microsporidia	Spores are extremely small and have been recovered from all body organs.	Histology results vary; acid-fast, trichrome, and Calcofluor white stains recommended for spores. Animal inoculation not recommended. Enteric infections in AIDS patients difficult to diagnose by examining stool specimens.
Toxoplasma gondii	Trophozoite (tachyzoite): crescent shaped; 4–6 µm long by 2–3 µm wide.	Diagnosis is most frequently based on clinical history and serologic evidence of infection.
	Cyst (bradyzoite); generally spherical; 200 µm to 1 mm in diam	
Sarcocystis spp.	Oocyst with thin wall contains 2 mature sporocysts, each containing 4 sporozoites; oocyst frequently ruptures; ovoid sporocysts, each 9–1 6 µm long and 7.5–12 µm wide	Thin-walled oocyst or ovoid sporocysts occur in stool.

[a]Data from L. S. Garcia, *Diagnostic Medical Parasitology,* 4th ed., ASM Press, Washington, D.C., 2001.
[b]GI, gastrointestinal.

Table 7.7 Morphological characteristics of protozoa found in blood[a]

Organism	Diagnostic stage
Malaria parasites	
Plasmodium vivax (benign tertian malaria)	Ameboid rings; presence of Schiiffner's dots; all stages seen in peripheral blood; mature schizont contains 16–18 merozoites; infects young RBCs[b]
Plasmodium ovale (ovale malaria)	Nonameboid rings; presence of Schiiffner's dots; all stages seen in peripheral blood; mature schizont contains 8–10 merozoites; RBCs may be oval and have fimbriated edges; infects young RBCs
Plasmodium malariae (quartan malaria)	Rings are thick; no stippling; all stages seen in peripheral blood; presence of band forms and rosette-shaped mature schizont; lots of malarial pigment; infects mature RBCs
Plasmodium falciparum (malignant tertian malaria)	Multiple rings; applique/accole forms; no stippling (rare Maurer's clefts); rings and crescent-shaped gametocytes seen in peripheral blood (no other developing stages, with rare exception of mature schizont); infects all RBCs
Babesia spp.	Ring forms only (resemble *P. falciparum* rings); seen in splenectomized patients; endemic in the United States (no travel history necessary); if present, "Maltese cross" configuration diagnostic
Trypanosoma brucei gambiense (West African sleeping sickness)	Trypomastigotes long and slender, with typical undulating membrane; lymph nodes and blood can be sampled; microhematocrit tube concentration helpful; examine spinal fluid in later stages of infection

(continued)

Parasitic Diagnosis

Table 7.7 Morphological characteristics of protozoa found in blood[a] *(continued)*

Organism	Diagnostic stage
Trypanosoma brucei rhodesiense (East African sleeping sickness)	Trypomastigotes long and slender, with typical undulating membrane; lymph nodes and blood can be sampled; microhematocrit tube concentration helpful; examine spinal fluid in later stages of infection
Trypanosoma cruzi (Chagas'' disease, South American trypanosomiasis)	Trypomastigotes short and stumpy, often curved in C shape; blood sampled early in infection; trypomastigotes enter striated muscle (heart, GI* tract) and transform into amastigote form
Leishmania spp. (cutaneous; not actually a blood parasite but presented for comparison with *Leishmania donovani*)	Amastigotes found in macrophages of skin; presence of intracellular forms containing nucleus and kinetoplast diagnostic
Leishmania braziliensis (mucocutaneous; not actually a blood parasite but presented for comparison with *L. donovani*)	Amastigotes found in macrophages of skin and mucous membranes; presence of intracellular forms containing nucleus and kinetoplast diagnostic
Leishmania donovani (visceral)	Amastigotes found throughout reticuloendothelial system and in spleen, liver, bone marrow, etc.; presence of intracellular forms containing nucleus and kinetoplast diagnostic

[a]Data from L. S. Garcia, *Diagnostic Medical Parasitology,* 4th ed., ASM Press, Washington, D.C., 2001.
[b]GI, gastrointestinal; RBCs, erythrocytes.

Table 7.8 Morphological characteristics of blood and tissue nematodes

Parasite	Adult worm	Microfilaria
Brugia malayi	Threadlike; males 13–25 mm by 70–80 μm; females 43–55 mm by 130–170 μm	177–230 by 5–6 μm; sheathed; tail tapered; gap between subterminal and terminal nuclei
Loa loa	Males 30–35 mm by 350–430 μm; females 50–70 mm by 500 μm	230–250 by 6–8 μm; sheathed; tail tapered; nuclei extends to tip of tail
Mansonella perstans	Males 45 mm by 60 μm; females 70–80 mm by 120 μm	190–200 by 4 μm; unsheathed; tail tapered; nuclei extends to tip of tail
Mansonella ozzardi	Threadlike; males 24–28 mm by 70–80 μm; females 32–62 mm by 130–160 μm	163–203 by 3–4 μm; unsheathed; long, slender tail with nuclei at tip
Onchocerca volvulus	Males 19–42 mm by 130–210 μm; females 33–50 mm by 270–400 μm	304–315 by 5–9 μm; unsheathed; tail tapered; nuclei not at tip of tail
Wuchereria bancrofti	Threadlike; males 40 mm by 100 μm; females 80–100 mm by 250 μm	244–296 by 7–10 μm; sheathed; tail tapered; nuclei not at tip of tail

Parasitic Diagnosis

Table 7.9 Morphological characteristics of helminths[a]

Helminth	Diagnostic stage
Nematodes (roundworms) *Ascaris lumbricoides*	Egg: both fertilized (oval to round with thick, mammillated or tuberculate shell) and unfertilized (tend to be more oval or elongate, with bumpy shell exaggerated) eggs found in stool. Adult worms: 10–12 in. (ca. 25–30 cm), found in stool. Rarely (in severe infections), migrating larvae can be found in sputum.
Trichuris trichiura	Egg: barrel shaped with two clear, polar plugs. Adult worm: rarely seen. Eggs should be quantitated (rare, few, etc.), since light infections may not be treated.
Enterobius vermicularis	Egg: football shaped with one flattened side. Adult worm: about 3/8 in. (ca. 1 cm) long, white with pointed tail. Female migrates from anus and deposits eggs on perianal skin.
Ancylostoma duodenale, Necator americanus	Egg: eggs of two species identical; oval with broadly rounded ends, thin shell, and clear space between shell and developing embryo (8–16 cell stage). Adult worms: rarely seen in clinical specimens.
Strongyloides stercoralis	Rhabditiform larvae (noninfective) usually found in stool; short buccal cavity or capsule with large, genital primordial packet of cells ("short and sexy"). In very heavy infections, larvae are occasionally found in sputum, and/or filariform (infective) larvae can be found in stool (slit in tail).
Ancylostoma braziliensis	Humans are accidental hosts. Larvae will wander through outer layer of skin, creating tracks (severe itching and eosinophilia). There are no practical microbiological diagnostic tests.
Toxocara cati or *Toxocara canis*	Humans are accidental hosts. Dog or cat ascarid eggs are ingested with contaminated soil; larvae wander through deep tissues (including eye); can be mistaken for cancer of eye; serologic tests helpful for confirmation; eosinophilia
Cestodes (tapeworms) *Taenia saginata*	Scolex (4 suckers, no hooklets) and gravid proglottid (>12 branches on single side) are diagnostic; eggs indicate *Taenia* spp. only (thick, striated shell, containing 6–hooked embryo or oncosphere); worm usually approx 12 ft (ca. 3.7 m) long
Taenia solium	Scolex (4 suckers with hooklets) and gravid proglottid (< 12 branches on single side) are diagnostic; eggs indicate *Taenia* spp. only (thick, striated shell, containing 6-hooked embryo or oncosphere); worm usually approx 12 ft (ca. 3.7 m) long

Diphyllobothrium latum	Scolex (lateral sucking grooves) and gravid proglottid (wider than long, reproductive structures in center, "rosette"); eggs operculated
Hymenolepis nana	Adult worm not normally seen; egg round to oval with thin shell, containing 6-hooked embryo or oncosphere with polar filaments lying between embryo and egg shell
Hymenolepis diminuta	Adult worm not normally seen; egg round to oval with thin shell, containing 6-hooked embryo or oncosphere with no polar filaments lying between embryo and egg shell
Echinococcus granulosus	Adult worm found only in carnivores (dog); hydatid cysts develop (primarily in liver) when humans accidentally ingest eggs from dog tapeworms; cyst contains daughter cysts and many scolices. Laboratory should examine fluid aspirated from cyst at surgery.
Echinococcus multilocularis	Adult worm found only in carnivores (fox or wolf); hydatid cysts develop (primarily in liver) when humans accidentally ingest eggs from carnivore tapeworms. Cyst grows like metastatic cancer with no limiting membrane.
Trematodes (flukes) *Fasciolopsis bush*	Eggs found in stool; very large and operculated (morphology like that of *F. hepatica* eggs)
Fasciola hepatica	Eggs found in stool; cannot be differentiated from those of *F. buski*
Clonorchis (Opisthorchis) sinensis	Eggs found in stool; very small (<35 um); operculated, with shoulders into which operculum fits
Paragonimus westermani	Eggs coughed up in sputum (brownish "iron filing" = egg packets); can be recovered in sputum or stool (if swallowed); eggs operculated, with shoulders into which operculum fits
Schistosoma mansoni	Eggs recovered in stool (large lateral spine); specimens should be collected with no preservatives (to maintain egg viability); worms in veins of large intestine
Schistosoma haematobium	Eggs recovered in urine (large terminal spine); specimens should be collected with no preservatives (to maintain egg viability); worms in veins of bladder
Schistosoma japonicum	Eggs recovered in stool (very small lateral spine); specimens should be collected with no preservatives (to maintain egg viability); worms in veins of small intestine

[a]Data from L. S. Garcia, *Diagnostic Medical Parasitology,* 4th ed., ASM Press, Washington, D.C., 2001.

