RESPIRATORY CULTURES – SPUTUM PROCEDURE
Student laboratory use only, not for use during rotations

I. Principle
Expectorated sputum specimens are cultured in an attempt to identify the etiologic agents of bacterial pneumonia (lower respiratory infection). Expectorated sputum samples can often be contaminated by normal oropharyngeal flora as part of the collection process making diagnosis difficult. In addition, if improperly collected, samples may actually represent spit rather than lung secretions. An evaluation of the specimen gram stain is made to determine the acceptability of a sputum sample. A poorly collected specimen will contain many squamous epithelial cells, which are indicative of oral contamination. Most pathogens of the lower respiratory tract can also be normal flora of the upper respiratory tract. Normal flora exists commensally with the host. Normal flora is isolated from the patient in the absence of disease. Under normal conditions a balance of organism is maintained that limits both quantity and predominance of any one organism. Normal flora plays an important role in protecting the host from pathogenic organisms.

II. Specimen Collection, Transport and Handling
A. Specimen type and collection
1. Sputum - a specimen resulting from a deep cough (first morning specimen preferred), frequently contaminated with oropharyngeal flora

B. Specimen transport and handling
1. Specimens should be placed in sterile containers with tightly fitted lids and transported to the lab as soon as possible

III. Direct Examination
A. Gram stain
1. A gram stain is performed on all sputum specimens before plating to determine acceptability of the specimen (presence of many squamous epithelial cells indicates poorly collected specimen containing spit rather than lung secretions).
2. Method
   a. Using a sterile swab, sample from the sputum container and roll swab onto a slide. Allow to air dry.
   b. Stain slide using Gram stain procedure
   c. Evaluate slide on Low Power for squamous epithelial cells
      i. If >25 epithelial cells/LPF are seen, the doctor is notified that the sputum is being rejected due to oral contamination. A new specimen is requested.
      ii. If <25 epithelial cells/LPF are seen, proceed with setting up culture.
   d. Evaluate slide under oil immersion for bacteria, PMN's, and other cells
3. Report gram stain results as part of respiratory culture

B. The sputum specimen gram stain result is helpful in determining the significance of the isolates present. Characteristics of the specimen gram stain can help distinguish between colonization and infection.
   1. A good sputum specimen will contain many PMN's (in a person with a normal immune system) and relatively few squamous epithelial cells.
   2. A poorly collected specimen will contain squamous epithelial cells, which are indicative of oral contamination.

IV. Culture Setup
A. Inoculate plates
   1. Sputum should be plated on the following:
      a. Blood agar
      b. Chocolate agar
c. MacConkey agar (or other selective/differential gram negative medium)
d. Use a swab to inoculate plates using representative areas of the specimen. When plating
the specimen, sample areas that are purulent or bloody. Avoid areas that look like saliva.
Inoculate the media using a swab to making the primary streak then use a sterile loop to
streak for isolation. Non inhibitory media should always be inoculated first.

B. Incubate media
1. Temperature: 35ºC
2. Atmosphere: BAP and CHOC - CO2, MAC - ambient air
3. Time: overnight incubation 18-24 hours, incubated a minimum total of 48 hours

C. Potential Bacterial Pathogens
1. Lower respiratory tract
   a. *Streptococcus pneumoniae*
   b. *Haemophilus influenzae*
   c. *Klebsiella pneumoniae* and other enteric bacilli
   d. *Staphylococcus aureus*
   e. *Pseudomonas aeruginosa* and other gram negative bacilli
   f. Yeast
   g. *Mycobacteria* species (special request culture)
   h. *Nocardia* species (usually a special request)
   i. *Moraxella (Branhamella) catarrhalis*
   j. *Legionella pneumophila* (special request culture)
   k. Anaerobic organisms

D. Normal Flora
1. Upper respiratory tract
   a. Coagulase negative *Staphylococcus* species
   b. *Streptococcus* species viridans group
   c. *Neisseria* species - other than N. gonorrhoeae
   d. *Enterococcus* species and Non-Enterococcus (non-hemolytic) species
   e. *Micrococcus* species
   f. *Stomatococcus* species
   g. *Capnocytophaga* species
   h. Diphtheroids
   i. Anaerobic organisms
   j. Yeast (in rare to few amounts)
   k. Enteric gram-negative rods (in rare amounts)
   l. *Haemophilus* species (in rare amounts)
   m. *Staphylococcus aureus* (in rare amounts)
   n. *Streptococcus pneumoniae* (in rare amounts)

