

AG

Dr. Ahmed Gaber

# Gaber Notes

## BOC (Micro)

**Dr. Ahmed Gaber Sharaf Eddin**  
MSc. MLS (ASCPi)



### *Micrococcus* species

- Gram Stain
  - Gram positive cocci
  - Characteristically in tetrads
  - Usually larger than *Staphylococcus* species
- Colony morphology
  - *Micrococcus luteus* = yellow pigment
  - *Micrococcus roseus* = pink pigment
- Biochemical tests
  - Catalase positive
  - Modified oxidase positive



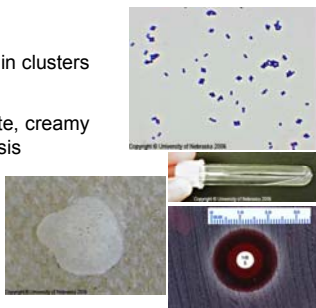
### *Staphylococcus aureus*

- Gram Stain
  - Gram positive cocci in clusters
- Colony morphology
  - Round, smooth, white, creamy colonies with beta-hemolysis
  - May have yellow pigment with extended incubation
- Biochemical tests
  - Catalase positive
  - Coagulase positive
- Pathology
  - Wound & skin infections, toxic shock syndrome, food poisoning, pneumonia, osteomyelitis, bacteremia, etc.



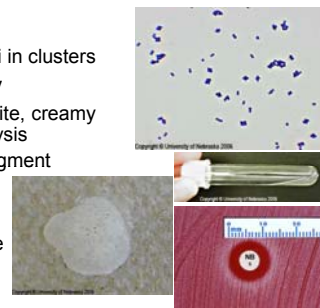
### *Staphylococcus epidermidis*

- Gram Stain
  - Gram positive cocci in clusters
- Colony morphology
  - Round, smooth, white, creamy colonies, no hemolysis
- Biochemical tests
  - Catalase positive
  - Coagulase negative
  - Novobiocin "S"
- Pathology
  - Normal skin flora, nosocomial infections, prosthetic valve endocarditis, catheters, septicemia



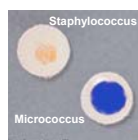
### *Staphylococcus saprophyticus*

- Gram Stain
  - Gram positive cocci in clusters
- Colony morphology
  - Round, smooth, white, creamy colonies, no hemolysis
  - May have yellow pigment
- Biochemical tests
  - Catalase positive
  - Coagulase negative
  - Novobiocin "R"
- Pathology
  - UTI's in young, sexually active females



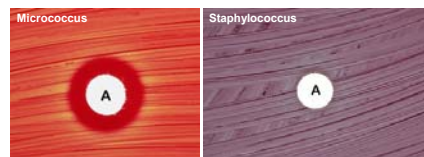
### Microdase (Modified Oxidase) Test

- Rapid test
  - Two minutes for reaction
  - Detects enzyme – cytochrome oxidase
  - Based on oxidase reaction but contains DMSO to release cytochrome oxidase
- Used to differentiate between
  - *Staphylococcus* species
    - No color change = negative for cytochrome C
  - *Micrococcus* species
    - Blue color = positive for cytochrome C



### Bacitracin ("A", "BC") Disk

- Overnight disk diffusion test
- Concentration of bacitracin is 0.04 UI
- Used to differentiate between
  - *Micrococcus* species = "S" (zone of no growth)
  - *Staphylococcus* species = "R" (growth right up to disk)



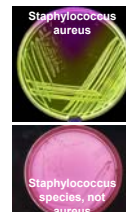
### Coagulase Tube Test

- Tests for free coagulase – an extracellular enzyme that causes a clot to form when bacterial cells are incubated in rabbit plasma
- Used to differentiate between
  - *Staphylococcus aureus* = positive (clot at 4 hours)
    - Some strains of *Staph. aureus* may lyse the clot (due to staphylokinase) so it is important to check reaction every ½ hour for the first 4 hours
  - Other *Staphylococcus* species = negative (no clot at 24 hours)
    - Report as “Coagulase negative *Staphylococcus* species”