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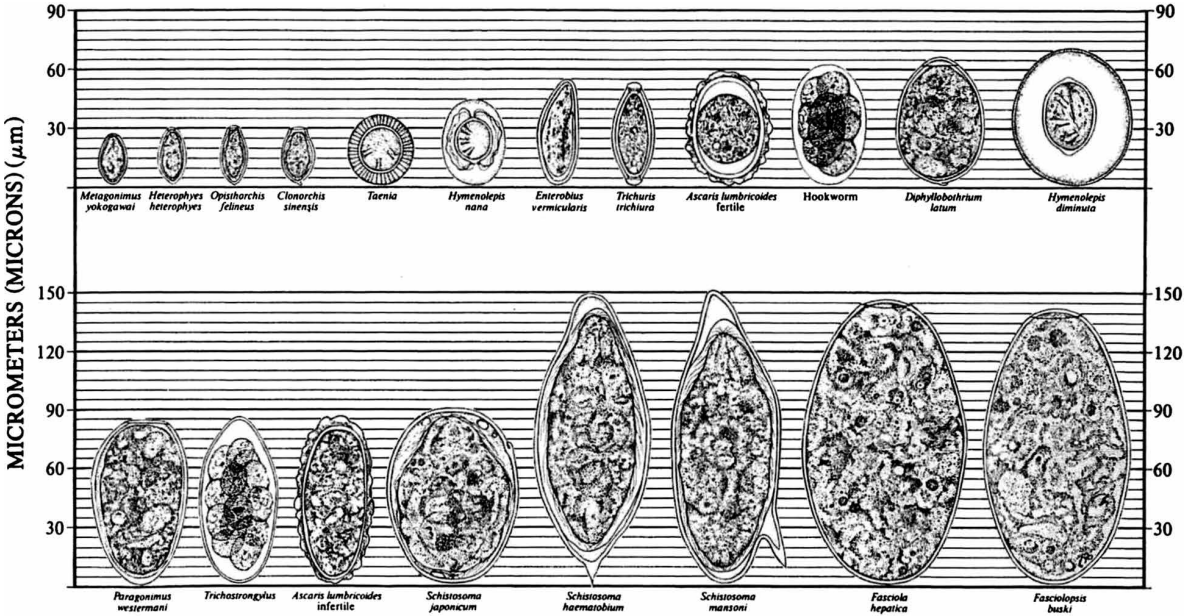
MICROMETERS (MICRONS) (μm)
90
60
30
150
120
90
60
30
Metagonimus yokogawai
Heterophyes heterophyes
Opisthorchis felineus
Clonorchis sinensis
Taenia
Hymenolepis nana
Enterobius vermicularis
Trichuris trichiura
Ascaris lumbricoides fertile
Hookworm
Diphyllobothrium latum
Hymenolepis diminuta
Paragonimus westermani
Trichostrongylus
Ascaris lumbricoides infertile
Schistosoma japonicum
Schistosoma haematobium
Schistosoma mansoni
Fasciola hepatica
Fasciolopsis buski

Figure 7.3 Relative sizes of helminth eggs (from CDC). *Schistosoma mekongi* and *Schistosoma intercalatum* have been omitted. From M. Brooke and D. Melvin, *Morphology of Diagnostic Stages of Intestinal Parasites of Humans*, 2nd ed., U.S. Department of Health and Human Services publication (CDC) 84-8116, Centers for Disease Control and Prevention, Atlanta, GA, 1984.

SECTION 8

Vaccines, Susceptibility Testing, and Methods of Organism Identification

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Two important control measures for infectious diseases are vaccination to prevent infection and use of antimicrobial therapy to eradicate infections. This section provides information for both approaches. Tables 8.1 and 8.2 summarize immunization recommendations for pediatric and adult patients. These recommendations are published periodically in the *Morbidity and Mortality Weekly Report* and at the Centers for Disease Control and Prevention (CDC) website (hppt://www.cdc.gov/nip). The tables are a summary of the recommendations of the Advisory Committee on Immunization Practices (ACIP), the American Academy of Family Physicians (AAFP), the American College of Obstetricians and Gynecologists (ACOG), the American College of Physicians-American Society of Internal Medicine (ACP-ASIM), and the Infectious Diseases Society of America (IDSA).

Information regarding antimicrobial agents is intended to be used as a quick reference guide for the practicing clinical microbiologist. Included within are tables that outline common methods of antimicrobial susceptibility testing, key information regarding clinical breakpoint criteria as presented by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). These tables should help the clinical microbiologist easily determine what breakpoints exist for commonly encountered organisms as well as commonly used antimicrobials. Lastly, several tables regarding intrinsic mechanisms of resistance and important mechanisms of resistance have been included. A notable difference from previous editions of the Pocket Guide is the omission of antibiogram tables. Due to the regional differences seen in antimicrobial resistance, it was determined that it would be impossible to present generalizable data that would be relevant to all users of this Pocket Guide. Readers are encouraged to consult contemporary data published by the numerous ongoing surveillance programs that present antibiogram data that is organism, patient, and region-specific.

Table 8.1 Recommended pediatric immunization schedule[a]

Vaccine	Minimum age for first dose	Recommended age for this dose	Recommended interval before next dose	Minimum interval before next dose
Hepatitis B	Birth			
First dose		Birth-2 mo	1–4 mo	4 wk
Second dose		1–4 mo	2–17 mo	8 wk
Third dose		6–18 mo		
Rotavirus	6 wk			
First dose		2–4 mo	8 wk	4 wk
Second dose		4–6 mo	8 wk	4 wk
Third dose		6–9 mo		
Diphtheria-tetanus-pertussis	6 wk			
First dose		2 mo	2 mo	4 wk
Second dose		4 mo	2 mo	4 wk
Third dose o		6 mo	6–12 mo	6 mo
Fourth dose		15–18 mo	3 yr	6 mo
Fifth dose		4–6 yr		
Haemophilus influenzae **type b**	6 wk			
First dose		2 mo	2 mo	4 wk
Second dose		4 mo	2 mo	4 wk
Third dose		6 mo	6–9 mo	8 wk
Fourth dose		12–15 mo		

(continued)

Table 8.1 Recommended pediatric immunization schedule[a] *(continued)*

Vaccine	Minimum age for first dose	Recommended age for this dose	Recommended interval before next dose	Minimum interval before next dose
Inactivated poliovirus	6 wk			
First dose		2 mo	2 mo	4 wk
Second dose		4 mo	2–14 mo	4 wk
Third dose		6–18 mo	3.5 yr	6 yr
Fourth dose		4–6 yr		
Pneumococcal	6 wk			
First dose		2 mo	2 mo	4 wk
Second dose		4 mo	2 mo	4 wk
Third dose		6 mo	6 mo	8 wk
Fourth dose		12–15 mo		
Measles, mumps, rubella	12 mo			
First dose		12–15 mo	3–5 yr	4 wk
Second dose		4–6 yr		

Varicella	12 mo			
First dose		12–15 mo	3–5 yr	12 wk
Second dose		4–6 yr		
Hepatitis A	12 mo			
First dose		12–23 mo	6–18 mo	6 mo
Second dose		18 mo		
Influenza	6 mo		4 wk	4 wk
Human Papillomavirus	9 yr			
First dose		11–12 yr	8 wk	4 wk
Second dose		11–12 yr (+2 mo)	4 mo	12 wk
Third dose		11–12 yr (+6 mo)		

[a]Data from Centers for Disease Control and Prevention, Advisory Committee on Immunization Practices: General best practices for immunization. September 2017

Table 8.2 Recommended adult immunization schedule[a]

	Age group (yr)		
Vaccine	**19–49**	**50–64**	**>65**
Tetanus/diphtheria	One booster dose every 10 yr	One booster dose every 10 yr	One booster dose every 10 yr
Influenza	One dose annually	One dose annually	One dose annually
Pneumococcal (PCV13 and PPSV23)	Recommended for those with certain medical conditions. One dose of PCV13 and 1, 2, or 3 doses of PPSV23. PCV13 should be administered first and PPSV23 should not be administered during the same visit. There should be a 5-year interval between PPSV23 doses.	Same as for 19–49-yr age group	Immunocompetent adults should receive PCV13 followed by PPSV23 at least 1 year after PCV13
Hepatitis A	Two or three doses (depending on vaccine) for persons with medical, behavioral, occupational, or other indications. Timing vaccine dependent.	Same as for 19–49-yr age group	Same as for 19–49-yr age group
Hepatitis B	Three doses (at 0, 1, and 6 mo intervals) for persons with medical, behavioral, occupational, or other indications	Same as for 19–49-yr age group	Same as for 19–49-yr age group

Measles-mumps-rubella (MMR)	One dose if vaccination history is unreliable; two doses for persons with occupational, geographic, or other indications	Up to 60 yr, same as for 19–49 yr age group. Not indicated for >60 yr	Not indicated
Varicella	Two doses (1 to 2 mo apart) for susceptible persons (without a reliable history of natural disease, vaccination, or antibody response)	Same as for 19–49-yr age group	Same as for 19–49-yr age group
Herpes Zoster	Not recommended	Adults >60 yr should receive one dose of herpes zoster vaccine regardless of whether they have had a prior episode of herpes zoster.	Adults >60 yr should receive one dose of herpes zoster vaccine regardless of whether they have had a prior episode of herpes zoster.
Meningococcal polysaccharide (MenACWY or MPSV4)	One or more dose for persons with medical or other indications	Same as for 19–49-yr age group	Same as for 19–49-yr age group
HPV—female	Three doses through age 26 yr at 0, 1–2 mo, and 6 months	Not recommended	Not recommended
HPV—male	Three doses through age 21 yr at 0, 1–2 mo, and 6 months	Not recommended	Not recommended

[a]Data from Centers for Disease Control and Prevention, Recommended Immunization Schedule for Adults Aged 19 Years or Older, United States, 2017 (https://www.cdc.gov/vaccines/schedules/downloads/adult/adult-combined-schedule.pdf).

