RESPIRATORY CULTURES PROCEDURE – FOR ROTATIONS

I. Principle
Respiratory specimens are cultured in an attempt to identify the etiologic agents of bacterial pneumonia (lower respiratory infection). Expectorated sputum samples are commonly collected to diagnosis the etiologic agent of pneumonia. 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. If a specimen is grossly contaminated a new specimen should be collected.

In addition to determining specimen acceptability, the specimen gram stain can assist in determination of clinical significance of a particular isolate since most pathogens of the respiratory tract can also be normal flora or the respiratory tract. Bronchoalveolar lavage, washings or brushings minimizes the contamination with upper respiratory flora and therefore increases isolation of the etiologic agent. However, the procedure is invasive. If bronchoalveolar lavage, washing or brushings fail to diagnosis the etiologic agent of pneumonia, a percutaneous transtracheal aspiration or lung biopsy may be collected. These are extremely invasive procedures, but will yield a sterile specimen, free of contamination from the normal flora of the upper respiratory tract.

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.

Some respiratory pathogens can be easily overgrown by normal flora or require supplemented media for isolation, for this reason if a physicians suspects Bordetella pertussis, Legionella sp. and Corynebacterium diphtheria it must be noted on requisition or specific order placed.

II. Specimen Collection, Transport and Handling
A. Specimen type and collection
1. Sputum - a specimen resulting from a deep cough, frequently contaminated with oropharyngeal flora (first morning specimen preferred).
2. Bronchial washings and brushings - collected through the lumen of a bronchoscope that can be directed to the site of a lesion or drainage. This method minimizes contamination with upper respiratory flora.
3. Percutaneous transtracheal aspiration - bypasses oropharyngeal contamination completely. A needle is inserted through the skin into the trachea and a catheter is inserted to aspirate any secretions present. (This sample is sterile, it is acceptable for anaerobic culture, the specimen is usually processed following Body Fluid/Tissue procedures)
4. Needle or open biopsy of lung - also bypasses contamination of specimen. (This sample is sterile, it is acceptable for anaerobic culture, the specimen is usually processed following Body Fluid/Tissue procedures).

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.
2. Refrigerate if >2 hours and for no longer than 24 hours prior to culture setup
3. Specimens collected for anaerobic culturing should be processed immediately or held under anaerobic conditions until processed.
   a. Sputum, bronchial washings, throat and nasopharyngeal swabs are not suitable for anaerobic culture as they are contaminated with normal oral flora that includes anaerobes, making interpretation of results impossible.
   b. Only transtracheal aspirates and lung biopsies should be cultured for anaerobes and are usually ordered and processed as Body Fluid/Tissue cultures.
III. Direct Examination

A. Gram stain
   1. A gram stain is performed on all respiratory specimens. Sputum samples are evaluated by gram stain for acceptability of specimen (presence of many squamous epithelial cells indicates poorly collected specimen containing spit).
   2. Method
      a. Sputum
         i. Using a sterile swab, sample from the container and roll swab onto a slide. Select the most purulent or blood tinged portion of the specimen.
         ii. Stain slide using gram stain procedure.
         iii. Evaluate slide on Low Power for cells including squamous epithelial cells
         iv. 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.
            • Rejection criteria is dependent on site specific procedures, some labs use combination of presence of WBC’s and squamous epithelial cells to determine acceptability of specimen for culture.
         v. If <25 epithelial cells/LPF are seen, proceed with setting up culture
         vi. Evaluate slide under oil immersion for bacteria, PMN’s, and other cells using gram stain procedure.
      b. Bronchial specimens
         i. Prepare cytocentrifuge prep for Gram stain.
         ii. Using gram stain procedure, stain and evaluate slide.
      c. Transtracheal aspirates/Lung biopsy
         i. See Body Fluids/Tissues procedure.
   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 many 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.
   2. Bronchial washing should be plated to the following:
      a. Blood agar
      b. Chocolate agar
      c. MacConkey agar (or other gram negative media)
      d. Using a 0.001 calibrated loop streak the BAP, MAC and Choc.
         • Make a single streak down the center of the plates.
         • Using the same loop, use a sweeping motion to streak the plates perpendicular to the primary inoculum without lifting the loop.
3. Percutaneous transtracheal aspiration should be plated on the following:
   a. Blood agar
   b. Chocolate agar
   c. MacConkey agar (or other selective/differential gram negative medium)
   d. Thioglycollate broth
   e. Anaerobic media
   f. See Body fluid procedure for processing of fluids received for culture.
4. Needle or open biopsy of lung should be plated on the following:
   a. Blood agar
   b. Chocolate agar
   c. MacConkey agar (or other selective/differential gram negative medium)
   d. Thioglycollate broth
   e. Anaerobic media
   f. See Wounds procedure for the processing of Tissue samples.

B. Incubate media
   1. Temperature: 35ºC
   2. Atmosphere: BAP and CHOC - CO2, MAC and Thio- ambient air, Anaerobic media in anaerobic conditions
   3. Time: overnight incubation 18-24 hours, incubated a minimum total of 48 hours Anaerobic plates minimum of 72 hours.

C. Common Potential 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. Non-pathogenic Neisseria species
      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 amounts)
      k. Enteric gram-negative rods (in rare amounts)
      l. Haemophilus influenzae (in rare amounts)
      m. Haemophilus species, not influenzae
      n. Staphylococcus aureus (in rare amounts)
      o. Streptococcus pneumoniae (in rare amounts)

**Note:** The above list is only a general guideline and exceptions may occur.
2. *Neisseria meningitidis* is often found in non-sterile respiratory specimens (in adults), but not included in the term "normal flora". *Neisseria meningitidis* may exist in the "carrier state" in certain individuals. If *N. meningitidis* is found in addition to normal respiratory flora, no further work up is necessary. See site specific procedures for the reporting guidelines for this organism.