### Mannitol Salt Agar

- Media classified as selective and differential
  - Selective for *Staphylococcus* species ( $\uparrow$  NaCl)
  - Differential for the fermentation of mannitol, the sole carbohydrate in the media
    - Mannitol fermented  $\rightarrow$   $\downarrow$  pH  $\rightarrow$  indicator turns pink to yellow
- *Staphylococcus aureus* = positive, yellow colonies surrounded by yellow zone
  - Note: some coag neg *Staph* can be +
- Other *Staphylococcus* species & *Micrococcus* species = negative, reddish colonies – no color change of media



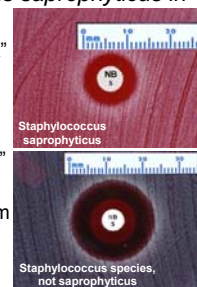
### DNase Agar

- Media classified as differential
- Inoculum is a dime-sized area of growth
- Used to detect the production of an active DNase exoenzyme by aerobic bacteria
- Used to differentiate
  - *Staphylococcus aureus* = positive, pink color around growth
  - Other *Staphylococcus* species = negative, no color change



### Novobiocin Disk

- Disk diffusion test using novobiocin (antibiotic) at a concentration of 5  $\mu$ g for the presumptive identification of *Staphylococcus saprophyticus* in urine cultures
- *Staphylococcus saprophyticus* = “R”
  - Organism grows up to the disk or a zone  $\leq$  16 mm
- Other *Staphylococcus* species = “S”
  - Organism’s growth is inhibited by antibiotic with a zone  $>$  16 mm



### Hemolysis

- Beta ( $\beta$ ) hemolysis
  - Complete lysis of RBC’s surrounding colony causing a clear zone or clearing of blood from the agar
- Alpha ( $\alpha$ ) hemolysis
  - Partial lysis of RBC’s surrounding colony causing a greenish discoloration in media
- Non-hemolytic
  - Also known as Gamma hemolysis ( $\delta$ )
  - No lysing of RBC’s and no color change of the medium surrounding colony



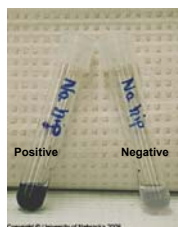
### Bacitracin (“A”, “BC) Disk

- Overnight disk diffusion test
- Concentration of bacitracin is 0.04 UI
- Used to differentiate between
  - *Streptococcus pyogenes* (Group A *Strep*) = “S” (any zone of no growth around the disk)
    - There are a small % of other beta-hemolytic *Strep* that are “S”
  - Most other beta-hemolytic *Strep* = “R” (growth up to edge of disk)



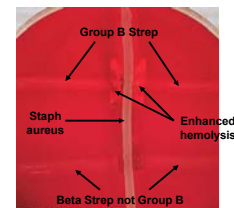
### Hippurate Hydrolysis Test

- A rapid test that detects the enzyme hippuricase
  - Na hippurate → benzoic acid + glycine → blue/purple color change
    - Grp B Strep hydrolyzes 2 hr, 35°C
    - Ninhydrin Reagent 15 min.
- Used to differentiate between
  - *Streptococcus agalactiae* (Group B *Strep*) = positive, blue/purple color
  - Other beta-hemolytic *Strep* = negative, no color change



### CAMP Test

- An overnight test that detects the extracellular CAMP factor
  - A streptococcal protein that acts synergistically with Staphylococcal beta-hemolysis on sheep RBC's
- Used to differentiate between
  - *Streptococcus agalactiae* (Group B *Strep*) = positive, "arrow" formation
  - Other beta-hemolytic *Strep* = negative, no "arrow" formation



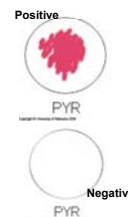
### Optochin ("P") Disk

- Overnight disk diffusion test
- Disk contains 5 µg ethyl-hydrocupreine hydrochloride
- Used to differentiate between
  - *Streptococcus pneumoniae* = "S" (>14 mm zone of no growth around the disk)
  - Other *Streptococcus* species = "R" (growth up to edge of disk)



### PYR Hydrolysis Test

- Rapid test
  - Enzyme L-pyrroglutamyl-aminopeptidase hydrolyzes the substrate L-pyrrolidonyl-beta-naphthylamide to produce a beta-naphthylamine
  - When the reagent cinnamaldehyde combines with the beta-naphthylamine, a bright red color is produced
- Used to differentiate
  - *Streptococcus pyogenes* = positive
  - *Enterococcus* species = positive
  - Other *Streptococcus* species = negative (no color change)