Antimicrobial Agents

Table 8.3 Clinical and Laboratory Standards Institute (CLSI) documents related to antimicrobial susceptibility testing from humans[a]

No.	Title
M2-A12	Performance Standards for Antimicrobial Disk Susceptibility Tests (2015)
M7-A10	Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically (2015)
M11-A8	Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria (2012)
M23-ED4	Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters (2016)
M24-A2	Antimycobacterial Susceptibility Testing of *Mycobacterium, Nocardiae,* and Other Aerobic Actinomycetes (2011)
M26-A	Methods for Determining Bactericidal Activity of Antimicrobial Agents (1999)
M27-ED4	Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts (2017)
M38-ED3	Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi (2017)
M39-A4	Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data (2014)
M43-A	Methods for Antimicrobial Susceptibility Testing for Human Mycoplasmas (2011)
M44-A2	Method of Antifungal Disk Diffusion Susceptibility Testing of Yeasts (2009)
M45-ED3	Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria (2016)
M51-S1	Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi (2010)
M52-ED1	Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems (2015)
M57-ED1	Principles and Procedures for the Development of Epidemiological Cutoff Values for Antifungal Susceptibility Testing (2016)
M59-ED1	Epidemiological Cutoff Values for Antifungal Susceptibility Testing (2016)
M60-ED1	Performance Standards for Antifungal Susceptibility Testing of Yeasts (2017)

Table 8.3 Clinical and Laboratory Standards Institute (CLSI) documents related to antimicrobial susceptibility testing from humans[a] *(continued)*

No.	Title
M61-ED1	Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi (2017)
M100-S27	Performance Standards for Antimicrobial Susceptibility Testing (2017)
SC21-L	Susceptibility Testing (collection of documents: M2, M7, M11, M21, M24, M27, M31, and M100)

[a]Documents available from CLSI (950 West Valley Road, Suite 2500, Wayne, PA 19087; telephone, 610-688-0100; FAX, 610-688-0700; website, http://www.clsi.org).

Table 8.4 Summary of CLSI antimicrobial susceptibility test methods for select bacteria, mycobacteria, and fungi[a]

Organism (Document)	Test method	Medium	Inoculum	Incubation conditions
Staphylococcus spp.	Disk diffusion	MHA	Direct	Air; 16–18 h (24 h CONS and cefoxitin); 35°C
(M100)	Broth/agar dilution	CAMHB/MHA (oxac + 2% NaCI) (supplement w/ 50 µg/ml calcium for daptomycin)	Direct	Air; 16–20 h (oxac, vanco: 24 h); 35°C
	Agar screen (*S. aureus*)	MHA + 2% NaCI + oxac	Direct	Air; 24 h; 35°C
Streptococcus pneumoniae	Disk diffusion	MHA + 5% sheep blood	Direct	5% CO_2; 20–24 h; 35°C
(M100)	Broth dilution	CAMHB + 2–5% LHB (supplement w/ 50µg/ml calcium for daptomycin)	Direct	Air; 20–24 h; 35°C
Streptococcus, other spp.	Disk diffusion	MHA + 5% sheep blood	Direct	5% CO_2; 20–24 h; 35°C
(M100)	Broth dilution	CAMHB + 2–5% LHB (supplement w/ 50µg/ml calcium for daptomycin)	Direct	Air; 20–24 h; 35°C
Enterococcus spp.	Disk diffusion	MHA	Direct; broth	Air; 16–18 h (vanco: 24 h); 35°C
(M100)	Broth/agar dilution	CAMHB/MHA (supplement w/ 50 µg/ml calcium for daptomycin)	Direct; broth	Air; 16–20 h (vanco: 24 h); 35°C
	Agar screen	BHIA + gent (500 µg/ml)	Direct; broth	Air; 24 h; 35°C
		BHIA + strep (2,000 µg/ml)	Direct; broth	Air; 24–48 h; 35°C
		BHIA + vanco (6 µg/ml)	Direct; broth	Air; 24 h; 35°C

Listeria monocytogenes (M45)	Broth dilution	CAMHB + 2–5% LHB	Direct	Air; 20–24 h; 35°C
Neisseria gonorrhoeae (M45)	Disk diffusion Agar dilution	GCA + 1% supplement GCA + 1% supplement	Direct Direct	5% CO_2; 20–24 h; 36°C 5% CO_2; 20–24 h; 36°C
Neisseria meningitidis (M45)	Disk diffusion Broth/agar dilution	MHA + 5% sheep blood CAMHB + 2–5% LHB MHA + 5% sheep blood	Direct Direct	5% CO_2; 20–24 h; 35°C 5% CO_2; 20–24 h; 35°C
Haemophilus spp. (M100)	Disk diffusion Broth dilution	HTM agar HTM broth	Direct Direct	5% CO_2; 16–18 h; 35°C Air; 20–24 h; 35°C
Enterobacteriaceae (M100)	Disk diffusion Broth/agar dilution	MHA CAMHB/MHA	Direct; broth Direct; broth	Air, 16–18 h; 35°C Air, 16–20 h; *(Yersinia pestis,* 24 h); 35°C
Vibrio spp. (including *V. cholerae* (M45)	Disk diffusion Broth/agar dilution	MHA CAMHB/MHA	Direct; broth Direct; broth	Air, 16–18 h; 35°C Air, 16–20 h; 35°C
Pseudomonas aeruginosa (M100)	Disk diffusion Broth/agar dilution	MHA CAMHB/MHA	Direct; broth Direct; broth	Air, 16–18 h; 35°C Air, 16–20 h; 35°C
Acinetobacter spp. (M100)	Disk diffusion Broth/agar dilution	MHA CAMHB/MHA	Direct; broth Direct; broth	Air, 16–18 h; 35°C Air, 16–20 h; 35°C

(continued)

Antimicrobial Agents

Table 8.4 Summary of CLSI antimicrobial susceptibility test methods for select bacteria, mycobacteria, and fungi[a] *(continued)*

Organism (Document)	Test method	Medium	Inoculum	Incubation conditions
Other Non-*Enterobacteriaceae* (M100)	Broth/agar dilution	CAMHB/MHA	Direct; broth	Air; 16–20 h; 35°C
Burkholderia cepacia complex (M100)	Disk diffusion Broth/agar dilution	MHA CAMHB/MHA	Direct; broth Direct: broth	Air; 20–24 h; 35°C (all methods)
Stenotrophomonas maltophilia (M100)	Disk diffusion Broth/agar dilution	MHA CAMHB/MHA	Direct; broth Direct: broth	Air; 20–24 h; 35°C (all methods)
Abiotrophia spp. and *Granulicatella* spp. (M45)	Broth dilution	CAMHB + 2–5% LHB +0.001% pyridoxal hydrochloride	Direct	Air; 20–24 h; 35°C
Aerococcus spp. (M45)	Broth dilution	CAMHB + 2–5% LHB	Direct	5% CO_2; 20–24 h; 35°C
Aeromonas spp. (M45)	Disk diffusion Broth dilution	MHA CAMHB	Direct	Air, 16–18 h; 35°C Air, 16–20 h; 35°C
Corynebacterium spp. and Coryneform genera (M45)	Broth dilution	CAMHB + 2–5% LHB (supplement w/ 50 µg/ml calcium for daptomycin)	Direct	Air, 24–48 h; 35°C

Erysipelothrix rhusiopathiae (M45)	Broth dilution	CAMHB + 2–5% LHB	Direct	Air, 20–24 h; 35°C
Pasteurella spp. (M45)	Disk diffusion Broth dilution	BMHA CAMHB + 2–5% LHB	Direct	Air, 16–18 h; 35°C Air, 18–24 h; 35°C
HACEK group (M45)	Broth dilution	CAMHB + 2–5% LHB Or HTM or Brucella broth with vitamin K, hemin, and 5% LHG	Direct	5% CO_2; 24–48 h; 35°C
Lactobacillus spp. (M45)	Broth dilution	CAMHB + 2–5% LHB (supplement w/ 50µg/ml calcium for daptomycin)	Direct	5% CO_2; 24–48 h; 35°C
Moraxella catarrhalis (M45)	Disk diffusion Broth dilution	MHA CAMHB	Direct	5% CO_2; 20–24 h; 35°C Air; 20–24 h; 35°C
Micrococcus spp. (M45)	Broth dilution	CAMHB	Direct	Air, 20–24 h; 35°C
Campylobacter jejuni/coli (M45)	Disk diffusion Broth dilution	MHA + 5% sheep blood CAMHB + 2–5% LHB	Direct	Microaerophilic; 24 h; 42°C Microaerophilic; 24 h; 42°C or 48 h; 36–37°C
Helicobacter pylori (M45)	Agar dilution	MHA + 5% aged sheep blood	Direct(72 h hold from BAP)	Microaerophilic; 3 days; 35°C

(continued)

Antimicrobial Agents

Table 8.4 Summary of CLSI antimicrobial susceptibility test methods for select bacteria, mycobacteria, and fungi[a] *(continued)*

Organism (Document)	Test method	Medium	Inoculum	Incubation conditions
Bacillus spp. (not *B. anthracis*) (M45)	Broth dilution	CAMHB	Direct	Air, 16–20 h; 35°C
Anaerobes (M100)	Broth (*Bacteroides fragilis* group) only)/agar (all anaerobes) dilution	Brucella broth/agar + hemin (5 pg/ml), vitamin K (1 µg/ml), 5% lysed sheep blood	Direct; broth	Anaerobic; 42–48 h (agar) 46–48 h (broth); 36°C
Aerobic actinomycetes (M24)	Broth dilution	CAMHB	Direct; broth	Air; 3–5 days; 35°C
Mycobacteria (M24)	Refer to CLSI M24	Refer to CLSI M24	Refer to CLSI M24	Refer to CLSI M24
Fungi (yeasts) (M27)	Disk diffusion Broth dilution	RPMI 1640 broth	Direct	Air; 24–48 h (*Cryptococcus,* 70–74 h); 35°C
Fungi (molds) (M51)	Disk diffusion Broth dilution	MHA	Direct from potato dextrose agar	Air; 16–24 h (zygomycetes); 24–48 h (*Aspergillus* spp.); 48–72 h (others); 35°C

[a]Inoculum can be prepared either directly with isolated colonies on an agar plate (direct) or after growth of the organism in a broth culture (broth).

Abbreviations: MHA, Mueller-Hinton agar, CAMHB, cation-adjusted Mueller-Hinton broth; NaCI, sodium chloride; LHB, lysed horse blood; BHIA, brain heart infusion agar, GCA, GC agar; HTM, Haemophilus test medium; CONS, coagulase-negative staphylococci; oxac, oxacillin; gent, gentamicin; strep, streptomycin; vanco, vancomycin.

