POLICY STATEMENT	Throat and nasopharyngeal cultures are important as an aid in the diagnosis of certain infections such as streptococcal pharyngitis, diphtheria, thrush and in the detection of the carrier state of such organisms as Beta -hemolytic Streptococci, *Hemophilus influenza*, *Neisseria meningitidis*, *Staphylococcus aureus*, and *Corynebacterium diphtheriae*. The tracheobronchial tree below the larynx is usually sterile or harbors only sparse or transient bacterial populations. Exceptions are patients with chronic bronchitis, cystic fibrosis, chronic pulmonary disease and patients with endotracheal tubes or tracheotomies who may harbor organisms in the trachea without evidence of infection. On the basis of these observations, specimens may be divided according to the likelihood of oropharyngeal contamination. Those in which some degree of contamination with normal respiratory flora is virtually inevitable are sputum, nasopharyngeal aspirates and brush biopsies. Cultures from specimens devoid of oropharyngeal contamination are more easily interpreted. They include trans-tracheal aspirates, and tissue removed surgically.
PURPOSE	This procedure provides technical instruction for the performance of the Respiratory Cultures.
SCOPE	This procedure applies to testing personnel authorized to perform testing. This group includes, but is not limited to Laboratory Technologists as well as leads and supervisory personnel.
RESPONSIBILITY	All the above personnel are responsible for following the Respiratory Cultures procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.
RELATED DOCUMENTS	MICR 6140 R Specimen Processing MICR 6305 R Bacterial Cultures

SPECIMEN HANDLING

See *MICR 6140 R Specimen Processing*

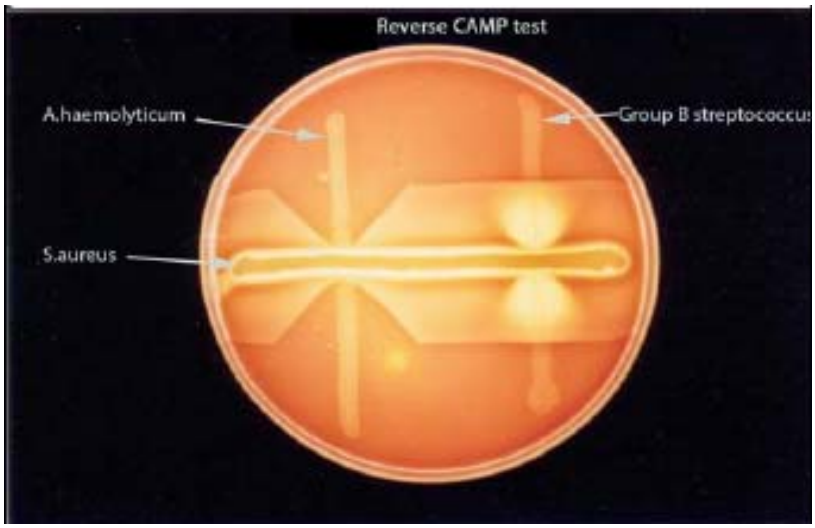
Trans-tracheal aspirates and surgically removed tissue are the only respiratory specimens suited for anaerobic culture.

CULTURE WORK UP

Upper Respiratory – nasal, nasopharyngeal and throat

1. Examine all plates for macroscopic growth at 24 and 48 hours.
2. Do not identify normal flora to genus or species level
    - Upper respiratory flora
        - Staphylococcus
        - Micrococcus
        - diphtheroids
        - alpha streptococci
        - Streptococcus Group F
        - Neisseria
        - Enterococcus
        - Enteric gram-negative rods
        - Yeast
3. Identify all organisms and perform antimicrobial susceptibility testing (AST) if appropriate.
    - Identify any amount
        - Beta Streptococcus Groups A, B (pediatric), C and G
        - *Neisseria gonorrhoeae*
    - Identify if heavy growth **and** predominate **and** in quantities greater than the normal flora. Lesser amounts are reported as normal flora. Sensitivities are not performed.
        - *Staphylococcus aureus* – rule out MRSA – perform PBP2
        - *Streptococcus pneumoniae*

- *Neisseria meningitidis*
- *Hemophilus influenzae*
- *Arcanobacterium haemolyticum*
  - Gram-positive rod -irregular, pleomorphic, coccobacillary (resembles a diptheroid on gram stain).
  - Colonies are beta hemolytic, <0.5mm at 48hours (resembles Beta Strep on culture)
  - Catalase negative
  - Inhibits the hemolysis of Staph aureus ATCC 25923 when set up perpendicular to Arcanobacterium. (Reverse Camp).



Lower Respiratory – sputum, endotracheal aspirates, bronchial washing, brush biopsy and transtracheal aspirate.