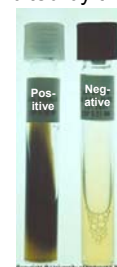
### 6.5% Sodium Chloride Test

- Certain GPC are able to grow in 6.5% NaCl whereas others are inhibited by this salt concentration
- Used to differentiate between
  - *Enterococcus* species = positive (growth, turbid)
  - Group D *Streptococcus* = negative (no growth, clear)
  - *Streptococcus* species *viridans* group = negative



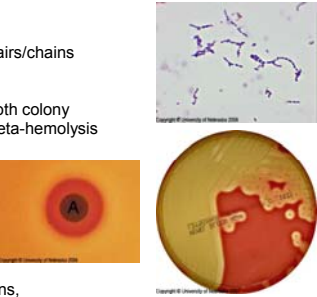
### Bile Esculin Hydrolysis

- Certain GPC are able to grow in the presence of bile and hydrolyze esculin to form a blackening of media, whereas others are inhibited by the bile
- Used to differentiate between
  - *Enterococcus* species = positive (black)
  - Group D *Streptococcus* = positive (black)
  - Other *Streptococcus* species = negative (no color change)



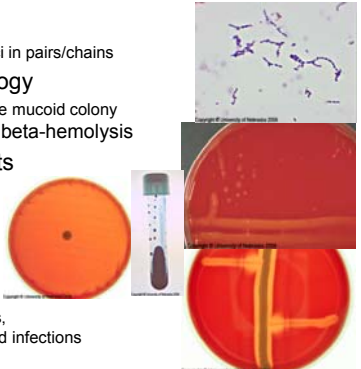
### *Streptococcus pyogenes* (Group A)

- **Gram Stain**
  - Gram positive cocci in pairs/chains
- **Colony morphology**
  - Small, transparent, smooth colony with well-defined zone beta-hemolysis
- **Biochemical tests**
  - Catalase negative
  - Bacitracin (A) "S"
  - PYR positive
- **Pathology**
  - Pharyngitis, skin infections, necrotizing fasciitis, toxic shock syndrome, post-streptococcal sequelae




### *Streptococcus agalactiae* (Group B)

- **Gram Stain**
  - Gram positive cocci in pairs/chains
- **Colony morphology**
  - Small, grayish-white mucoid colony with small zone beta-hemolysis
- **Biochemical tests**
  - Catalase negative
  - Bacitracin (A) "R"
  - Hippurate positive
  - CAMP positive
- **Pathology**
  - Neonate meningitis, endometritis, wound infections



### *Streptococcus pneumoniae*

- **Gram Stain**
  - Gram positive cocci in pairs (lancet-shaped)
- **Colony morphology**
  - Small, round, glistening, mucoid, dome-shaped colony with alpha-hemolysis
  - Autolytic changes (center collapses) when older
- **Biochemical tests**
  - Catalase negative
  - Optochin (P) "S"
  - Bile solubility positive
- **Pathology**
  - Pneumonia, sinusitis, otitis media, bacteremia, meningitis



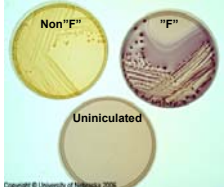
### *Streptococcus species viridans* group

- **Gram Stain**
  - Gram positive cocci in pairs (lancet-shaped)
- **Colony morphology**
  - Small, round, glistening, mucoid, dome-shaped colony with alpha-hemolysis
  - Autolytic changes (center collapses) when older
- **Biochemical tests**
  - Catalase negative
  - Optochin (P) "R"
  - Bile esculin negative
- **Pathology**
  - Normal flora, opportunistic, subacute bacterial endocarditis

### *Enterococcus* species

### MacConkey Agar

- **Classified as a selective and differential media**
  - Selective for Gram negative rods (Gram positive organisms are inhibited by bile salts)
  - Differential for lactose fermentation
    - Media contains lactose as its only carbohydrate
    - Indicator is neutral red (agar is pink)
- **Lactose Fermenter**
  - Pink colonies
- **Non-lactose Fermenter**
  - Clear colonies
  - Note: Proteus will not swarm on this media



