1. Purpose
   1.1. To provide accurate specimen collection information to the units as part of our on-line collection manual

2. Principles
   2.1. Proper selection, collection, and handling of specimens for microbiology is necessary to ensure quality results that have the greatest impact on patient care.

3. Procedure
   The following page(s) have been posted on the on-line collection manual.
   - Note that much of the collection information was taken from the Clinical Microbiology Procedures Handbook. The authors have written the following in their manual for collection procedures "the following can be coped for preparation of a specimen collection instruction manual for physicians and other caregivers"

4. References

Departments: Microbiology

Written By: Tonia Lowe
Effective Date: 12/06/2012
Date: 11/29/2012
Respiratory Specimens

- A physician should perform any collection method requiring an invasive technique. Only a physician specialist with advanced training and skills should perform some specimen collection techniques.
- All patient specimens must be handled as containing potential biohazards.

1. General Considerations:
   a. Upper respiratory specimens for bacterial culture (e.g. throat specimens, nasopharyngeal samples, nasal discharge) are usually contaminated with normal flora.
      1) Upper respiratory specimens do not provide clinically useful information for the diagnosis of lower respiratory infections.
      2) Upper respiratory specimens for bacterial culture must specify the pathogen suspected (e.g. beta strep culture, isolation culture for MRSA, or Candida spp. for thrush)
   b. Notify the laboratory when a specimen for the detection of diphtheria is being sent to the laboratory.
   c. Specimens should be obtained before antibiotics/antimicrobials are administered.

2. Appropriate respiratory specimen types for disease or condition:

<table>
<thead>
<tr>
<th>Disease or Condition</th>
<th>Acceptable Specimen(s)</th>
<th>Disease or Condition</th>
<th>Acceptable Specimen(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces</td>
<td>Needle and syringe aspirate or surgical drainage; lab will reject syringes with the needle attached</td>
<td>Fungal infection (not thrush)</td>
<td>Sputa Endotracheal aspirate Lung biopsy Lung aspirate Bronchoscopic specimens</td>
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<tr>
<td></td>
<td>Sputa Endotracheal aspirate Lung biopsy Lung aspirate Bronchoscopic specimens</td>
<td></td>
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<tr>
<td>Candidiasis (oral thrush)</td>
<td>Swab of buccal mucosa, tongue, or oropharynx</td>
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<tr>
<td>Cystic fibrosis</td>
<td>Deep throat swab Sputa Endotracheal aspirate Lung biopsy Lung aspirate Bronchoscopic specimens</td>
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<tr>
<td>Diphtheria</td>
<td>Nasopharyngeal swab – call lab</td>
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</tr>
<tr>
<td>Epiglottitis</td>
<td>Blood culture</td>
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<tr>
<td>Esophagitis</td>
<td>Biopsy sample</td>
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</tbody>
</table>

3. **Throat swab**
   a. Specimens should be obtained before antibiotics or antihistamines are administered.
   b. Choose transport media:
      1) Bacterial culture: use an aerobic (white top) culturette swab
      2) Viral culture: Throat swabs for viral culture have less recovery of pathogens than nasopharyngeal samples. Use M4 media.
   c. Collect sample
      1) Position the patient so the oral cavity is well lighted. Instruct the patient to breath deeply and depress the tongue with a tongue blade.
      2) Remove the swab and guide the swab to the posterior pharynx. **Do not touch the tongue, cheeks, or uvula.**

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<table>
<thead>
<tr>
<th>Disease or Condition</th>
<th>Acceptable Specimen(s)</th>
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</thead>
<tbody>
<tr>
<td>Lemierre’s disease</td>
<td>Blood culture</td>
</tr>
<tr>
<td>Meningococcal carriage</td>
<td>Oropharyngeal swab with comment to rule out N. meningitidis carriage</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
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<tr>
<td></td>
<td>Sputa</td>
</tr>
<tr>
<td></td>
<td>Endotracheal aspirate</td>
</tr>
<tr>
<td></td>
<td>Lung biopsy</td>
</tr>
<tr>
<td></td>
<td>Lung aspirate</td>
</tr>
<tr>
<td></td>
<td>Bronchoscopic specimens</td>
</tr>
<tr>
<td>Pertussis</td>
<td>Call laboratory for pertussis kit: Nasal wash, nasal aspirate, or nasopharyngeal swab. Media inoculated bedside.</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>Pleural fluid</td>
</tr>
<tr>
<td>Pneumonia, bronchitis</td>
<td>Sputa</td>
</tr>
<tr>
<td></td>
<td>Endotracheal aspirate</td>
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<tr>
<td></td>
<td>Lung biopsy</td>
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<td></td>
<td>Lung aspirate</td>
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<tr>
<td></td>
<td>Bronchoscopic specimens</td>
</tr>
<tr>
<td>Pneumonic tularemia</td>
<td>Sputa</td>
</tr>
<tr>
<td></td>
<td>Endotracheal aspirate</td>
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<td></td>
<td>Lung biopsy</td>
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<tr>
<td></td>
<td>Lung aspirate</td>
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<tr>
<td></td>
<td>Bronchoscopic specimens</td>
</tr>
<tr>
<td>Pneumonic plague</td>
<td>Oropharyngeal swab</td>
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<tr>
<td></td>
<td>Sputa</td>
</tr>
<tr>
<td></td>
<td>Endotracheal aspirate</td>
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<td></td>
<td>Lung biopsy</td>
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<tr>
<td></td>
<td>Lung aspirate</td>
</tr>
<tr>
<td></td>
<td>Bronchoscopic specimens</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>Maxillary sinus puncture and aspiration</td>
</tr>
<tr>
<td></td>
<td>Rigid endoscopy (not nasal wash nor drainage)</td>
</tr>
<tr>
<td>Staphylococcal carriage</td>
<td>Nasal swab ordered as an isolation culture to rule out “staph” or “MRSA”</td>
</tr>
<tr>
<td>Streptococcal carriage</td>
<td>Nasopharyngeal swab requested for beta strep culture</td>
</tr>
<tr>
<td>Vincent’s angina</td>
<td>Oropharyngeal swab</td>
</tr>
<tr>
<td>Viral infection</td>
<td>Sputa</td>
</tr>
<tr>
<td></td>
<td>Endotracheal aspirate</td>
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<tr>
<td></td>
<td>Lung biopsy</td>
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<td></td>
<td>Lung aspirate</td>
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<tr>
<td></td>
<td>Bronchoscopic specimens</td>
</tr>
<tr>
<td></td>
<td>Nasopharyngeal swab in M4 media</td>
</tr>
</tbody>
</table>