   **Note:** The above list is only a general guideline and exceptions may occur.

2. Lower respiratory tract, i.e., bronchi and lungs
   a. Normally sterile

V. Culture Interpretation
A. Quantitation
1. Guidelines for estimating amount of growth of an individual organism:
   a. rare - growth present in first quadrant only (<15 colonies)
   b. few - growth present in quadrants 1 and 2 (>15 colonies in quadrant 1 and <15 colonies
      in 2nd quadrant)
   c. moderate - growth in quadrants 1, 2, and 3 (>15 colonies in 2nd quadrant and <15
      colonies in quadrant 3)
   d. many - confluent growth, extending to all 4 quadrants.
B. Culture work up

The following is a list of general guidelines to follow when interpreting sputum cultures:

For all isolates, identify to the appropriate level per source. **Stop identification when you have ruled out potential pathogens.**

1. **Sputum** - as many organisms that are considered potential pathogens in a sputum culture are also part of the normal oropharyngeal flora, an attempt must be made to distinguish that which is pathogenic versus normal flora. Under normal conditions, a balance of organisms is maintained that limits both the quantity and the predominance of any one organism.
   a. Moderate or many of potential pathogens
      - Perform and report identification and appropriate susceptibility testing
   b. Rare to few amounts of potential pathogen
      - If other normal respiratory flora is present – report as normal flora
      - If no other normal respiratory flora is present – perform and report identification and appropriate susceptibility testing
   c. Pure culture or predominance of a potential pathogen
      - Perform and report identification and appropriate susceptibility testing
   d. Report total number of Normal respiratory flora that is present.

2. **Gram positive cocci** –
   a. See *Streptococcaceae* and *Staphylococcus* identification charts. **Stop identification process when you have ruled out significant pathogens from the source, S. aureus, Strep. pneumoniae, Beta-hemolytic strep.**
      - For **coagulase-positive, catalase-positive**, colonies with morphology consistent with *S. aureus* (beta-hemolytic, yellow colonies) in moderate to many amounts, report *S. aureus*. Susceptibility testing using growth from an 18-24 hour pure culture would typically be performed, but will not be done in student lab. Rare or few amounts can be reported as normal flora.
      - For **coagulase-negative, catalase-positive**, colonies with morphology consistent with coagulase negative staphylococcus in any amount report as Normal respiratory flora. No further testing needed.
      - Catalase negative
        o For **optochin-sensitive**, or **bile solubility positive** alpha hemolytic colonies with morphology consistent with *Streptococcus pneumoniae* report identification. KB susceptibility using Oxacillin disc to determine resistance to penicillin would typically be performed at this point, but will not be done in student lab. Rare or few amounts can be reported as normal flora.
        o For optochin-resistant, or bile solubility negative alpha hemolytic or gamma hemolytic colonies with morphologies inconsistent with *Streptococcus pneumoniae* report out as normal flora. No susceptibilities would be performed.

3. **Gram negative rods**
   a. Perform Oxidase and Glucose OF to determine if organism is an oxidizer, fermenter, or non fermenter unless organism has colony morphology consistent with Haemophillus species (gray mousy color)
      See Gram negative rod flowcharts. Susceptibility testing will not be performed in student lab.
      - **Enteric gram-negative rods**
        o Identify when present as the only isolate or in moderate to many amounts. Do not work up if present with large amounts of normal oral flora, or when there are many epithelial cells seen on the gram stain.
      - **Pseudomonas** and non-fermenting gram-negative rods-
        o Identify when present in moderate to many amounts or predominant.
        o Identify in any amount when no normal flora is present.
• **Haemophilus** –
  o Identify when isolated from immunocompromised patients, elderly patients, children, or when present as the predominant organism in a sputum in which many PMN's were seen on the gram stain. When present with mixed normal oral flora in adults that are not immunocompromised and without symptoms of pneumonia, *Haemophilus* species can be considered part of the normal flora.

4. Gram negative diplococci
   a. Identify if present in moderate to many amounts or predominant (See Gram negative Diplococci flowchart)
   b. Perform Oxidase
   c. If oxidase positive, suspect *Moraxella catarrhalis*, especially if the specimen gram stain shows intracellular gram negative diplococci

C. When any of the normal flora organisms are isolated from a sputum specimen, the amounts of each organism are combined and reported as “normal respiratory flora”.

D. **Report results**
   1. Correlate all information – Does it make sense?
   2. Preliminary report – include:
      a. As much information about organism identification as possible
      Example:
      Many Non-lactose fermenting oxidase positive gram negative rods (Identification and Susceptibility to follow)
      Moderate Normal Respiratory Flora
   3. Final report – include:
      a. Organism identification (if pathogen)
      b. Organism susceptibility results (if pathogen)
      Example:
      Many Pseudomonas aeruginosa, MIC results
      Moderate Normal Respiratory Flora

E. After 24 hours, culture plates are re-incubated. After 48 hours, culture plates are discarded (if all organism workups are completed).

**IX. References**
A. Textbook of Diagnostic Microbiology, Mahon & Manuselis, 2nd edition, Chapter 26, pages 877-918.