### Hektoen-Enteric (HE) Agar

- Classified as a selective and differential media
  - Selective for Gram negative rods (Gram positive organisms are inhibited by bile salts)
  - Differential for lactose & sucrose fermentation
    - Media contains lactose and sucrose
    - Indicator is bromthymol blue (agar is green)
- Lactose and/or Sucrose "F" (Yellow colonies)
- Lactose & Sucrose Non-"F" (Bluish-green colonies)
- H<sub>2</sub>S production = black precipitate



### Eosin Methylene Blue (EMB) Agar

- Classified as a selective and differential media
  - Selective for Gram negative rods (Gram positive organisms are inhibited by eosin dye)
  - Differential for lactose fermentation
    - Media contains lactose as its only carbohydrate
    - Indicators is eosin & methylene blue
- Lactose Fermenter
  - Dark-pink-centered colonies
  - Lowers pH which precipitates dye
  - Note: *E. coli* = metallic green sheen
- Non-lactose Fermenter
  - Clear colonies



*Escherichia coli*

### Xylose Lysine Desoxycholate (XLD) Agar

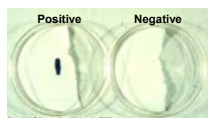
- Classified as a selective and differential media
  - Selective for Gram negative rods (Gram positive organisms are inhibited by bile salts)
  - Differential for lactose, sucrose and xylose fermentation
    - Media contains lactose, sucrose, and xylose
    - Indicator is phenol red (turns yellow when pH decreases)
  - Differential for lysine decarboxylation (LDC)
    - Media contains lysine
- Yellow colonies = Fermenter of carbohydrates
- Yellow colonies with black centers = "F" & H<sub>2</sub>S +
- Colorless/red colonies = Non "F"
- Red colonies with black centers = LDC + & H<sub>2</sub>S +

### Salmonella-Shigella (SS) Agar

- Classified as a selective and differential media
  - Selective for pathogenic Enterics (Gram positive organisms are inhibited by bile salts)
  - Differential for lactose fermentation
    - Media contains lactose as its only carbohydrate
    - Indicator is neutral red (agar is pink)
- Lactose "F" (Red/Pink colonies)
- Lactose Non-"F" (Colorless colonies)
- H<sub>2</sub>S production = black precipitate

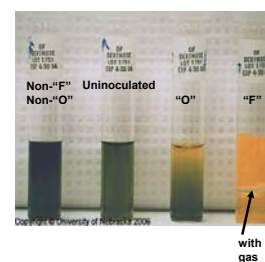
### Oxidase Test

- Enzyme cytochrome oxidase in the presence of the reagent tetra-methyl- (or dimethyl-) para-phenylene-diamine-dihydrochloride to form a blue color within 30 seconds
  - Positive = blue color development within 30 seconds
  - Negative = no color development
- Must use a platinum loop or sterile wooden stick, because nichrome loops can cause a false positive
- Tetra-methyl reagent is more sensitive than dimethyl reagent



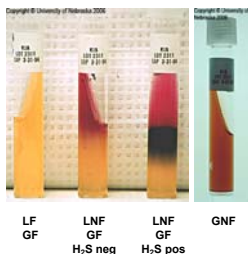
### Glucose OF Media

- Fermentative ("F") organism will produce an acid reaction (yellow) throughout tube
- Oxidative ("O") organism will produce an acid reaction in the upper 1/3 of media
- Non-fermenter & Non-oxidizer (Non-"F" & Non-"O") will produce an alkaline reaction (blue) in upper portion of tube due to peptone metabolism.



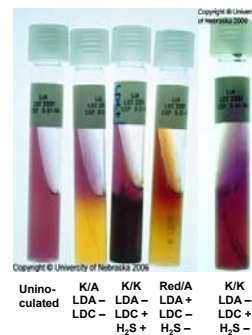
### Kligler's Iron Agar (KIA)

- **Lactose Fermenter, Glucose Fermenter**
  - Acid (yellow) slant & butt (A/A)
- **Lactose Non-Fermenter, Glucose Fermenter**
  - Alkaline (red) slant & acid butt (K/A)
- **Glucose Non-Fermenter**
  - Alkaline (red) slant & butt (K/K)
- **H<sub>2</sub>S**
  - Positive = Black precipitate
  - Negative = No precipitate



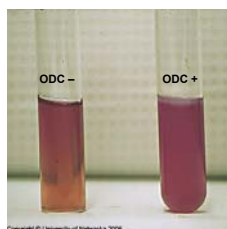
### Lysine Iron Agar (LIA)

- Detects deamination (LDA) and decarboxylation (LDC) of the amino acid lysine
- **Slant**
  - LDA positive = wine-red
  - LDA negative = purple (alkaline)
- **Butt**
  - LDC positive = purple (alkaline)
  - LDC negative = yellow (acid)
- **H<sub>2</sub>S**
  - Positive = Black precipitate
  - Negative = No precipitate
- **Note:** This test is invalid if the organism is unable to ferment glucose!