Table 8.5 Routes of administration and drug class for select antimicrobial agents[a]

Antimicrobial agent	Drug class	Route of administration			
		PO	IM	IV	Topical
Amikacin	Aminoglycoside		X	X	
Amoxicillin	Penicillin	X			
Amoxicilin-clavulanate	β-lactam/β-lactamase inhibitor	X			
Ampicillin	Penicillin	X	X	X	
Ampicillin-sulbactam	β-lactam/β-lactamase inhibitor			X	
Azithromycin	Macrolide	X		X	
Azlocillin	Penicillin			X	
Aztreonam	Monobactam		X	X	
Aztreonam-avibactam	β-lactam/β-lactamase inhibitor			X	
Carbenicillin	Penicillin		X	X	
Carbenicillin indanyl sodium	Penicillin	X			
Cefaclor	Cephem	X			
Cefadroxil	Cephem	X			
Cefamandole	Cephem		X	X	
Cefazolin	Cephem		X	X	
Cefepime	Cephem		X	X	
Cefdinir	Cephem	X			
Cefditoren	Cephem	X			
Cefixime	Cephem	X			
Cefmetazole	Cephem			X	
Cefonicid	Cephem		X	X	

(continued)

Table 8.5 Routes of administration and drug class for select antimicrobial agents[a] *(continued)*

Antimicrobial agent	Drug class	Route of administration			
		PO	IM	IV	Topical
Cefoperazone	Cephem		X	X	
Cefotaxime	Cephem		X	X	
Cefotetan	Cephem		X	X	
Cefoxitin	Cephem		X	X	
Cefpirome	Cephem		X	X	
Cefpodoxime	Cephem	X			
Cefprozil	Cephem	X			
Ceftaroline	Cephem			X	
Ceftaroline-avibactam	β-lactam/β-lactamase inhibitor			X	
Ceftazidime	Cephem		X	X	
Ceftazidime-avibactam	β-lactam/β-lactamase inhibitor			X	
Ceftibuten	Cephem	X			
Ceftizoxime	Cephem		X	X	
Ceftobiprole	Cephem			X	
Ceftolozane-tazobactam	β-lactam/β-lactamase inhibitor			X	
Ceftriaxone	Cephem		X	X	
Cefuroxime	Cephem		X	X	
Cefuroxime axetil	Cephem	X			
Cephalexin	Cephem	X			
Cephalothin	Cephem			X	
Cephapirin	Cephem			X	

Cephradine	Cephem	X			
Chloramphenicol	Phenicol	X		X	
Cinoxacin	Quinolone	X			
Ciprofloxacin	Fluoroquinolone	X		X	
Clarithromycin	Macrolide	X			
Clinafloxacin	Fluoroquinolone	X			
Clindamycin	Lincosamide	X		X	
Colistin	Lipopeptide			X	
Daptomycin	Lipopeptide			X	
Delafloxacin	Fluoroquinolone			X	
Dicloxacillin	Penicillin	X			
Dirithromycin	Macrolide	X			
Doripenem	Carbapenem			X	
Doxycycline	Tetracycline	X		X	
Ertapenem	Carbapenem		X	X	
Erythromycin	Macrolide	X		X	
Fidaxomicin	Macrocyclic	X			
Fleroxacin	Fluoroquinolone	X		X	
Fosfomycin	Fosfomycyin	X		X	
Fusidic acid	Steroidal	X		X	X
Gatifloxacin	Fluoroquinolone	X		X	
Gemifloxacin	Fluoroquinolone	X			
Gentamicin	Aminoglycoside		X	X	
Imipenem	Carbapenem			X	
Kanamycin	Aminoglycoside		X	X	
Levofloxacin	Fluoroquinolone	X		X	

(continued)

Table 8.5 Routes of administration and drug class for select antimicrobial agents[a] *(continued)*

Antimicrobial agent	Drug class	Route of administration			
		PO	IM	IV	Topical
Linezolid	Oxazolidinone	X		X	
Lomefloxacin	Fluoroquinolone	X			
Loracarbef	Cephem	X			
Meropenem	Carbapenem				
Meropenem-vaboractam	β-lactam/β-lactamase inhibitor			X	
Methicillin	Penicillin	X		X	
Metronidazole	Nitroimidazole	X		X	
Mezlocillin	Penicillin			X	
Minocycline	Tetracycline				
Moxifloxacin	Fluoroquinolone	X		X	
Mupirocin	Pseudomonic acid				X
Nafcillin	Penicillin			X	
Nalidixic acid	Quinolone	X			
Netilmicin	Aminoglycoside		X	X	
Nitrofurantoin	Nitrofurantoin	X			
Norfloxacin	Fluoroquinolone	X			
Ofloxacin	Fluoroquinolone	X	X	X	
Oritivancin	Lipoglycopeptide			X	
Oxacillin	Penicillin	X	X	X	
Pefloxacin	Fluoroquinolone	X		X	
Penicillin	Penicillin	X	X	X	

Piperacillin	Penicillin		X	X
Piperacillin-tazobactam	β-lactam/β-lactamase inhibitor			X
Polymyxin B	Lipopeptide			X
Quinupristin-dalfopristin	Streptogrammin			X
Rifampin	Ansamycin	X		X
Sparfloxacin	Fluoroquinolone	X		
Spectinomycin	Aminocyclitol		X	
Streptomycin	Aminoglycoside		X	
Sulfonamindes	Folate pathway inhibitor	X		X
Tedizolid	Oxazolidinone	X		X
Teicoplanin	Glycopeptide		X	X
Telithromycin	Ketolide	X		
Tetracycline	Tetracycline	X		X
Ticarcillin	Penicillin		X	X
Ticarcillin-clavulanate	β-lactam/β-lactamase inhibitor			X
Tigecycline	Glycylcline			X
Tinidazole	Nitroimidazoles	X		X
Tobramycin	Aminoglycoside		X	X
Trimethoprim	Folate pathway inhibitor	X		
TMP-SMX	Folate pathway inhibitor	X		X
Trovafloxacin	Fluoroquinolone			X
Vancomycin	Glycopeptide	X		X

[a]Clinical and Laboratory Standards Institute, M100–S27.

[b]PO, oral; IM, intramuscular; IV, intravenous.

Antimicrobial Agents

Table 8.6 Routes of administration and drug class for select antifungal agents

Antimicrobial agent	Drug class	Route of administration			
		PO	IM	IV	Topical
Amphotericin B	Polyene			X	
Flucytosine	Fluorinated pyrimidine	X			
Fluconazole	Azole	X		X	X
Ketoconazole	Azole	X			X
Itraconazole	Azole	X			
Voriconazole	Azole	X			X
Isavuconazole	Azole	X		X	
Posaconazole	Azole	X		X	
Caspofungin	Echinocandin			X	
Micafungin	Echinocandin			X	
Anidulafungin	Echinocandin			X	
Ciclopirox					X
Griseofulvin		X			
Terbinafine	Allylamine	X			X

PO, oral; IM, intramuscular; IV, intravenous.

Table 8.7 Routes of administration and drug class for select antiparasitic agents

		Route of administration			
Antimicrobial agent	**Indication**	**PO**	**IM**	**IV**	**Topical**
Albendazole	Antihelminthic, *Echinococcus,* pinworm, microsporidia, *Bayliascaris*	X			
Amphotericin B	*Acanthamoeba* spp., *Leishmania* spp., *Naegleria fowleri,*			X	
Atovaquone-proguanil	Malaria	X			
Artemether	Malaria		X		
Chloroquine	Malaria, Extraintestinal Amebiasis	X			
Clindamycin	*Babesia* spp.	X		X	
Dapsone	*Pneumocystis*	X			
Doxycycline	Malaria	X			
Diethylcarbamazine	Microfilaria	X			
Ivermectin	Microfilaria, Antihelminthic, Strongyloidiasis, *Bayliascaris*	X			
Pentamidine	*Acanthamoeba* spp., *Balamuthia* spp., *Leishmania* spp., *Pneumocystis, Trypanosoma brucei gambiense*		X	X	
Permethrin	Louse				X

(continued)

Table 8.7 Routes of administration and drug class for select antiparasitic agents *(continued)*

		Route of administration			
Antimicrobial agent	**Indication**	**PO**	**IM**	**IV**	**Topical**
Praziquantel	Antihelminthic	X			
Pyrimethamine	Malaria, Toxoplasmosis	X			
Malarone	Malaria	X			
Mebendazole	Antihelminthic, Microfilaria	X			
Mefloquine	Malaria	X			
Metronidazole	*Balantidium coli*, Amebiasis, *Dracunculus*, Giardiasis, *Trichomonas* spp.	X			
Quinine	Malaria, *Babesia* spp.	X		X	
Trimethoprim/ Sulfamethoxazole	*Cyclospora, Isospora*, *Pneumocystis*	X		X	

PO, oral; IM, intramuscular; IV, intravenous.

Table 8.8 Antibacterial agents for specific bacteria

	Antibiotics	
Organism	**Generally active[a]**	**Unpredictable activity**
Acinetobacter spp.	**Carbapenems**	β-Lactam-β-lactamase inhibitors
Actinobacillus spp.	**Cephalosporins**, rifampin, aminoglycosides, tetracyclines	Penicillins
Actinomyces spp.	**Penicillin**, doxycycline, broad-spectrum cephalosporins	Clindamycin, macrolides
Aeromonas hydrophila	**Broad-spectrum cephalosporins**, carbapenems, fluoroquinolones	Macrolides, aminoglycosides, tetracyclines
Anaplasma phagocytophila	**Doxycycline**	Chloramphenicol
Arcanobacterium haemolyticum	**Penicillin,** cephalosporins, carbapenems, vancomycin, macrolides, clindamycin, tetracyclines, fluoroquinolones	
Bacillus anthracis	**Penicillin,** ciprofloxacin	
Bacillus cereus	**Vancomycin**, carbapenems, macrolides, clindamycin, fluoroquinolones, gentamicin	Sulfonamides, tetracycline
Bacteroides fragilis group	**Metronidazole**	Clindamycin, carbapenems, cefoxitin, β-lactam–β-lactamase inhibitors

(continued)