3) Vigorously swab the posterior pharynx, tonsils, and inflamed areas to remove organisms adhering to the mucosal membrane. Having the patient say “Ah” lifts the uvula and decreases the gag reflex.

4) Return the swab to the tube.

d. A minimum of two patient identifiers are required on all specimens. Follow all labeling policies.

e. Upper respiratory specimens for bacterial culture must specify the pathogen suspected (e.g. beta strep culture).

f. Do not delay delivery to the lab. While the culturette transport media maintains viability of many organisms for 24 hours, overgrowth of upper respiratory flora may affect results.

4. **Nasopharyngeal swab**

a. Specimens should be obtained before antimicrobials or antihistamines are administered.

b. Choose transport media:
   
   1) Bacterial culture: use an aerobic (white top) culturette swab
   2) Viral culture: Nasopharyngeal swabs for viral culture have less recovery of pathogens than nasopharyngeal aspirates or washes. Use M4 media.

c. Collect sample
   
   1) With the patient’s head tilted back, remove the swab and guide the swab up through the nasal passages to the posterior nasopharynx.
   2) For culture
      
      a) Return the swab to the tube.

d. Upper respiratory specimens for bacterial culture must specify the pathogen suspected (e.g. beta strep culture).

e. A minimum of two patient identifiers are required on all specimens. Follow all labeling policies.

f. Do not delay delivery to the lab. While the culturette transport media maintains viability of many organisms for 24 hours, overgrowth of upper respiratory flora may affect results.

5. **Nasopharyngeal aspirate**

a. NPak method: Syringe aspiration kits are available through distribution.

   1) Position the patient - The patient should lie on their back with neck extended to allow pooling of the aspirate in the nasopharynx.
   2) Attach the leur catheter and (for regular culture) generously lubricate. RSV and influenza testing is affected by lubricant in the sample (increases indeterminate results), do not use generous amounts of lubricant for these samples.

   3) Expel the appropriate volume of saline from the syringe according to age or patient comfort.
      
      a) 0-3 years old: 1 cc
      b) 3-10 years old: 2 cc
      c) 10 – adult: 3 cc
4) Instruct the patient to hold their breath. Advance the catheter along the floor of the nose to the age appropriate marking that are on the catheter or until resistance is met abutting the nasopharynx.

5) The syringe plunger is then pushed and pulled. The aspirate sample is adequate if at least 1cc is collected.

6) For RSV, influenza, or viral culture, place 2-3 cc of the sample in the appropriate transport media (M4 media)

7) A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay.

b. Non-NPak method

1) Attach mucus trap to suction outlet and sterile catheter, leaving wrapper on catheter

2) Without applying suction and with the patient’s head tilted approximately 70°, remove the wrapper and insert the catheter into the nose, directed posteriorly and toward the opening of the external ear.

3) Apply suction as follows:

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Catheter Size* (French)</th>
<th>Suction Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature infant</td>
<td>6</td>
<td>80-100 mmHg</td>
</tr>
<tr>
<td>Infant</td>
<td>6</td>
<td>80-100 mmHg</td>
</tr>
<tr>
<td>Toddler/Preschooler</td>
<td>8</td>
<td>100-120 mmHg</td>
</tr>
<tr>
<td>School age</td>
<td>8</td>
<td>100-120 mmHg</td>
</tr>
<tr>
<td>Adolescent/Adult</td>
<td>8</td>
<td>100-120 mmHg</td>
</tr>
</tbody>
</table>

*The depth of insertion necessary to reach the posterior pharynx is equal to the distance between the anterior nares and the external opening of the ear. The catheter should remain in the nasopharynx for a minimal period of time, not to exceed 10 seconds.