### Ornithine Decarboxylase Media

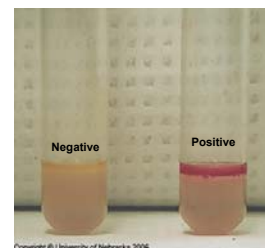
- Detects decarboxylation (ODC) of the amino acid ornithine
- Top 1/3 of media always purple
- **Butt**
  - ODC positive = purple
  - ODC negative = yellow (lower 2/3 or tube)
- **Note:** This test is invalid if the organism is unable to ferment glucose!



### Indole Test

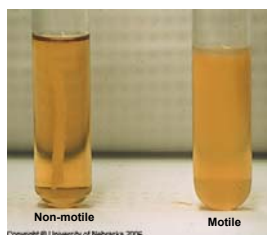
- Detects the enzyme Tryptophanase
 

Tryptophane  $\xrightarrow{\text{tryptophanase}}$  Indole  $\xrightarrow{\text{Para-dimethylamino-benzaldehyde}}$  Cherry Red color  
Kovac's Reagent
- **Indole positive**
  - Cherry red color after adding Kovac's Reagent
- **Indole negative**
  - No color reaction after adding Kovac's Reagent



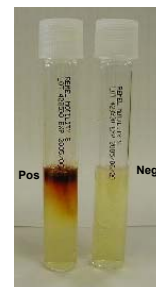
### Motility S Media

- **Motile**
  - Diffuse clouding away from the line of inoculation
- **Non-motile**
  - Growth only along the line of inoculation



### Nitrate Reduction Test

- Detects organism's ability to reduce nitrate (NO<sub>3</sub>) to nitrite (NO<sub>2</sub>) or nitrogen gas (N<sub>2</sub>↑)
- Add 2 reagents to the Motility S tube
  - Alpha-naphthylamine & sulfanilic acid
- NO<sub>3</sub> → NO<sub>2</sub>
  - Add reagents = Red color
- NO<sub>3</sub>
  - Add reagents = No color reaction
  - Add zinc dust = Red color
- NO<sub>3</sub> → N<sub>2</sub>↑
  - Add reagents = No color reaction
  - Add zinc dust = No color reaction



### Simmon's Citrate Media

- Detects organism's ability to use citrate as its sole source of carbon
- Positive
  - Blue color – alkaline end products, bromothymole blue indicator turns blue when pH increases
  - Growth on slant – with or without color change
- Negative
  - No color change and no growth on slant



### Christiansen's Urea Media

- Detects the enzyme urease  

$$\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{urease}} \text{ammonium carbonate}$$
- If the organism can hydrolyze urea, there will be an increase in pH due to the alkaline end product. The indicator, phenol red, will turn a bright pink color.
- Positive = pink color
- Negative = no color change



### Gelatin

- Detects proteolytic enzymes which hydrolyze large protein molecules into smaller peptides and peptones
- Reagent = acidic mercuric solution
- Positive
  - Clear zone adjacent to line of growth after addition of reagent
- Negative
  - A milky color in area adjacent to growth after addition of reagent

### *Klebsiella pneumonia*

- Growth on sheep blood agar is very mucoid



### *Escherichia coli*

- Growth on sheep blood agar is very often beta-hemolytic



### *Proteus mirabilis*

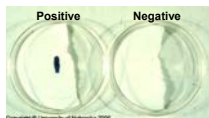
- Growth on sheep blood agar swarms across the entire plate



One colony swarms to edge of plate

### Oxidase Test

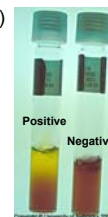
- Enzyme cytochrome oxidase in the presence of the reagent tetra-methyl- (or dimethyl-) para-phenylene-diamine-dihydrochloride to form a blue color within 30 seconds
  - Positive = blue color development within 30 seconds
  - Negative = no color development
- Must use a platinum loop or sterile wooden stick, because nichrome loops can cause a false positive
- Tetra-methyl reagent is more sensitive than dimethyl reagent



