FASTIDIOUS GRAM-NEGATIVE RODS

Objectives: After review of the Clinical Microbiology Study Manual and completion of the clinical rotation, the Clinical Laboratory Science/Medical Technology student will be able to:

1. Define the term fastidious, as used in clinical microbiology.
2. Discuss appropriate specimen collection for the isolation of *Bordetella pertussis*.
3. Evaluate the following media, including purpose, proper use, inhibitory or selective properties and colonial appearance:
   a. Blood cysteine
   b. Regan-Lowe
   c. Bordet-Gengou
   d. Buffered charcoal yeast extract (BCYE)
4. Describe specific specimen processing requirements, including special media, incubation times and any characteristic colonial morphology for each of the following:
   a. *Brucella* species
   b. *Francisella* species
   c. *Bordetella* species
   d. *Legionella* species
   e. *Capnocytophaga* species
   f. *Eikenella corrodens*
5. List the organisms referred to as the HACEK group.
6. Discuss the following techniques used to enhance Gram stain morphology:
   a. Extended safranin
   b. Substitution of carbol fuschin
7. Assess the use of immunologic methods for direct specimen detection and/or organism identification of the following organisms:
   a. *Francisella tularensis*
   b. *Bordetella pertussis*
   c. *Brucella* species
   d. *Legionella* species
8. Identify *Eikenella corrodens* based on the following characteristics:
   a. Colony morphology
   b. Glucose utilization
   c. Oxidase reaction
   d. Catalase reaction
9. Identify *Capnocytophaga* species based on the following characteristics:
   a. Colony morphology
   b. Gram stain morphology
10. Correlate the clinical, epidemiological and laboratory findings associated with the following infections:
    a. *Francisella tularensis*
       1) Tularemia
    b. *Bordetella pertussis*
       1) Pertussis (whooping cough)
    c. *Brucella* species
       1) Brucellosis (undulant fever)
    d. *Legionella* species
       1) Legionnaire’s disease
       2) Pontiac fever
    e. *Eikenella corrodens*
       1) Human bite wound
    f. HACEK group
       1) Subacute bacterial endocarditis
11. Evaluate the appropriateness of susceptibility testing of the organisms listed in objective #4.
FASTIDIOUS GRAM NEGATIVE BACILLI

General Information

- Fastidious = an organism with complex or extensive nutritional requirements
- Organisms appear as faintly staining gram negative rods
- Two methods to enhance the gram reaction
  - Extend time for safranin counterstain to at least 2 minutes
  - Substitute carbolfuschin for safranin
- Serologic methods are useful in direct detection, identification and diagnosis of disease
  - Fluorescent antibody
  - Direct agglutination
  - DNA probes
  - Enzyme immunoassay

Genus *Bordetella* (Mahon, 2nd edition, pages 457-462)

*Bordetella pertussis*

Clinical Significance

- *Bordetella pertussis* causes Whooping Cough or Pertussis in man
- Strict human pathogen, spread by airborne droplets
- Lives in ciliated epithelium of the upper respiratory tract
- Produces an exotoxin and has a cell wall endotoxin
- The major virulence factor is the pertussis toxin

Specimen collection, transport, and processing

- Nasopharyngeal swab or aspirate is the specimen of choice
- Specimen should be plated at bedside and a smear made for DFA screening
  **The number of organisms present in the secretions limits sensitivity of the screen**
- If the specimen must be transported, Regan-Lowe media is recommended for transport. It contains charcoal, horse blood and cephalaxin
- The organism is a fastidious obligate aerobe
- Need special media for isolation:
  - Bordet-Gengou = contains potato-glycerol-blood agar
  - Regan-Lowe = contains charcoal and horse blood
  - Methicillin or cephalaxin can be added to inhibit normal flora
  - Media should be incubated in a humid environment at 35°C with 5-10% CO2 for at least 7 days