4) Using a rotating motion, slowly withdraw the catheter. Hold the trap upright to prevent secretions from going into the pump.

5) Rinse the catheter with approximately 2.0 mL physiologic saline.

6) Place 2-3 cc of the sample in the appropriate transport media (M4 media).

7) A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay

6. Sputum – expectorated

a. DO NOT have the patient rinse mouth and gargle prior to sputum collection.

b. Instruct the patient not to expectorate saliva or post nasal discharge into the container

c. Collect specimen resulting from deep cough in a sterile leakproof cup.

d. A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay

7. Sputum – induced (for detection of *Pneumocystis carinii* or *Mycobacterium tuberculosis*)

a. Using a wet toothbrush and sterile water or saline, brush the buccal mucosa, tongue, and gums for 5 to 10 minutes prior to the procedure. Do not use tap water nor toothpaste.

b. Rinse the patient’s mouth thoroughly with sterile water or saline.
c. Using an ultrasonic nebulizer, have the patient inhale 20 to 30 mL of 3% NaCl solution.
d. Collect the induced sputum in a sterile leakproof cup.
e. A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay

8. **Tracheostomy and endotracheal aspirate**
   a. Aspirate the specimen into a sterile sputum trap.
   b. Replace the luki tube with the extra cap for pneumatic tube transport.
   c. Tracheosomy aspirate culture is only to be performed when clinical pneumonia is present.
   d. A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay

9. **Bronchoscopy** – collected by pulmonologist or other trained physician.
   a. To reduce excess recovered blood, obtain bronchial wash and BAL samples before brushings or biopsies.
   b. Avoid suctioning through the working channel before retrieving specimens to avoid contamination of the specimens with upper respiratory flora.
   c. Avoid the injection of topical anesthetic agents as much as possible, as injection may lead to the contamination of the specimen. Aerosol application of anesthetics is preferred.
   d. **Bronchial washing and Bronchial lavage (BAL)**
      1) Pass the bronchoscope transnasally or transorally in nonintubated patients or via the endotracheal tube in intubated patients.
      2) Inject sterile nonbactrostatic 0.85% NaCl (generally 5 to 20 mL aliquots) from a syringe through a biopsy channel of the bronchoscope.
      3) Collect sample
         a) Collect BAL sample by carefully wedging the tip of the broncosope into an airway lumen and instilling a large volume of sterile, nonbacterostatic (greater than 140 mL). The sample returned contains secretions distal to the bronchioles and alveoli.
         b) For bronchial washing, sample the major airways, the same area sampled by endotracheal aspirate.
      4) Gently suction the recovered specimen into a sterile container before administering the next aliquot. In general 50% to 75% of the saline instilled in recovered in the lavage effluent. Keep aliquots separate.
      5) Discard the initial fluid as contaminated and submit the rest for culture and staining. Aliquots from the same site may be combined for microbiology cultures and smear. Consult with the physician before combining aliquots for different sites.
      6) Do not send these samples through the tube station.
      7) A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay
e. **Bronchial brush**
   1) Instill a brush to collect cellular material from the airway wall. This is the best specimen for viral culture and cytology studies.
   2) Only protected specimen brushes are acceptable for bacterial culture. Obtain by inserting a telescoping double catheter plugged with polyethylene glycol at the distal end (to prevent contamination of the bronchial brush) through a biopsy channel of the bronchoscope.
   3) Collect in a sterile leak-proof cup.
   4) A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay.

f. **Transbronchial biopsy samples**
   1) Obtain the biopsy sample through the biopsy channel of the bronchoscope, and transport it in a sterile container with a small amount of nonbacteriostatic sterile saline.
   2) Collect sample in a sterile leakproof cup.
   3) A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay.

10. **Lung aspirate** – collected by trained physician
   a. Use a computed tomography scan to obtain lung aspirates by inserting a needle through the chest wall into a pulmonary infiltrate.
   b. Aspirate material from the lesion.
   c. If the lesion is large or if there are multiple lesions, collect multiple specimens from representative sites.
   d. Transfer sample in a sterile leak-proof cup. Note: lab will reject syringes with the needle attached.
   e. A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay.

11. **Lung biopsy** – collected by trained physician
   a. Obtain a 1cm to 3cm piece of tissue.
   b. If the lesion is large or if there are multiple lesions, collect multiple specimens from representative sites.
   c. Send sample in a sterile leak-proof cup with enough 0.85% NaCl saline to keep moist. Do not send in formalin.
   d. A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay.

12. **Pleural fluid**
   a. Clean the needle puncture site with alcohol, and disinfect it with an iodine solution (1 to 2% tincture of iodine or a 10% solution of povidone-iodine (1% free iodine). If tincture of iodine is used, remove with 70% alcohol after the procedure to prevent burns.
   b. Aseptically perform percutaneous aspiration with syringe and needle to obtain pleural fluid.
c. Transfer sample in a sterile leak-proof cup. Note: lab will reject syringes with the needle attached

d. A minimum of two patient identifiers are required on all specimens. Follow all labeling policies and send to the laboratory without delay