Copyright © University of Nebraska 2006

### CTA Sugars for *Neisseria* ID

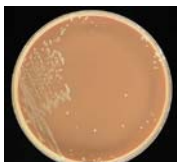
- Detects oxidation of carbohydrates (CHO)
- Indicator is phenol red
  - CHO oxidized
    - Decrease in pH, indicator turns yellow
  - CHO not utilized
    - No pH change, indicator stays red



Organism	Glucose	Maltose	Lactose	Sucrose	DNase	Nitrate
<i>N. gonorrhoeae</i>	+	-	-	-	-	-
<i>N. meningitidis</i>	+	+	-	-	-	-
<i>N. lactamica</i>	+	+	+	-	-	-
<i>M. catarrhalis</i>	-	-	-	-	+	+

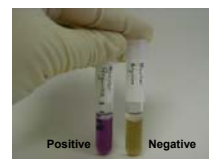
### Thayer-Martin (TM) Media

- A selective agar for *Neisseria gonorrhoeae*, *N. meningitidis*, and *N. lactamica* (normal flora)
- Agar contains antibiotics
  - Colistin – inhibits Gram negative rods
  - Vancomycin – inhibits Gram positive organisms
  - Nystatin – inhibits yeast
- Modified TM agar
  - Contains antibiotic trimethoprim/sulfamethoxazole to inhibit *Proteus* species



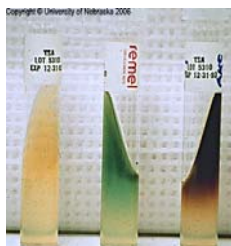
### Moeller's Decarboxylase Tests

- Detects enzymatic ability of organism to decarboxylate or hydrolyze an amino acid to form an amine
- pH increases (alkaline) and changes indicator to purple
- Amino acids tested include:
  - Arginine, Lysine and Ornithine
- Positive
  - Purple color
- Negative
  - Yellow/purple or green/purple
- Note: this media works with glucose "F", glucose "O", and glucose Non-"F" Non-"O"



### Pigment Production for *Pseudomonas*

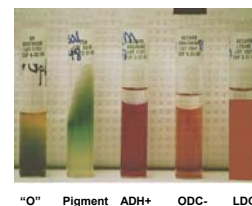
- Use Trypticase Soy Agar (TSA slant) to view pigmentation
- Pigments
  - Pyoverdinin – yellow-green / yellow-brown
  - Pyocyanin – blue
  - Combination of pyoverdinin & pyocyanin – green
  - Pyorubin – red
  - Pyomelanin – brown / black



Copyright © University of Nebraska 2006

### *Pseudomonas aeruginosa*

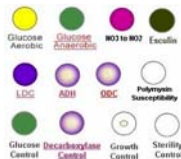
- Gram negative rod
- BAP
  - Beta-hemolytic
  - Flat, spreading colony
  - Fruity, grape-like smell
- Oxidase positive
- Glucose "O"
- Arginine dihydrolase positive
- Lysine decarboxylase negative
- Ornithine decarboxylase negative
- Nitrate variable
- Esculin hydrolysis negative
- Gelatin variable
- DNase negative
- Growth at 42°C
- Sometimes produces green or red pigment



"O" Pigment ADH+ ODC- LDC-

### *Stenotrophomonas maltophilia*

- Gram negative rod
- Oxidase negative
- Glucose "O"
- Arginine dihydrolase negative
- Lysine decarboxylase positive
- Ornithine decarboxylase negative
- Nitrate variable
- Esculin hydrolysis variable
- Gelatin positive
- DNase positive
- No growth at 42°C
- Sometimes can develop a lavender-green to light purple pigment



### X & V Quad Plate

- Used for the speciation of *Haemophilus*
- Determines requirement of X and V factors and hemolysis on horse or rabbit blood
- X Factor
  - Hemin, heat stable
- V Factor
  - NAD, heat labile
- Quadrants
  - I contains X factor
  - II contains V factor
  - III contains X & V factors
  - IV contains horse blood