Laboratory identification

- Colony morphology:
  - Bordet-Gengou = slightly beta hemolytic smooth, shiny, resembling a mercury droplet
  - Regan-Lowe = domed and shiny with a white mother-of-pearl opalescence
  - BAP = no growth
  - MAC = no growth
- Gram Morphology: small faintly staining gram negative coccobacilli
- Oxidase = positive
- Nitrate = negative
- Urea = negative
- Nonmotile
Serologic identification
• Usually identified by fluorescent antibody
• Agglutination methods are also available
• Probes are available for direct detection in the specimen and for culture confirmation

Treatment & Prevention
• Erythromycin is the drug of choice for treatment
• Vaccination is the best protection

*Bordetella parapertussis*
• Found on the mucous membranes of humans
• Causes acute respiratory tract infection resembling mild whooping cough in man

*Bordetella bronchiseptica*
• Found on the mucous membranes of animals and occasionally man
• Causes respiratory infections in animals
• Human infection seen primarily in immunocompromised patients = wound, blood, respiratory

<table>
<thead>
<tr>
<th>Gram stain morphology</th>
<th><em>Bordetella pertussis</em></th>
<th><em>Bordetella parapertussis</em></th>
<th><em>Bordetella bronchiseptica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on Bordet-Gengou</td>
<td>&quot;Mercury droplet&quot; Beta hemolytic (3-4 days)</td>
<td>Gray-brown Beta hemolytic (2-3 days)</td>
<td>Large, flat, dull Beta hemolytic (24 hrs)</td>
</tr>
<tr>
<td>Growth on Regan Lowe</td>
<td>Shiny white &quot;Mother of pearl&quot;</td>
<td>Similar to Bordet-Gengou</td>
<td>Similar to Bordet-Gengou</td>
</tr>
<tr>
<td>BAP</td>
<td>No growth</td>
<td>Growth</td>
<td>Growth</td>
</tr>
<tr>
<td>MAC</td>
<td>No growth</td>
<td>Variable</td>
<td>Growth</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>+ (24 hr)</td>
<td>+ (3-4 hrs)</td>
</tr>
<tr>
<td>Motility</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate utilization</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Genus **Brucella** (Mahon, 2nd edition, pages 443-444)

**Clinical significance**
- Four species are pathogenic to man: *B. abortus* (cattle), *B. canis* (dogs), *B. melitensis* (goats), *B. suis* (pigs)
- *Brucella* causes brucellosis (undulant fever/Malta fever), a relapsing febrile illness
- Transmission is via: Direct contact with infected animals, Ingestion of contaminated meat or dairy products, Inhalation of the aerosolized organism
- Incidence in US has declined due to vaccination of animals and pasteurization processes. Imported cheeses and candies have been implicated in US cases
- Brucella is a facultative intracellular organism

**Specimen collection, transport, and processing**
- Specimen of choice is blood, bone marrow or tissue
- **There is a risk of laboratory acquired infection**
  - Always work in a hood
  - Grows on BAP, CHOC, and BCYE (used for isolation of Legionella)
  - Blood culture mediums support growth of Brucella
  - Media should be incubated at 35°C with 5-10% CO2 with a humid atmosphere
  - Organism is very slow to grow – **hold cultures at least 21 days**

**Laboratory identification**
- Colony morphology: smooth glistening, translucent colonies that become brown with age
- Gram Morphology: tiny faint staining gram negative coccobacilli
- Oxidase: positive
- Nitrate: positive
- Catalase: positive
- Glucose oxidizer
- Non-motile

<table>
<thead>
<tr>
<th></th>
<th>Brucella abortus</th>
<th>Brucella melitensis</th>
<th>Brucella suis</th>
<th>Brucella canis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO2 requirement</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H2S (Pb acetate strip)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Urea</td>
<td>+ (&gt;2 hr)</td>
<td>Variable (&gt;2 hrs)</td>
<td>+ (0-30 min)</td>
<td>+ (0-30 min)</td>
</tr>
<tr>
<td>Growth on dye medium:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic fuchsin</td>
<td>Growth</td>
<td>Growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Growth on dye medium:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thionine</td>
<td>No growth</td>
<td>Growth</td>
<td>Growth</td>
<td>Growth</td>
</tr>
</tbody>
</table>

