# Department of Microbiology
# Lower Respiratory Tract Culture Procedure

## I. Specimen Types

Bronchial alveolar lavage (BAL)
Bronchial brush
Bronchial wash
Bronchoscopy
Endotracheal aspirate
Expectorated sputum
Gastric secretions from newborns
Lung aspirate
Tracheal aspirate (leukens)
Tracheal sites
Transtracheal aspirate

## II. Mixed Flora

Alpha-hemolytic strep
Beta hemolytic strep not group A
*Corynebacterium sp.*
*Neisseria sp.* not *meningitidis*
Non-hemolytic strep (except newborn gastrics)
*Staph* sp. not *aureus*
*Haemophilus* species, other than *influenzae*

## III. Potential Pathogens

Gram-negative rods
Group A strep
Group B strep (in newborn gastrics; bring up other cases on Rounds)
*Haemophilus influenzae*
*Moraxella catarrhalis*
*Staph aureus*
*Strep pneumoniae*
Yeast (only from BAL, bronch brush, lung or transtracheal aspirate)
Neisseria meningitidis

Note: If there is an average of < 5 colonies of a potential pathogen, do not work-up the potential pathogen. Report as **Mixed flora**. This guideline does not apply to BAL specimens. All potential pathogens should be worked up in BAL specimens regardless of quantity.

## IV. Culture Workup and Reporting

A. At 24 h of incubation:
   1. Note the quality score of the specimen (the "Q") and the bacteria seen on Gram stain.
   2. Determine the number of potential pathogens.
      a. If the potential pathogens are ≤ Q:
         i. Speciate and report the presumptive isolate(s) according to the identification charts**,** and record in the computer.
         ii. Perform susceptibility testing on organisms, if appropriate.
         iii. Re-incubate the plates.
      b. If the potential pathogens are >Q, the potential pathogens are correlated with the direct smear.

i. If the correlating potential pathogens do not exceed the score, correlating potential pathogens are worked up (see IV.A.2.a.) and a gross generic report is given for non-correlating isolates by reporting those isolates as: **Mixed flora including (list non-correlating potential pathogens)**.
   a) Attach the comment: **This is a mixed culture of potential pathogens. Correlation of the culture results with the gram stained direct smear indicates one or more isolate is more significant than others. The organisms seen only in culture may not relate to infection and may represent colonization or contamination. [MXSIG]**
   b) Hold the plates for 7 days on the shelf.

ii. If the potential pathogens that correlate exceed the score, a gross generic report of isolates is given.
   a) Report the isolates generically: **Mixed flora including (list potential pathogens)**.
   b) Attach the comment: **This is a mixed culture of potential pathogens. Correlation of culture results with the gram stained direct smear does not identify any isolate as more significant than another. Bacteria may not relate to infection and may represent colonization or contamination. [MXNSIG]**
   c) Hold the plates for 7 days.

iii. If Q0 a "gross" report is given.
   a) Report the pathogens generically: **Mixed flora including (list potential pathogens)**. Identification testing should be limited to tests that can be completed on that same day (e.g., gram stain, motility, spot tests, etc.).
   b) If potential pathogens are listed, add the comment: **This is a mixed culture suggesting the probability of contamination. Collection of another specimen is suggested, avoiding superficial sources of contamination. [SWCONT]**
   c) Hold the plates for 7 days on the shelf.

3. Record the presence or absence of mixed flora.
4. Perform bile solubility testing on all alpha hemolytic colonies, and record the results in the computer.
   a. If positive, report: ***S. pneumoniae***.
   b. If negative, report: **Mixed flora**.
      Exception: If *S. pneumoniae* was seen in the Gram stain, transfer all suspicious alpha-hemolytic colonies to a BAP with an optochin disc.
   c. For Q1, Q2, or Q3 specimens, if questionable colonies are present, transfer them to a BAP with an optochin disc to rule

out *S. pneumoniae*. Do not perform subcultures for Q0 specimens.

5. If beta hemolytic strep is present, perform a latex agglutination for group A strep only.
   a. If positive, report: **Group A streptococcus.**
   b. If negative, report: **Mixed flora**.
6. Issue a preliminary report of the status of any potential pathogen and mixed flora, if present.
7. If the plates are sterile, report: **No Growth to Date.**

B. At 48 h of incubation:

1. See **IV.A.**, but do not reincubate plates.
2. If a A-jar was set up, at 48 h:
   a. Screen all alpha-hemolytic colonies for *S. pneumoniae*.
   b. Screen all beta-hemolytic colonies for group A strep.
3. If there is no growth at 48 h, report: **No Growth**.
4. If no potential pathogens are isolated and mixed flora is present, report: **Mixed flora**.
   a. If potential pathogens are present, see **IV.A**.
5. If yeast is isolated:
   a. On BAL, bronchial brush, lung or transtracheal aspirate specimens only, perform germ tube test and report: ***Candida albicans*** or ***Candida* not *albicans***. Perform and report routine antimicrobial susceptibility testing.
   b. Do not report the presence of yeast from other lower respiratory tract secretions due to the potential for superficial contamination with oropharyngeal secretions during the collection process.

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Revisions & Updates: 11/04/2010 Under list of potential pathogens, M. catarrhalis – removed “(only if reported in Gram Stain)” per AR. 12/28/2010 Changed reporting for Q0 specimens – add SWCONT comment if potential pathogens are listed. 06/11/2011 Updated reporting for yeast isolates on BAL specimens. Instead of reporting “yeast,” perform germ tube and report either C. albicans or Candida spp., not albicans and perform AST. 11/01/2011 Added limiting testing of isolates in Q0 specimens to tests that can be performed same day. Subbing to r/o S. pneumo on Q0 specs is not necessary. Guidelines for not working up potential pathogens, < 5 colonies does not apply to BAL specimens. Deleted verbiage for Quant. Culture (see separate procedure).
9/23/14 Added *Haemophilus* species, other than *influenzae,* to list of mixed flora.