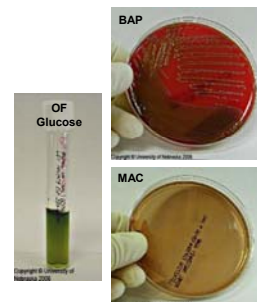
### *Haemophilus* species

- Gram negative coccobacilli
- Fastidious
  - Will not grow on sheep blood agar
  - Must use Chocolate agar
  - Requires CO<sub>2</sub>
- Porphyrin test
  - Delta-aminolevulinic acid (ALA) is substrate
  - Determines if organism has enzyme required to produce hemin (X factor)
  - If positive, organism does not require X factor

Organism	Requires X	Requires V	Hemolysis on horse blood	ALA-Porphyrin test
<i>H. Influenzae</i>	Positive	Positive	None	Negative
<i>H. Parainfluenzae</i>	Negative	Positive	None	Positive
<i>H. Hemolyticus</i>	Positive	Positive	Beta	Negative
<i>H. parahemolyticus</i>	Negative	Positive	beta	Positive

### *Pasteurella multocida*

- Gram negative rod
  - Small (can be coccoid), can exhibit bipolar staining
- No growth on MAC
- Oxidase positive
- Glucose "F" weak (apple green color)
- Nitrate positive
- ODC positive
- Indole negative
- Urease negative
- "S" to 2 units of Penicillin



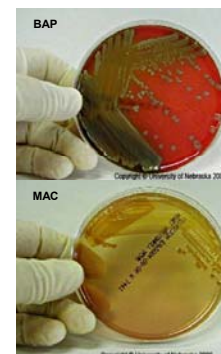
### *Campylobacter jejuni*

- Curved Gram negative rod
- Grows at both 37°C & 42°C in 10% CO<sub>2</sub> (microaerophilic)
- Oxidase positive
- Darting motility on wet prep
- Hippurate hydrolysis positive
- Nalidixic Acid "S"
- Cephalothin "R"



### *Aeromonas hydrophila*

- Gram negative rods
- Beta-hemolytic on BAP
- Grows on MAC
  - Lactose variable
- Oxidase positive
- Glucose "F"
- LDC variable
- ODC negative
- Indole positive
- Gelatin positive
- VP positive
- Esculin positive
- DNase positive



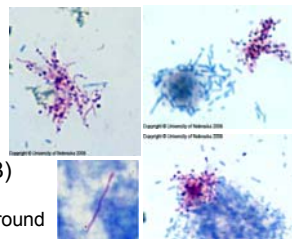
### Acinetobacter species

- Gram negative coccobacilli
- Oxidase negative
- *Acinetobacter baumannii*
  - Glucose “O”
  - Lactose “F”
  - Grows at 42°C
- *Acinetobacter Iwoffii*
  - Glucose Non-“F”, Non-“O”
  - Lactose Non-“F”
  - No growth at 42°C
- Nitrate negative
- Non-motile

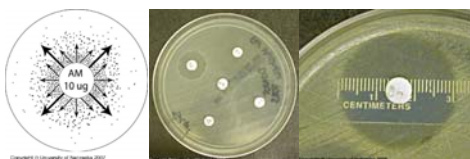


### Acid Fast Stain / Ziehl-Neelsen Stain

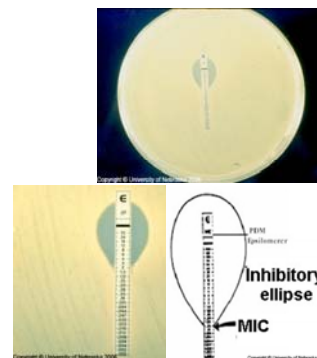
- Mycobacteria are readily stained with 1° stain, which binds to mycolic acid in their lipid-rich cell wall. This stain cannot be removed (decolorized) and organism is referred to as being acid-fast.
- Primary Stain is carbo-fuscin
- Decolorizer is acid alcohol
- Counterstain is methylene blue
- Acid Fast Bacilli (AFB)
  - Red branching rods against a blue background



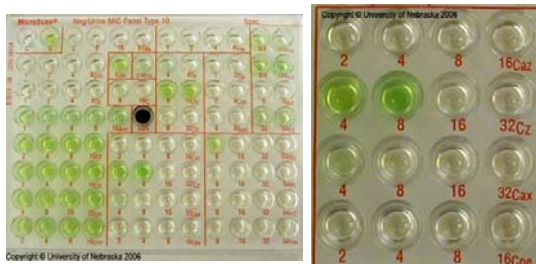
### Kirby Bauer Susceptibility



### E-Test Susceptibility



### MIC



### Bacteroides fragilis group

- Most common anaerobic organism isolated in the clinical laboratory
- Pleomorphic Gram negative rod
- Grows in 20% bile
- Hydrolyzes esculin
- Penicillin “R”
- Beta-lactamase positive