**Serologic identification**
- Tube agglutination test most commonly used
- Single titer of >=1:160 or a fourfold rise in titer between acute and convalescent specimens is considered significant
- Does not detect *Brucella canis*

**Treatment & Prevention**
- Recommended treatment: doxycycline and rifampin for 6 weeks
Genus **Capnocytophaga** (Mahon, 2nd edition, page 440)

**Clinical Significance**
- Part of normal oropharyngeal flora
- Associated with periodontal disease, sepsis in patients with granulocytopenia and malignancies, endocarditis, and animal bites

**Specimen Collection, Transport and Processing**
- Most frequently isolated from respiratory specimens
- Requires CO₂ for growth (capnophilic)
- Facultative anaerobe

**Laboratory Identification**
- Colony morphology
  - BAP & CHOC = slight yellow, nonhemolytic, spreading over agar surface (at 48 hrs), center of colony has moist, mottled appearance
  - MAC = no growth
- Gram stain morphology = fusiform, filamentous gram neg. rod, size and shape vary with age
- Oxidase: negative
- Catalase: negative
- Motility: “gliding motility” = do not have flagella but move by twitching

Genus **Eikenella** (Mahon, 2nd edition, page 439)

**Clinical significance**
- Only one species: *Eikenella corrodens*
- Normal flora of mouth, respiratory tract and GI tract of man.
- Opportunistic pathogen in man
- Associated with dental/periodontal and head/neck infections/abscesses, human bite wounds, septicemia following tooth extraction and endocarditis

**Specimen Collection, Transport and Processing**
- No special collection requirements
- Needs hemin in media for growth

**Laboratory identification:**
- BAP & CHOC = tiny colonies at 48 hrs
- MAC = no growth
- Colonies "pit" (corrode) the agar and are often sunk into small craters in the agar
  - Usually have a pale yellow pigment
  - May have greening around colony
  - May have a characteristic bleach odor
- Gram morphology: small, slender GNR
- Oxidase: positive
- Glucose non-oxidizer (asaccharolytic)
- Catalase: negative
- Nitrate positive
- Non-motile

**Treatment & Prevention**
- Susceptibility testing is not routinely performed
- Treat with penicillins, 3rd generation cephalosporins, tetracycline, and quinolones
Genus *Francisella* (Mahon, 2nd edition, page 444)

**Clinical Significance**
- *Francisella tularensis* causes tularemia, an acute febrile, HIGHLY INFECTIOUS disease
- Acquire by:
  - Direct contact with infected animals (rabbits)
  - Bite from an insect
  - Inhalation of infectious aerosols

**Specimen Collection, Transport and Processing**
- Specimen should be inflammatory material from the infected site (lesion, lymph node aspirate, sputum, tissue biopsy)
- Wear gloves and perform all work in a biosafety hood
- Do not aerosolize or allow contact with skin or mucous membranes
  - Organism can penetrate unbroken skin—be careful!!!
- Requires cysteine / cystine for growth
- Glucose-cystine blood (Francis') agar preferred for isolation
- Grows on Chocolate agar, buffered charcoal yeast extract (BCYE) and modified Thayer-Martin (MTM)
- Incubate at 35 C with 5-10% CO2 for 7 days (strict aerobe)

**Laboratory identification**
- Colony morphology
  - BAP = No growth
  - Choc = Small, gray alpha hemolytic (if on blood containing media) colony at 2-5 days
  - MAC = No growth
- Gram morphology: pale staining gram negative coccobacilli
- Oxidase: negative
- Catalase: negative-weak pos
- Ferments glucose
- Non-motile

**NOTE:** Usually identified by DFA or direct agglutination tests due to risk of laboratory acquired infection. Biochemical identification is usually performed in reference laboratories.