Antimicrobial Agents

Table 8.8 Antibacterial agents for specific bacteria *(continued)*

	Antibiotics	
Organism	**Generally active[a]**	**Unpredictable activity**
Bartonella henselae	**Erythromycin**, doxycycline	Penicillins, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole
Bordetella pertussis	**Macrolides**, trimethoprim-sulfamethoxazole, fluoroquinolones	
Borrelia burgdorferi	**Doxycycline or broad-spectrum cephalosporins**, penicillins, macrolides	Fluoroquinolones
Brucella spp.	**Doxycycline**, trimethoprim-sulfamethoxazole	Cephalosporins
Burkholderia cepacia complex	**Trimethoprim-sulfamethoxazole**	Fluoroquinolones, Tigecycline
Burkholderia pseudomallei	**Carbapenems, penicillins**	
Fluoroquinolones, broad-spectrum cephalosporins		
Campylobacter jejuni	**Macrolides**, tetracyclines	Fluoroquinolones
Campylobacter fetus	**Carbapenem**, aminoglycosides	Ampicillin, macrolides
Capnocytophaga spp.	**Clindamycin**, β-lactam–β-lactamase inhibitors	Fluoroquinolones, carbapenems

Cardiobacterium hominis	**Penicillin**, cephalosporins, carbapenems, tetracycline	
Chlamydia trachomatis	**Doxycycline**, erythromycin	Fluoroquinolones
Chlamydophila pneumonia	**Doxycycline**, macrolides, fluoroquinolones	
Chlamydophila psittaci	**Doxycycline**, macrolides, fluoroquinolones	
Citrobacter freundi	**Broad-spectrum cephalosporins**, carbapenems, fluoroquinolones	
Citrobacter koseri	**Broad-spectrum cephalosporins**, carbapenems, fluoroquinolones	
Clostridium botulinum	**Penicillin**	
Clostridium difficile	**Metronidazole**, vancomycin	
Clostridium perfringens	**Penicillin**, cephalosporins, tetracyclines, clindamycin	
Clostridium tetani	**Penicillin**	
Corynebacterium diphtheria	**Erythromycin**, penicillin, clindamycin, cephalosporins, vancomycin, fluoroquinolones, aminoglycosides	Trimethoprim-sulfamethoxazole
Corynebacterium jeikeium	**Vancomycin**	Fluoroquinolones
Corynebacterium urealyticum	**Vancomycin**	Fluoroquinolones, macrolides, tetracyclines

(continued)

Table 8.8 Antibacterial agents for specific bacteria *(continued)*

	Antibiotics	
Organism	**Generally active[a]**	**Unpredictable activity**
Coxiella burnetii	**Doxycycline**, macrolides, fluoroquinolones	
Ehrlichia chaffeensis	**Doxycycline**	Chloramphenicol
Ehrlichia ewingii	**Doxycycline**	Chloramphenicol 3
Eikenella corrodens	**Penicillin**, broad-spectrum cephalosporins, tetracyclines, fluoroquinolones	Aminoglycosides
Enterobacter aerogenes	Carbapenems	Broad-spectrum cephalosporins, fluoroquinolones
Enterobacter cloacae	Carbapenems	Broad-spectrum cephalosporins, fluoroquinolones
Enterococcus faecalis	**Penicillin, ampicillin, or vancomycin with gentamicin**	Imipenem, fluoroquinolones
Enterococcus faecium	Oxazolidinones	**Penicillin**, ampicillin, or vancomycin with gentamicin
Erysipelothrix rhusiopathiae	**Penicillin**, cephalosporins, carbapenems, macrolides, clindamycin, fluoroquinolones	

Escherichia coli	**β-Lactam–β-lactamase inhibitors**, fluoroquinolones, broad-spectrum cephalosporins	
Francisella tularensis	**Streptomycin** or gentamicin, carbapenems, fluoroquinolones	Tetracyclines
Fusobacterium spp.	**Metronidazole**, carbapenems, penicillin, clindamycin	Broad-spectrum cephalosporins
Hafnia alvei	**β-Lactam–β-lactamase inhibitors,** fluoroquinolones, broad-spectrum cephalosporins	
Haemophilus aphrophilus	Penicillin, broad-spectrum cephalosporins	
Haemophilus ducreyi	**Broad-spectrum cephalosporins**, fluoroquinolones, macrolides	Penicillins, β-lactam–β-lactamase inhibitors, tetracyclines, trimethoprim-sulfamethoxazole
Haemophilus influenza	Broad-spectrum cephalosporins, trimethoprim sulfamethoxazole	Ampicillin
Kingella kingae	Cephalosphorins, penicillins, carbapenems, aminoglycosides, macrolides	Clindamycin
Klebsiella granutomatis	**Tetracyclines**, macrolides, trimethoprimsulfamethoxazole, aminoglycosides, fluoroquinolones	

(continued)

Table 8.8 Antibacterial agents for specific bacteria *(continued)*

	Antibiotics	
Organism	**Generally active[a]**	**Unpredictable activity**
Klebsiella oxytoca	**Broad-spectrum cephalosporins,** fluoroquinolones, carbapenems	
Klebsiella ozaenae	**Fluoroquinolones**, carbapenems	
Klebsiella pneumonia	**Broad-spectrum cephalosporins**, fluoroquinolones, carbapenems	
Lactobacillus spp.	**Penicillin** or ampicillin with aminoglycoside	
Legionella pneumophila	**Macrolides**, fluoroquinolones, rifampin	Penicillins, cephalosporins, aminoglycosides
Leptospira interrogans	**Penicillin**, doxycycline, cephalosporins	
Leuconostoc spp.	**Carbapenems**, aminoglycosides, tetracyclines	Penicillin, cephalosporins
Listeria monocytogenes	**Penicillin** or ampicillin with aminoglycoside, vancomycin	Fluoroquinolones, broad-spectrum cephalosporins
Moraxella catarrhalis	**Cephalosporins**, fluoroquinolones, carbapenems, tetracyclines, macrolides	
Morganella morganii	**Carbapenems**	Broad-spectrum cephalosporins
Mycoplasma pneumoniae	**Macrolides**, tetracyclines	Aminoglycosides, fluoroquinolones

Neisseria gonorrhoeae	**Broad-spectrum cephalosporins**	Penicillin, tetracyclines, macrolides, fluoroquinolones
Neisseria meningitides	**Penicillin**, broad-spectrum cephalosporins	
Nocardia spp.	**Sulfonamides**, carbapenems, amikacin, linezolid	Fluoroquinolones, broad-spectrum cephalosporins
Pasteurella multocida	**Penicillin**, cephalosporins, carbapenems, fluoroquinolones, tetracyclines	Macrolides, clindamycin
Plesiomonas shigelloides	**Cephalosporins**, carbapenems, β-lactam–β-lactamase inhibitors, fluoroquinolones	
Porphyromonas spp.	**Metronidazole**, carbapenems, clindamycin	Broad-spectrum cephalosporins
Prevotella spp.	**Metronidazole**, carbapenems, clindamycin	Broad-spectrum cephalosporins
Proteus mirabilis	**Ampicillin**, broad-spectrum cephalosporins, carbapenems	
Proteus vulgaris	Broad-spectrum cephalosporins, carbapenems, fluoroquinolones	
Providencia spp.	Broad-spectrum cephalosporins, carbapenems, fluoroquinolones	
Pseudomonas aeruginosa	**Carbapenems**	Broad-spectrum cephalosporins, fluoroquinolones

(continued)

Antimicrobial Agents

Table 8.8 Antibacterial agents for specific bacteria *(continued)*

	Antibiotics	
Organism	**Generally active**[a]	**Unpredictable activity**
Rhodococcus equi	**Carbapenems**, vancomycin, aminoglycosides, fluoroquinolones	Macrolides, clindamycin, tetracyclines
Rickettsia spp.	**Doxycycline**, fluoroquinolones	Erythromycin
Rothia mucilaginosa	**Penicillin**, cephalosporins, carbapenems, vancomycin	Aminoglycosides, clindamycin, macrolides
Salmonella enterica serovar Typhi	**Fluoroquinolones**, broad-spectrum cephalosporins	Chloramphenicol, amoxicillin, trimethoprimsulfamethoxazole
Salmonella spp.	**Fluoroquinolones**, broad-spectrum cephalosporins	Chloramphenicol, amoxicillin, trimethoprim-sulfamethoxazole
Serratia marcescens	**Broad-spectrum cephalosporins**, carbapenems, fluoroquinolones	
Shigella spp.	Fluoroquinolones, azithromycin	Trimethoprim-sulfamethosxazole, ampicillin
Staphylococcus spp. (methicillin-susc.)	**Oxacillin**, vancomycin, cephalosporins, imipenem, macrolides, clindamycin, fluoroquinolones	
Staphylococcus spp. (methicillin-res.)	Vancomycin	Carbapenems, fluoroquinolones
Stenotrophomonas maltophilia	**Trimethoprim-sulfamethoxazole**	Fluoroquinolones

Streptobacillus moniliformis	**Penicillin**, tetracyclines	
Streptococcus agalactiae (group B)	**Penicillin**, cephalosporins, carbapenems, vancomycin	
Streptococcus, anginosus group	**Penicillin**, cephalosporins, carbapenems, vancomycin	
Streptococcus, mitis group	**Cephalosporins**, carbapenems, vancomycin	Penicillin (drug of choice if susc.)
Streptococcus pneumoniae	**Cephalosporins**, carbapenems, vancomycin	Penicillin (drug of choice if susc.)
Streptococcus pyogenes (group A)	**Penicillin**, cephalosporins, carbapenems, vancomycin	
Treponema pallidum	**Penicillin**, broad-spectrum cephalosporins, tetracyclines	Macrolides
Tsakamurella spp.	**Carbapenems**, aminoglycosides, fluoroquinolones	Tetracyclines, trimethoprim-sulfamethoxazole
Vibrio cholerae	**Doxycycline**, fluoroquinolones	Trimethoprim-sulfamethoxazole
Vibrio vulnificus	**Doxycycline** with ceftazidime	Aminoglycosides
Yersinia enterocolitica	**Fluoroquinolones**, trimethoprim-sulfamethoxazole	Broad-spectrum cephalosporins
Yersinia pestis	**Streptomycin** or gentamicin	Chlorampenicol, ciprofloxacin, doxycycline

[a]Therapy of choice in bold type.

