The sputum culture is an important part of the diagnostic evaluation of potential lower respiratory tract infections. However, expectorated sputum specimens are variably contaminated by colonizing oropharyngeal flora, making results hard to interpret. Proper collection of the specimen is crucial to the recovery of the etiological agent.

**SPECIMEN CRITERIA:**

1. If possible, specimen should be collected before antimicrobial treatment.
2. First morning specimen is best.
3. Specimen must be collected in a sterile container.
4. Expectorated sputum specimens will be evaluated by means of a gram stain to determine the acceptability of a specimen for culture. If the specimen is unacceptable, the nursing unit will be requested to obtain a new specimen.
5. If a good specimen cannot be obtained, an aspirated specimen via Lukens trap may be obtained. These specimens are accepted for culture without an evaluation.
6. If multiple cultures are ordered they should be collected at least 24 hours apart.

**EXPECTORATED SPUTUM**

1. Specimen collection should be supervised by a trained professional.
2. Request the patient to remove any dentures and to rinse the mouth or gargle with plain water before specimen collection.
3. Tell the patient to provide a specimen from a deep cough, avoiding, as much as possible, mixing the specimen with saliva or nasal secretions.
4. Make sure the patient understands the difference between saliva (from mouth) and sputum (from chest).

**INDUCED SPUTUM**

1. Using a wet toothbrush, brush the buccal mucosa, tongue, and gums prior to the procedure.
2. Rinse the patient's mouth thoroughly with water.
3. Using ultrasonic nebulizer, have the patient inhale approximately 20 to 30 ml of 3 to 10% 0.85 NaCl.
4. Collect the induced sputum in a sterile screw-cap cup or other suitable collection assembly.

**TRACHEOSTOMY AND ENDOTRACHEAL ASPIRATIONS**

Aspirate the specimen into a sterile sputum trap.

**BRONCHOSCOPY**

Precautions:

- To avoid excess blood in the recovered fluid, obtain bronchial wash and BAL specimens before brushing or biopsy specimen.
- Avoid suctioning through the working channel before retrieval of specimens to avoid contamination of the specimens.
- Avoid the injection of topical anesthetic agents as much as possible, as the injection method may lead to contamination of the specimen. Aerosol application of anesthetic agents is preferred.
Collection:

**BRONCHOALVEOLAR WASHING or BAL:**
NOTE: The difference between a BAL sample and a bronchial washing is not apparent from the appearance of the specimen. The BAL sample is from the distal respiratory bronchioles and alveoli. Bronchial washings sample the major airways, which is the same area sampled by an endotracheal aspirate. **Please make sure to have correct specimen type on requisition.**

1. Pass the bronchoscope transnasally or transorally in nonintubated patients or via the endotracheal tube in intubated patients.
2. Inject sterile nonbacteriostatic 0.85% NaCl from a syringe through a biopsy channel of the bronchoscope.
3. Collect BAL sample by carefully wedging the tip of the bronchoscope into an airway lumen and instilling a large volume of sterile, nonbacteriostatic saline (greater than 140 ml). The sample returned contains secretions distal to the bronchioles and alveoli.
4. Gently suction the recovered specimen into a sterile container before administering the next aliquot. Keep aliquots separate during collection.
5. Discard the initial fluid as contaminated and submit the rest for culture and staining.
   NOTE: Aliquots from same site may be combined for cultures, but aliquots from separate sites should be combined only after consultation with the physician.

**BRONCHIAL BRUSH:**

1. Instill a brush to collect cellular material from the airway wall.
2. Only protected specimen brushing (PSB) are acceptable for bacterial culture. Obtain by inserting a telescoping double catheter plugged at the distal end (to prevent contamination of the bronchial brush) through the biopsy channel of the bronchoscope.
3. Place the brush (with protected covering removed) in a sterile cup for transport. If specimen cannot be transported to laboratory within 1 hour, place small amount of sterile saline in specimen cup with the brush.

**TRANSBRONCHIAL BIOPSY:**

1. Obtain the biopsy sample through the biopsy channel of the bronchoscope, and transport it in a sterile container with a small amount of sterile saline.

**TRANSPORT:**
Specimens should be promptly transported to the laboratory after collection. If a specimen has been at room temperature for more than 2 hours a new specimen will be requested.
LEGIONELLA SPP
Bacteria in the genus Legionella primarily cause respiratory illness, i.e., Legionnaires' disease or Pontiac fever. Legionella can also cause a variety of other illnesses, including wound infections, prosthetic valve endocarditis, and sinusitis. In Legionella pneumonia, sputum may be scant and watery with few or no cells. Sputum for Legionella culture is not evaluated prior to processing. Testing for Legionella spp. is sent to a reference laboratory.

LEGIONELLA CULTURE AND DFA SMEAR
Culture is the most sensitive detection method and should always have priority over the direct detection methods. Submit 5 to 10 ml of sputum, bronchial washing, body fluids, or 1 gm tissue in sterile container. Add a small amount of sterile, distilled water to prevent drying, if necessary. Do not add saline, because it may be inhibitory.

FUNGUS CULTURE
MYCOBACTERIA (AFB) CULTURE
Collect specimen as stated above. At least 5 ml of specimen is optimum for recovery of Mycobacterium spp. Transport promptly to the laboratory.