1. Examine all plates for macroscopic growth at 24 and 48 hours.
2. Do not identify normal flora to genus or species level
   - Lower respiratory flora
     - Staphylococcus coagulase negative
     - alpha streptococci
     - Neisseria

- Enterococcus
- diphtheroids
- Low numbers of Gram negative rods
- Yeast

4. Identify organisms and perform antimicrobial susceptibility testing (AST) according to the following criteria utilizing the Q/2-3-4 system.

- Gram stains are utilized to evaluate the quality of the specimen and guide the work up of the culture.
  - WBCs indicate infection or inflammation
  - SEC indicate superficial contamination
- Potential Pathogens (PP)
  - Beta Streptococcus Group A, B, C and G
  - *Streptococcus pneumoniae*
  - *Staphylococcus aureus*
  - *Hemophilus influenzae*
  - *Neisseria meningitidis*
  - *Moraxella catarrhalis*
  - *Cryptococcus neoformans*
  - Gram-negative rods - If gram negative rods are seen on Gram stain and there is more then one morphology, work up the predominant morphology. If there is not a predominant morphology, report as mixed gram negative rods.
- Culture work up is based on number potential pathogens (PP) present:
  - 1 or 2PP = full work up with ID and AST
  - 3 PP = refer to gram stain
    - 1 or 2 PP seen on gram stain full work up with ID and AST
    - 3 PP are seen on gram stain; minimal ID all 3 PPs
  - 4 PP = minimal ID
  - If mixed/normal flora > PPs = minimal ID all PPs

5. Physicians may request additional work up by ordering “MICRO ADD ON TO CULTURE WORKUP” and specifying their request.

REPORTING

- Enter preliminary reports daily until final at 48 hours.
- Enter preliminary report of “No growth after <24 hours” or “Culture in Progress” if the culture was processed after 4pm.
- Report all normal flora with quantitation.
- Report potential pathogens with quantitation and AST according to work up guidelines.
- Enter all results in the appropriate spot in the Meditech workcard.

REFERENCES

Garcia, Lynne, *Clinical Microbiology Procedure Handbook,* 3rd Edition, 2010, Volume 1, Section 3.11 Lower Respiratory Tract Cultures. 2004

Susan Sharp and Yvette McCarter, ASCP (American Society for Clinical Pathology) Workshop, Clinical Microbiology- Review and Update, 2011

Cumitech 7B Lower Respiratory Tract Infections ASM Press

Cumitech 10A Laboratory Diagnosis of Upper Respiratory Tract Infections ASM Press.

Yvette McCarter and Susan Sharp, Clinical Microbiology Newsletter, March 1, 2013 vol. 35 no. 5, *Best Practices for Respiratory Cultures.*

BAL – quantitative cultures

Inoculation (Loop method):

1. Cytocentrifuge the gram stain
2. Media
   - 2 Chocolates (x100 and x1000) – quantitative culture
   - Chocolate, BAP and MAC – isolation plates
3. Vortex specimen
4. Inoculate the x100 CHOC with the blue (0.01 ml or 10 µl) loop. Place a place a blue loop sticker on this plate.
5. Inoculate the x1000 CHOC with the white (0.001 ml or 1 µl) loop.
6. Spread the inoculum over the surface of each plate using a sterile L-shaped spreader. Starting with the most dilute (x1000) plate and then using the same spreader proceed to the least dilute (x100) plate.
7. Add 2 drops to each of the remaining CHOC, BAP and MAC and streak for isolation
8. Incubate all plates at 35°C in CO_2.

Interpretation and Reporting:

1. Gram stain:
   - SEC – squamous epithelial cells - presence is indicative of oral contamination
   - WBC
   - Bacteria - any organism seen on cytocentrifuge smear is indicative of bacterial pneumonia.
2. Culture:

Count the colonies on the CHOC from the dilution with the greatest number of colonies without confluence. Multiply by the dilution factor (x100 and x1000).

- From the x100 CHOC with the 0.01 ml loop (10 µl) 1 colony = 100 CFU/ml
- From the x1000 CHOC with the 0.001 ml loop (1 µl) 1 colony = 1000 CFU/ml
- Count each morphotype individually
  - ≥ 10^4 CFU/ml – ID and AST of each primary pathogen
  - ≥ 10^4 CFU/ml of normal Oral Flora
    - If SEC are absent on the gram stain report ≥ 10^4 CFU/ml with minimum ID
    - If SEC are present on the gram stain report as ≥ 10^4 CFU/ml Oral Flora
  - <10^4 CFU/ml – considered contamination – Minimum ID unless pure culture
- Candida species is often a contaminant of the procedure and should be considered part of the normal oral flora. Do not work up.