Antimicrobial Agents

Table 8.9 Intrinsic resistance of selected Gram-negative bacteria[a]

Organism	Ampicillin	1st Generation Cephalosporin	2nd Generation Cephalosporin	3rd Generation Cephalosporin	Piperacillin	Imipenem	Ertapenem	Aztreonam	Tetracycline	Tigecycline	Colistin Polymixin B	Nitrofurantoin	Aminoglycosides	Cotrimoxazole	Chloramphenicol	Fosfomycin
Citrobacter freundii	R	R	R													
Citrobacter koseri	R				R											
Enterobacter cloacae complex	R	R	R													
Escherichia coli																
Hafnia alvei	R	R	R													
Klebsiella pneumoniae	R															
Morganella morganii	R	R	R			*				R	R	R				
Proteus mirabilis						*			R	R	R	R				
Proteus penneri	R	R	R			*			R	R	R	R				
Proteus vulgaris	R	R	R			*			R	R	R	R				

Providencia stuarti	R					*			R	R	R	R	**				
Salmonella spp.																	
Shigella spp.																	
Serratia marcescens	R	R	R								R	R					
Yersinia enterocolitica	R	R															
Acinetobacter baumannii complex	R						R	R								R	R
Burkholderia cepacia complex	R	R	R	***	R	R	R	R			R	R	R				R
Pseudomonas aeruginosa	R	R	R	***			R		R	R				R	R	R	
Stenotrophomonas maltophilia	R	R	R	***	R	R	R	R	R				R				R

[a]Clinical and Laboratory Standards Institute, M100-S27.

*May have elevated MICs by mechanisms other than carbapenemase production.

**Resistant to most aminoglycosides but not amikacin.

***Resistant to ceftriaxone but not ceftazidime.

Table 8.10 Intrinsic resistance of selected Gram positive bacteria[a]

Organism	Novobiocin	Fosfomycin	Fusidic Acid	Cephalosporins	Vancomycin	Aminoglycosides	Clindamycin	Quinupristin-dalfopristin	Cotrimoxazole
Staphylococcus aureus									
Staphylococcus lugdunensis	There is no intrinsic resistance.								
Staphylococcus epidermidis									
Methicillin Resistant *Staphylococcus* spp.				R*					
S. saprophyticus	R	R	R						
S. cohnii	R								
S. xylosus	R								
S. capitis		R							
Enterococcus faecalis			R	R**		R**	R**	R	R**
Enterococcus faecium			R	R**		R**	R**		R**

Enterococcus gallinarum/E. casseliflavus	R	R**	R	R**	R**	R	R**
Clostridium spp.				R			
Clostridium innocuum			R	R			
Erysipelothrix rhusiopathiae			R				
Leuconostoc spp.			R				
Pediococcus spp.			R				
Lactobacillus spp.			R***				

[a]Clinical and Laboratory Standards Institute, M100-S27.

*Methicillin resistant staphylococci are resistant to all cephalosporins except for the so-called antistaphylococcal cephalosporins such as ceftaroline.

**May appear active *in vitro* but are not effective clinically.

***Some, but not all, species are resistant to vancomycin.

Table 8.11 Important mechanisms of multidrug resistance in bacteria.

Mechanism of resistance	Category	Primary organism(s)	Typical resistance profile	Detection
mecA (Penicillin Binding Protein 2a' (PBP2a'))	Altered target	*Staphylococcus* spp.	All beta-lactams except ceftaroline	Phenotypic (oxacillin and cefoxitin), molecular, and detection of PBP2a'
Klebsiella pneumoniae Carbapenemase (KPC)	Beta-lactamase	*Enterobacteriaceae, Pseudomonas* spp., *Acinetobacter* spp.	All beta-lactams. New beta-lactamase inhibitors (such as avibactam) inhibit activity	Phenotypic (modified hodge test, modified carbapenemase inactivation method (mCIM), carbaNP), molecular
Extended Spectrum Beta-Lactamases (ESBL)	Beta-lactamase	*Enterobacteriaceae*	All beta-lactams except cephamycins and carbapenems, variable resistance to cefepime	Phenotypic, molecular
New Delhi Metalo Beta-Lactamase (NDM)	Beta-lactamase	*Enterobacteriaceae, Pseudomonas* spp., *Acinetobacter* spp.	All beta-lactams except aztreonam	Phenotypic (modified hodge test performs poorly), molecular
AmpC	Beta-lactamase	*Enterobacteriaceae, Pseudomonas* spp., *Acinetobacter* spp., *Aeromonas* spp.	All beta-lactams except cefepime and carbapenems	Molecular
AmpC + Porin Mutation	Beta-lactamase and cell wall changes	*Enterobacteriaceae, Pseudomonas* spp., *Acinetobacter* spp.	All beta-lactams except cefepime	None

IMP	Beta-lactamase	*Enterobacteriaceae, Pseudomonas* spp., *Acinetobacter* spp.	All beta-lactams	Molecular
VIM	Beta-lactamase	*Enterobacteriaceae, Pseudomonas* spp., *Acinetobacter* spp.	All beta-lactams	Molecular
cfiA	Beta-lactamase	*Bacteroides* spp.	All beta-lactams	Molecular
erm	Inducible or constitutive methylation of ribosomal binding site	*Staphylococcus* spp., *Streptococcus* spp.	Macrolides, lincosamides, streptogrammins	Phenotypic (D-test)
vanA/B	Altered binding site	*Enterococcus* spp. (most commonly *E. faecium*), Vancomycin Resistant *Staphylococcus aureus* (rare)	Glycopeptides	Phenotypic, molecular
mcr	Phosphoethanolamine transferase	*Enterobacteriaceae*	Polymyxins	Molecular
cfr	Methyltransferase	*Enterococcus* spp., *Staphylococcus* spp.	Oxazolidinones, Macrolides, lincosamides, streptogrammins, phenicols, pleuromutilin	Molecular

(continued)

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Table 8.11 Important mechanisms of multidrug resistance in bacteria. *(continued)*

Mechanism of resistance	Category	Primary organism(s)	Typical resistance profile	Detection
optrA	ABC transporter	*Enterococcus* spp., *Staphylococcus* spp.	Oxazolidinones, Phenicols	Molecular
Efflux pumps	Efflux pumps	Predominantly Gram-negative organisms	Macrolides, beta-lactams, tetracyclines, quinolones, aminoglycosides	Molecular
Plasmid mediated quinolone resistance	Target modification	Gram-negatives	Quinolones	Molecular
Aminoglycoside modifying enzymes (AME)	Enzymatic inactivation	Gram-positives, Gram-negatives, Mycobacteria	Aminoglycosides	Molecular

Table 8.12 Organisms included in CLSI and EUCAST breakpoint tables

Organism	Clinical and Laboratory Standards Institute (CLSI) (Document number)	European Committee on Antimicrobial Susceptibility Testing (EUCAST)
Enterobacteriaceae	Y (M100)	Y
Pseudomonas spp.	Y (M100)	Y
Acinetobacter spp.	Y (M100)	Y
Burkholderia cepacia	Y (M100)	Y
Stenotrophomonas maltophilia	Y (M100)	Y
Miscellaneous non-*Enterobacteriaceae*	Y (M100)	Y (see specific examples below)
Staphylococcus spp.	Y (M100)	Y
Enterococcus spp.	Y (M100)	Y
Haemophilus influenzae and *H. parainfluenzae*	Y (M100)	Y (*H. influenzae* only)
Neisseria gonorrhoeae	Y (M100)	Y
Neisseria meningitidis	Y (M100)	Y
Streptococcus pneumoniae	Y (M100)	Y
Viridans group *Streptococcus* spp.	Y (M100)	Y
Beta-hemolytic *Streptococcus* spp.	Y (M100)	Y (Groups A, B, C, and G)
Anaerobes	Y (M100)	Y (see specific examples below)
Gram-positive anaerobes		Y
Clostridium difficile		Y
Gram-negative anaerobes		Y
Helicobacter pylori	Y (M45)	Y
Listeria monocytogenes	Y (M45)	Y
Pasteurella multocida	Y (M45)	Y

(continued)

Table 8.12 Organisms included in CLSI and EUCAST breakpoint tables *(continued)*

Organism	Clinical and Laboratory Standards Institute (CLSI) (Document number)	European Committee on Antimicrobial Susceptibility Testing (EUCAST)
Campylobacter jejuni and *coli*	Y (M45)	Y
Moraxella catarrhalis	Y (M45)	Y
Corynebacterium spp.	Y (M45)	Y
Aerococcus spp.	Y (M45)	Y (*Aerococcus sanguinicola* and *S. urinae* only)
Kingella kingae	Y (M45— See HACEK group)	Y
Aeromonas spp.	Y (M45)	Y
Abiotrophia spp. and *Granulicatella* spp.	Y (M45)	N
Bacillus spp. (not anthracis)	Y (M45)	N
Erysipelothrix rhusiopathiae	Y (M45)	N
Gemella spp.	Y (M45)	N
HACEK group: *Aggregatibacter* spp., *Cardiobacterium* spp., *Eikenella corrodens*, and *Kingella* spp.	Y (M45)	N (Except for *Kingella kingae*) (see above)
Lactobacillus spp.	Y (M45)	N
Lactococcus spp.	Y (M45)	N
Leuconostoc spp.	Y (M45)	N

Micrococcus spp.	Y (M45)	N
Pediococcus spp.	Y (M45)	N
Rothia mucilaginosa	Y (M45)	N
Vibrio spp. (including *V. cholerae*)	Y (M45)	N
Bacillus anthracis	Y (M45)	N
Yersinia pestis	Y (M45)	N
Burkholderia mallei and *B. pseudomallei*	Y (M45)	N
Francisella tularensis	Y (M45)	N
Brucella spp.	Y (M45)	N
Candida spp	Y (M27)	Y
Cryptococcus spp.	N	N
Aspergillus spp.	N	Y
Other Filamentous fungi	N	N
Mycobacterium tuberculosis	Y (M24)	Y
Mycobacterium avium complex	Y (M24)	N
Mycobacterium kansasii	Y (M24)	N
Mycobacterium marinum	Y (M24)	N
Rapidly growing mycobacteria	Y (M24)	N
Miscellaneous slowly growing mycobacteria	Y (M24)	N
Aerobic actinomycetes	Y (M24)	N
Nocardia spp.	Y (M24)	N

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Table 8.13 Guide to interpretive criteria for select organisms for commonly tested antimicrobials