**Serologic testing**
- Most cases diagnosed serologically
- DFA tests may be performed on specimen
- Antibody titers can be determined by ELISA and agglutination tests
- A four-fold rise in titer between acute and convalescent specimen is considered diagnostic
- A single acute phase titer of 1:160 or greater is considered presumptive

**Treatment & Prevention**
- Streptomycin is drug of choice for treatment.
Genus **LEGIONELLA**  (*Mahon, 2nd edition, pages 447-456*)

**Clinical Significance**
- There are two forms of Legionellosis:
  - **Legionnaires’ disease**  Characterized by malaise, myalgia with rapid onset of a dry cough and fever, and development of pneumonia
    Illness is fatal in 15-30% of cases not treated
  - **Pontiac Fever**  Characterized by fever, headache, myalgia and malaise
    Lacks symptoms of pneumonia and is not fatal
- Disease occurs most frequently in men, cigarette smokers, people with underlying disease, immunosuppressed/immunocompromised, people who drink alcohol
- Major cause of nosocomial pneumonia
- Organism exists in natural/man-made water systems and in soil
- Transmission: inhalation of the organism in aerosols
- **Legionella pneumophila**  serogroup 1 causes most human infection

**Specimen Handling and Processing**
- Appropriate specimens: BAL, bronchial washing, lung biopsy, pleural fluid
- Avoid aerosolization and transport ambient temperature
- Organism requires cysteine and iron salts for growth
- Buffered Charcoal Yeast Extract agar (BCYE) is most widely used
- BCYE + antibiotics is used as a selective media for contaminated specimens
- Incubate at 35 C in 5-10% CO2 with increased humidity for 10 days
- Organisms usually take 2-4 days to grow

**Laboratory Identification**
- Colony Morphology:
  - BAP & MAC = no growth
  - CHOC = may grow very slowly
  - BCYE = convex, grayish white, glistening with an entire edge at 2-4 days
    When viewed with dissecting microscope they have a "ground glass" appearance with a pink or blue-green tint
- Gram stain morphology: thin, faintly staining gram negative short to filamentous rods
- Oxidase and Catalase: weakly positive
- Gelatin: positive (most species)
- Motility: positive by polar flagella
- Does not oxidize or ferment carbohydrates = biochemically inert
- Some species fluoresce under UV light, color varies depending on the species

**Serologic diagnosis**
- Direct screen of specimen can be done with DFA stain and DNA probe
- Identify isolate with DFA or DNA probe
- IFA test of choice
  - Need to demonstrate a four-fold rise in titer to at least 1:128
  - Draw acute specimen within 7 days of onset
  - Draw convalescent specimen 3-5 weeks after onset

**Treatment & Prevention**
- Susceptibility testing is not routinely performed
- Drug of choice: Erythromycin alone or with Rifampin
HACEK Group  (*Mahon, 2nd edition, pages 436-440*)

**Organisms include**
- *Haemophilus aphrophilus*
- *Actinobacillus actinomycetemcomitans*
- *Cardiobacterium hominis*
- *Eikenella corrodens*
- *Kingella kingae*

**Clinical Significance**
- Infective endocarditis
- Usually diagnosed in Blood Cultures
- Hold cultures for extended period beyond 1 week and make blind subcultures to several enriched media, including buffered charcoal-yeast extract
GRAM NEGATIVE ROD (GNCB)

Glucose fermentation

(+) Oxidase (-) Glucose oxidation

(+)

OX MOT MAC
Aeromonas + + + Enterobacteriaceae
Vibrio + + +
Plesiomonas + + +
Pasteurella + - -
Actinobacillus +/- - +/-

(-)

OX MOT MAC
Pseudomonas + + + Eikenella + - -
**Flavobacterium + - +/- Moraxella + - -
Acinetobacter - - + Alcaligenes + + +
Stenotrophomonas - + + Acinetobacter lwoffii - - +

**Some species capable of weak glucose fermentation