### *Clostridium perfringens*

- Anaerobic organism
- Large Gram positive rods
  - Blunt, rounded ends ("boxcar" shaped)
  - May see spores
- BAP – double zone of beta-hemolysis
- Catalase negative
- Lecithinase positive on egg yolk agar
- Causes
  - Gas gangrene
  - Food poisoning

### *Bacillus* species

- Large Gram positive rods
  - With age can appear Gram variable or Gram negative
  - Can see spores (if organism is stressed)
- Blood Agar
  - Large colonies
  - Rough edges
  - Beta-hemolytic
- Motile
- Considered a contaminant

### *Bacillus anthracis*

- Large Gram positive rods
  - Subterminal spores (not at end or in the middle)
- Blood Agar
  - Non-hemolytic
- Non-motile
- Causes anthrax

### *Listeria monocytogenes*

- Small Gram positive rods (can be coccobacilli)
- Blood agar
  - Grayish white colony
  - Small zone beta-hemolysis
- Catalase positive
  - Demonstrated on T-soy agar slant – tube catalase
- Esculin hydrolysis positive
- Hippurate hydrolysis positive
- Motility
  - Wet prep = tumbling motility
  - 25°C semisolid media = inverted umbrella

### CTA Sugars for *Corynebacterium* ID

- Detects oxidation of carbohydrates (CHO)
- Indicator is phenol red
- CHO oxidized
  - Decrease in pH, indicator turns yellow
- CHO not utilized
  - No pH change, indicator stays red

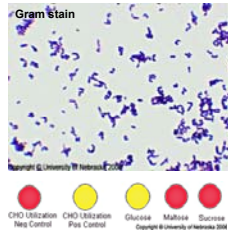
Organism	Glucose	Maltose	Sucrose	Urease	Nitrate
<i>C. diphtheriae</i>	+	+	-	-	+
<i>C. jeikeium</i>	+	+/-	-	-	-
Diphtheroids	+/-	+/-	+/-	+/-	+/-

### *Corynebacterium diphtheriae*

- Pleomorphic Gram positive rods
  - Often palisading
  - Can be club-shaped
- BAP
  - May have a very small zone beta-hemolysis
- Catalase positive
- Nitrate positive
- Glucose positive
- Maltose positive
- Sucrose negative
- Urease negative
- Note: must test for the diphtheria toxin

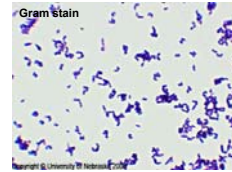
### *Corynebacterium jeikeium*

- Pleomorphic Gram positive rods
  - Often palisading
  - Can be club-shaped
- BAP
  - Non-hemolytic
  - Opaque colonies
- Catalase positive
- Nitrate negative
- Glucose positive
- Maltose variable
- Sucrose negative
- Urease negative
- Key to identification – resistant to most antibiotics used to treat Gram-positive infections. It is "S" to vancomycin
- Causes infections in immunocompromised persons



### Diphtheroids

- Pleomorphic Gram positive rods
  - Often palisading
  - Can be club-shaped
- BAP
  - Non-hemolytic
  - Opaque colonies
- Catalase positive
- Nitrate variable
- Glucose variable
- Maltose variable
- Sucrose variable
- Urease variable
- Considered normal skin flora or contaminant



### Albert's Stain

- Methylene blue stain
- Stains *Corynebacterium* species irregularly, giving a beaded appearance
- Metachromatic areas of cell stain more intensely (dark purple/black) than rest of cell and are referred to as Babès-Ernst granules (or metachromatic granules)

### Cystine-Tellurite Blood Agar (CTBA)

- Classified as a selective and differential media
- Selective for *Corynebacterium*
  - Potassium tellurite inhibit many non-coryneform bacteria
- Differential for tellurite reduction
  - *Corynebacterium* species (also Staph & Strep)
    - Colonies appear gray or black
  - *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, & *Corynebacterium pseudotuberculosis*
    - Brown halo surrounding colony due to cystinase activity