		CLSI		EUCAST		
Organism	**Antibiotic**	**Disk diffusion**	**Minimum inhibitory concentration**	**Disk diffusion**	**Minimum inhibitory concentration**	**Comments**
Enterobacteriaceae						
	Ampicillin	Y	Y	Y	Y	
	Ampicillin-sulbactam	Y	Y	Y	Y	
	Amoxicillin	Inferred	Inferred	Inferred	Y	
	Amoxicillin-clavulanate	Y	Y	Y	Y	
	Piperacillin	Y	Y	Y	Y	
	Piperacillin-tazobactam	Y	Y	Y	Y	
	Ticarcillin-clavulanate	Y	Y	Y	Y	
	Ceftolozane-tazobactam	N	Y	Y	Y	MIC and DD breakpoints available in the FDA package insert.
	Cefazolin	Y	Y	N	N	
	Cefazolin (Urine)	Y	Y	N	N	
	Ceftaroline	Y	Y	Y	Y	
	Cefepime	Y	Y	Y	Y	
	Ceftriaxone	Y	Y	Y	Y	
	Cefotaxime	Y	Y	Y	Y	
	Ceftazidime	Y	Y	Y	Y	

Ceftazidime-avibactam	N	N	Y	Y	MIC and DD breakpoints available in the FDA package insert.
Cefuroxime	Y	Y	Y	Y	
Aztreonam	Y	Y	Y	Y	
Doripenem	Y	Y	Y	Y	
Meropenem	Y	Y	Y	Y	
Ertapenem	Y	Y	Y	Y	
Imipenem	Y	Y	Y	Y	
Gentamicin	Y	Y	Y	Y	
Tobramycin	Y	Y	Y	Y	
Amikacin	Y	Y	Y	Y	
Ciprofloxacin	Y	Y	Y[a]	Y	
Levofloxacin	Y[a]	Y	Y	Y	
Tetracycline	Y	Y	Y	Y	
Minocycline	Y	Y	Y	Y	
Tigecycline	N	N	Y	Y	MIC and DD breakpoints available in the FDA package insert.
Trimethoprim-Sulfamethoxazole	Y	Y	Y	Y	
Colistin	N	N	N	Y	
Nitrofurantoin	Y	Y	Y	Y	

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Table 8.13 Guide to interpretive criteria for select organisms for commonly tested antimicrobials *(continued)*

		CLSI		EUCAST		
Organism	**Antibiotic**	**Disk diffusion**	**Minimum inhibitory concentration**	**Disk diffusion**	**Minimum inhibitory concentration**	**Comments**
	Fosfomycin	Y[b]	Y[b]	Y[c]	Y	Agar dilution MIC and disk diffusion breakpoints available from FDA package insert.
	Chloramphenicol	Y	Y	Y	Y	
Pseudomonas aeruginosa						
	Piperacillin	Y	Y	Y	Y	
	Piperacillin-Tazobactam	Y	Y	Y	Y	
	Ceftolozane-tazobactam	Y	Y	Y	Y	
	Ticarcillin-clavulanate	Y	Y	Y	Y	
	Ceftazidime	Y	Y	Y	Y	
	Cefepime	Y	Y	Y	Y	
	Aztreonam	Y	Y	Y	Y	
	Doripenem	Y	Y	Y	Y	
	Meropenem	Y	Y	Y	Y	
	Imipenem	Y	Y	Y	Y	
	Colistin	N	Y	N	Y	

	Gentamicin	Y	Y	Y	Y
	Tobramycin	Y	Y	Y	Y
	Amikacin	Y	Y	Y	Y
	Ciprofloxacin	Y	Y	Y	Y
	Levofloxacin	Y	Y	Y	Y
Acinetobacter spp.					
	Piperacillin	Y	Y	N	N
	Piperacillin-Tazobactam	Y	Y	N	N
	Ticarcillin-clavulanate	Y	Y	N	N
	Ceftazidime	Y	Y	N	N
	Cefepime	Y	Y	N	N
	Ceftriaxone	Y	Y	N	N
	Doripenem	Y	Y	Y	Y
	Meropenem	Y	Y	Y	Y
	Imipenem	Y	Y	Y	Y
	Colistin	N	Y	N	Y
	Gentamicin	Y	Y	Y	Y
	Tobramycin	Y	Y	Y	Y
	Amikacin	Y	Y	Y	Y
	Doxycycline	Y	Y	N	N
	Minocycline	Y	Y	N	N
	Ciprofloxacin	Y	Y	Y	Y
	Levofloxacin	Y	Y	Y	Y
	Trimethoprim-Sulfamethoxazole	Y	Y	Y	Y

(continued)

Table 8.13 Guide to interpretive criteria for select organisms for commonly tested antimicrobials *(continued)*

		CLSI		EUCAST		
Organism	**Antibiotic**	**Disk diffusion**	**Minimum inhibitory concentration**	**Disk diffusion**	**Minimum inhibitory concentration**	**Comments**
Staphylococcus spp.						
	Penicillin	Y	Y	Y	Y	
	Methicillin	Inferred	Inferred	Inferred	Inferred	
	Nafcillin	Inferred	Inferred	Inferred	Inferred	
	Oxacillin[d]	N	Y	N	Y	
	Cefoxitin[d]	Y	Y	Y	Y	
	Amoxicillin-Clavulanate	Inferred	Inferred	Inferred	Inferred	
	Ampicillin-Sulbactam	Inferred	Inferred	Inferred	Inferred	
	Piperacillin-Tazobactam	Inferred	Inferred	Inferred	Inferred	
	Oral cephalosporins	Inferred	Inferred	Inferred	Inferred	
	Parenteral cephalosporins	Inferred	Inferred	Inferred	Inferred	
	Carbapenems	Inferred	Inferred	Inferred	Inferred	
	Ceftaroline	Y[e]	Y[e]	Y	Y	
	Vancomycin[d]	N	Y	Y[d]	N	
	Oritavancin	N	Y	N	Y	
	Televancin	N	Y	N	Y	
	Dalbavancin	N	N	N	Y	

Daptomycin	N	Y	N	Y	
Gentamicin	Y	Y	Y[d]	Y	
Tobramycin	Y	Y	Y[d]	Y	
Amikacin	Y	Y	Y[d]	Y	
Tetracycline	Y	Y	Y	Y	
Doxycycline	Y	Y	Y	Y/Inferred	
Tigecycline	N	N	Y	Y	MIC and DD breakpoints available in the FDA package insert.
Ciprofloxacin	Y	Y	Y[d]	Y	
Levofloxacin	Y	Y	Y[d]	Y	
Trimethoprim-Sulfamethoxazole	Y	Y	Y	Y	
Nitrofurantoin	Y	Y	Y	Y	
Cindamycin	Y	Y	Y	Y	
Chloramphenicol	Y	Y	Y	Y	
Rifampin	Y	Y	Y	Y	
Quinipristin-dalfopristin	Y	Y	Y	Y	
Linezolid	Y	Y	Y	Y	
Tedizolid	N	Y	N/Inferred	Y	
Fosfomycin	N	N	N	Y	Agar dilution MIC and disk diffusion breakpoints available from FDA package insert.

(continued)

Table 8.13 Guide to interpretive criteria for select organisms for commonly tested antimicrobials *(continued)*

		CLSI		EUCAST		
Organism	**Antibiotic**	**Disk diffusion**	**Minimum inhibitory concentration**	**Disk diffusion**	**Minimum inhibitory concentration**	**Comments**
Enterococcus spp						
	Penicillin	Y	Y	N	N	
	Ampicillin	Y	Y	Y	Y	
	Amoxicillin	Inferred	Inferred	Inferred	Y	
	Piperacillin-Tazobactam	Inferred	Inferred	Inferred	Inferred	
	Imipenem	Inferred	Inferred	Y	Y	
	Vancomycin	Y	Y	Y	Y	
	Teicoplanin	Y	Y	Y	Y	
	Oritavancin	N	Y	N	N	
	Televancin	N	Y	N	N	
	Dalbavancin	N	N	N	N	
	Daptomycin	N	Y	N	N	
	Tetracycline	Y	Y	N	N	
	Doxycycline	Y	Y	N	N	
	Minocycline	Y	Y	N	N	
	Tigecycline	N	N	Y	Y	MIC and DD breakpoints available in the FDA package insert for vancomycin-susceptible *E. faecalis* only.

	Ciprofloxacin	Y	Y	Y	Y
	Levofloxacin	Y	Y	Y	Y
	Nitrofurantoin	Y	Y	Y[f]	Y[f]
	Rifampin	Y	Y	N	N
	Fosfomycin	Y[f]	Y[f]	N	N
	Chloramphenicol	Y	Y	N	N
	Quinipristin-dalfopristin	Y	Y	Y	Y
	Linezolid	Y	Y	Y	Y
	Tedizolid	N	Y[f]	N	N
	Amikacin	N	N	Inferred	Inferred
	Gentamicin	Y[g]	Y[g]	Y[g]	Y[g]
	Streptomycin	Y[g]	Y[g]	Y[g]	Y[g]
	Tobramycin	N	N	Y[g]	Y[g]
	Trimethoprim-Sulfamethoxazole	N	N	Y	Y
Streptococcus pneumoniae					
	Penicillin	Inferred from oxacillin	Y[h]	Inferred from oxacillin	Y[h]
	Ampicillin	Inferred	Inferred	Y	Inferred
	Amoxicillin	Inferred	Y/Inferred	Inferred	Inferred
	Amoxicillin/Clavulanate	Inferred	Y/Inferred	Inferred	Inferred
	Cefepime	Inferred	Y[h]/Inferred	Y	Inferred from oxacillin

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Table 8.13 Guide to interpretive criteria for select organisms for commonly tested antimicrobials *(continued)*

		CLSI		EUCAST		
Organism	**Antibiotic**	**Disk diffusion**	**Minimum inhibitory concentration**	**Disk diffusion**	**Minimum inhibitory concentration**	**Comments**
	Ceftriaxone	Inferred	Y[h]/Inferred	Inferred from oxacillin	Y	
	Cefuroxime	Inferred	Y[h]/Inferred	Inferred from oxacillin	Y	
	Ceftaroline	Inferred	Y/Inferred	Inferred from oxacillin	Y	
	Meropenem	N	Y	Inferred from oxacillin[h]	Y	
	Ertapenem	N	Y	Inferred from oxacillin	Y	
	Imipenem	N	Y	Inferred from oxacillin	Y	
	Doripenem	N	Y	Inferred from oxacillin	Y	
	Vancomycin	Y	Y	Y	Y	
	Teicoplanin	N	N	Y	Y	
	Azithromycin	Y	Y	Inferred from erythromycin	Y	
	Tetracycline	Y	Y	Y	Y	

	Doxycycline	Y	Y	Inferred	Y	
	Tigecycline	N	N	N	N	MIC and DD breakpoints available in the FDA package insert.
	Levofloxacin	Y	Y	Y	Y	
	Trimethoprim-Sulfamethoxazole	Y	Y	Y	Y	
	Chloramphenicol	Y	Y	Y	Y	
	Rifampin	Y	Y	Y	Y	
	Clindamycin	Y	Y	Y	Y	
	Quinipristin-dalfopristin	Y	Y	N	N	
	Linezolid	Y	Y	Y	Y	
	Tedizolid	N	N	N	N	

[a]For all *Enterobacteriaceae* except *Salmonella* spp.
[b]For *E. coli* from the urinary tract only.
[c]For *E. coli* only.
[d]There are specific recommendations for various staphylococci species. Consult current document for species-specific guidance.
[e]For reporting on methicillin resistant *S. aureus* only.
[f]For *E. faecalis* only.
[g]For synergy only.
[h]Infection-site specific breakpoints exist. Consult current guideline document for up-to-date breakpoint criteria.