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

V. Culture Interpretation
   A. Quantitation (Sputum Culture work-up)
      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. Sputum 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 pathogens in a sputum culture are also part of the normal oropharyngeal 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 unless CF patient
            • If no other normal respiratory flora is present – perform and report identification and appropriate susceptibility testing
         c. Pure culture or predominant organism is present of a potential pathogen perform and report identification and appropriate susceptibility testing
         d. **Cystic Fibrosis Patients** - organisms commonly isolated as pathogens include *Pseudomonas aeruginosa* and *Staphylococcus aureus*, these are worked up with an identification and sensitivity whenever present regardless of the quantitation. Other organisms that may be present as pathogens include *Haemophilus influenzae*, *Burkholderia cepacia* and other *Pseudomonas* species. It is important to note on the requisition the colony morphology of all *Pseudomonas* species (i.e., rough, smooth, mucoid).
         e. 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.
      • **Staphylococcus aureus**
        o Identify and perform appropriate susceptibility testing when:
          ▪ Present in moderate to many amounts or predominant
          ▪ Present in any amount when no normal flora is present
          ▪ Present in any amount when isolated from CF patient
        o For **coagulase-positive, catalase-positive**, colonies with morphology consistent with *S. aureus* (beta-hemolytic, yellow colonies) report *S. aureus* and perform susceptibility testing using growth from an 18-24 hour pure culture.
o Rare or few amounts can be reported as normal flora unless no other normal flora is present.

- **Coagulase negative staphylococcus**
  o 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.

- **Streptococcus pneumoniae**
  o Identify and perform appropriate susceptibility testing when:
    ▪ Present in moderate to many amounts or predominant
    ▪ Present in any amount when no normal flora is present
  o For **catalase-negative, optochin-sensitive, or bile solubility positive** alpha hemolytic colonies with morphology consistent with Streptococcus pneumonia perform KB susceptibility using Oxacillin disc to determine resistance to penicillin.
  o For optochin-resistant, or bile solubility negative alpha hemolytic or gamma hemolytic colonies with morphologies inconsistent with **Streptococcus pneumonia** report out as normal flora. No susceptibilities performed.

- **Beta-hemolytic streptococcus**
  o Identify when:
    ▪ Present in moderate to many amounts or predominant
    ▪ Present in any amount when no normal flora is present
  o No susceptibilities performed as beta-hemolytic streptococcus are still routinely susceptible to penicillin.

3. **Gram negative rods**
   a. Perform Glucose OF to determine if organism is an oxidizer, fermenter, or non fermenter unless organism has colony morphology consistent with Haemophilus species (gray mousy color)
      See Gram negative rod flowcharts.
   - **Enteric gram-negative rods** - perform identification and perform appropriate susceptibility testing when present as the only gram negative rod 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 other Non-fermenting gram-negative rods**
     o Identify and perform appropriate susceptibility testing when:
       ▪ Present in moderate to many amounts or predominant
       ▪ Present in any amount when no normal flora is present
       ▪ Isolated in few amounts as the only gram negative rod
   - **Haemophilus**
     o Identify when:
       ▪ Present in moderate to many amounts
       ▪ Isolated from immunocompromised patients, elderly patients or children
       ▪ Present as the predominant organism in a sputum in which many PMN’s were seen on the gram stain
     o Perform Beta-lactamase testing on all reported isolates of **Haemophilus influenzae**
     o **Haemophilus** species other than influenzae can be considered part of the normal flora
     o 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
     - Perform Oxidase
• If oxidase positive, suspect *Moraxella catarrhalis*, especially if the specimen gram stain shows intracellular gram negative diplococci – perform appropriate identification tests (See Gram negative Diplococci flowchart)
• Perform Beta-lactamase testing on all reported isolates of *Moraxella catarrhalis*

5. **Yeast** - when yeast is in moderate to many amounts or the only isolate, perform a germ tube. For germ tube negative yeasts, isolate and send to mycology for identification. A small amount of yeast present with mixed oral flora is normal.

6. 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”.

C. **Bronchial Specimens Culture Work-up**
For all isolates, identify to the appropriate level per source. **Stop identification when you have ruled out potential pathogens.**
1. Bronchial Wash specimens should have less contamination of oropharyngeal flora than sputum samples.
2. Colony counts are determined
   a. Multiply number of colonies (0.001 ml loop) times 1000 to arrive at colony count
   b. Quantitate the amount of normal respiratory flora and potential pathogens.
   c. If >10,000 col/mL of a potential pathogen is present report colony count and identification and appropriate susceptibility.
   d. Report colony count of total number of colonies that are normal respiratory flora.
   e. See above for identification of isolates.

D. **Percutaneous transtracheal aspiration and Needle or open biopsy specimens Work-up**
1. Specimens should be sterile, all potential pathogens identified and appropriate susceptibilities performed.
3. See above for identification of isolates.

E. **Report results**
1. Correlate all information – Does it make sense?
   a. Preliminary report – include:
      i. As much information about organism identification as possible
         Example:
         Many *Staphylococcus* species (Identification and Susceptibility to follow)
         Moderate Normal Respiratory Flora
   b. Final report – include:
      i. Organism identification (if pathogen)
      ii. Organism susceptibility results (if pathogen)
         Example:
         Many *Staphylococcus aureus*, MIC results
         Moderate Normal Respiratory Flora

F. After 24 hours, culture plates are reincubated. After 48 hours, culture plates are discarded (if all organism workups are completed).

VI. **References**
A. Textbook of Diagnostic Microbiology, Mahon & Manuselis, 3rd edition, Chapter 32, pages 899-934.