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Table 8.14 Summary of MALDI-TOF MS identification of bacteria, mycobacteria, and fungi

Organism	Reliable genus level identification	Reliable species level identification	Comments
Gram-negative bacteria			
Aeromonas spp.	+	–	Cannot differentiate between *A. hydrophila* and *A. caviae*
Achromobacter spp.	+	+/–	Cannot differentiate between *A. xylosoxidans* and *A. rhulandii*.
Acinetobacter spp.	+	+	
Aggregatibacter spp.	+	+	Minimal data
Alcaligenes faecalis	+/–	+/–	Minimal data. One of 12 isolates in clinical trial misidentified to Genus level.
Bacteroides spp.			
Brucella spp.	+	+	May not be included in databases. May be misidentified as *Ochrabactrum* spp.
Burkholderia cepacia complex	+	+	Databases do not include *B. mallei* and *B. pseudomallei*.
Burkholderia gladioli	+	+	
Campylobacter spp.	+	+	
Cardiobacterium hominis		+/–	Minimal data. May not be present in some databases.
Chryseobacterium spp.	+/–	+/–	Frequently fails to produce an identification.
Citrobacter spp.	+	+/–	
Cronobacter sakazakii	+	+	

Edwardsiella spp.	+	+/–	
Eikenella corrodens	+	+	
Enterobacter spp.	+	+/–	Cannot differentiate with *E. cloacae* complex.
Escherichia coli	+	+	Cannot differentiate from *Shigella* spp.
Escherichia spp. (non *E. coli)*	+	+	
Francisella spp.	+	+	Minimal data.
Fusobacterium spp.	+	+	
Haemophilus spp.	+	+	
Inquilinus limosus	+	+	May fail to produce an identification. Not present in some databases.
Kingella kingae	+	+	
Klebsiella spp.	+	+	
Moraxella catarrhalis	+	+	
Morganella spp.	+	+	
Neisseria spp.	+	+	Some systems may not readily differentiate between *N. meningitidis* and nonpathogenic *Neisseria* spp.
Oligella urethralis	+	+	
Pandorea spp.	+	+/–	Not present in some databases.
Pantoea agglomerans	+/–	+/–	
Pasteurella spp.	+	+	
Porphyromonas spp.			
Prevotella spp.			
Proteus spp.	+	+/–	Some systems may not differentiate between *P. penneri* and *P. vulgaris.*

(continued)

Antimicrobial Agents

Table 8.14 Summary of MALDI-TOF MS identification of bacteria, mycobacteria, and fungi *(continued)*

Organism	Reliable genus level identification	Reliable species level identification	Comments
Providencia spp.	+	+	
Pseudomonas aeruginosa group	+	+	Mucoid strains may fail to produce an identification.
Pseudomonas putida group	+	+	
Pseudomonas fluorescens group	+	+	
Raoutella spp.	+	+	
Ralstonia pickettii	+	+	
Roseomonas spp.	+	+	Not present in some databases.
Salmonella spp.	+	+	Cannot differentiate *S.* TYPHI
Serratia spp.	+	+	
Spingomonas spp.	+	+/−	Databases may not contain *S. anopheles.*
Stenotrophomonas maltophilia	+	+	
Vibrio spp.	+	+	
Yersinia spp.	+	+/−	Databases do not include *Y. pestis.*
Gram-positive bacteria			
Abiotrophia defective	+	+	Minimal data
Actinomyces spp.	+	+	
Aerococcus spp.	+	+/−	Minimal data for species differentiation.

Arcanobacterium spp.	+	+	
Bacillus spp.	+	+/–	May not differentiate within the *B. cereus* group of organisms, which includes *B. anthracis.*
Corynebacterium spp.	+	+	
Clostridium spp.	+	+	
Enterococcus spp.	+	+	
Finegoldia magna	+	+	
Gemella spp.	+	+	
Gordonia spp.	+/–	+/–	Minimal data suggests unreliable performance.
Lactobacillus spp.	+	+	
Listeria spp.	+	-	Does not readily differentiate between *L. monocytogenes* and other species.
Micrococcus spp.	+	+	
Nocardia spp.	+	+	
Peptostreptococcus spp.	+	+	
Propionibacterium spp.	+	+	
Rothia mucilaginosa	+	+	
Staphylococcus spp.	+	+/–	Some systems may not differentiate between the coagulase negative staphylococci.
Streptococcus, Viridans Group	+	+/–	Some systems cannot differentiate *S. mitis/oralis* from *S. pneumoniae.*
Streptococcus, Large colony, beta-hemolytic	+	+/–	May not always differentiate between *S. pyogenes* and *S. dysgalactiae.*
Turicella otitidis	+	+	Minimal data.

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Table 8.14 Summary of MALDI-TOF MS identification of bacteria, mycobacteria, and fungi *(continued)*

Organism	Reliable genus level identification	Reliable species level identification	Comments
Mycobacteria—Slow Growers			
M. tuberculosis complex	+	+	Does not differentiate species within the MTB complex.
M. avium complex	+	+	
M. gordonae	+	+	
M. kansasii	+	+	
M. haemophilum	+	+	
M. marinum complex	+	+	
M. scrofulaceum	+	+	
M. simiae	+	+	
M. szulgai	+	+	
M. xenopi	+	+	
Mycobacteria—Rapid Growers			
M. chelonae	+	+	
M. abscessus	+	+	
M. fortuitum complex	+	+	
M. mucogenicum complex	+	+	
M. immunogenum	+	+	
M. smegmatis complex	+	+	

Fungi			
Alternaria spp.	+	**No data**	
Aspergillus spp.	+	+	
Bipolaris spp.	+	**No data**	
Blastomyces dermatitidis	+	+	
Candida spp.	+	+	*C. auris* may be misidentified as *C. duobushaelmulonii* and *C. haelmulonii*.
Cladosporium spp.	+	**No data**	
Curvularia spp.	+/-	**No data**	
Cryptococcus spp.	+	+	Can differentiate between *C. neoformans* and *C. gattii*.
Fusarium spp.	+	**No data**	
Histoplasma capsulatum	+	+	
Mucor spp.	+	**No data**	
Paecilomyces spp.	+	**No data**	
Penicillium spp.	+	**No data**	
Rhizopus spp.	+	**No data**	
Rhodotorula spp.	+	+	
Saacharomyces spp.	+	+	
Scopulariopsis spp.	+	**No data**	
Trichosporon spp.	+	+	
Trichophyton spp.	+	+	

Table 8.15 Gene sequencing targets for organism identification[a]

Gene Target	Organisms Identified	Criteria for Genus-level Identification	Criteria for Species-level Identification	Alternative targets	Limitations in Species-Level Differentiation
16S rRNA	Bacteria	>97.0% identity	>99.0% identity with >0.8% separation from other species For *Klebsiella, Enterobacter, Citrobacter, Pantoea-* >99.5% identity with >0.5% separation from other species *Campylo-bacter*—>99.4% identity Aerobic actinomycetes—>99.6% identity with >0.4% separation from other species	Staphylococci—*dnaJ, sodA, tuf, rpoB* Streptococci—*dnaJ, tuf, rpoB, gyrB* Enterococci—*tuf* *Bacillus* spp.—16S-23S region *Enterobacteriaceae—gyrB,* Elongation factor Tt *Corynebacterium* spp.—*rpoB* *Acinetobacter, Haemophilus, Aggregatibacter*—16S-32S region, *gyrB* *Pseudomonas* spp.—*gyrB* *Bordetella, Burkholderia—recA* *Neisseria* spp.—16S-32S region	Gram-positive coagulase negative staphylococci, *Streptococcus pneumoniae/mitis* group, *S. bovis* group, *S. salivarius* group, *Corynebacterium* spp., *Bacillus* spp. Gram-negative *Klebsiella, Enterobacter, Citrobacter, Pantoea, Escherichia, Shigella, Yersinia, Salmonella, Acinetobacter*

				Campylobacter spp.—*cpn60* *Brucella*—*recA, gyrB* *Nocardia* spp.—*secA1* *Rhodococcus equi*—*choE*	Aerobic Actinomycetes *Nocardia* spp., *Rhodococcus equi*
16S rRNA	Mycobacteria	99.0–99.9% identity	100% identity	*M. tuberculosus* complex—*gyrB* *M. kansasii*—*gyrB, rpoB, secA1, dnaA, hsp65, ITS* *M. marinum, M. ulcerans*—*gyrB, hsp65, secA1, dnaA* *M. fortuitum* complex—*rpoB* *M. chelonae, M. abscessus*—*rpoB, hsp65, secA1, ITS*	*M. tuberculosus* complex, *M. kansasii, M. marinum, M. ulcerans, M. chelonae, M. abscessus, M. fortuitum* complex
Intergenic Spacer Region (ITS)	Fungi			*Phaeoacremonium*—beta tubulin *Fusarium* spp.—elongation factor (EF) Yeast and yeast-like fungi—D1/D2 Zygomycetes, Dimorphic fungi—D1/D2 *Aspergillus*—beta tubulin Dermatophytes—28S D2	*Phaeoacremonium, Fusarium* spp., zygomycetes, *Alternaria* spp.

[a]Clinical and Laboratory Standards Institute—MM18AE. Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline

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