PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC32300 – Respiratory Culture	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
Pneumonia may be categorized as: i) Community acquired pneumonia (CAP), ii) Nosocomial or Hospital acquired pneumonia (NAP / HAP), iii) Aspiration pneumonia and iv) Pneumonia in immunocompromised patients. The most common organisms to cause CAP include *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Legionella pneumophila*. HAP is more commonly due to aerobic gram-negative bacilli, anaerobes, *Staphylococcus aureus*, *Streptococcus pneumoniae* and others. Aspiration pneumonia may be due to a mixture of oral aerobes and anaerobes.

PURPOSE/RATIONALE:
To determine the presence or absence of bacterial pathogens in respiratory specimens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for respiratory culture.

SAMPLE INFORMATION:

Type	Sterile container
Source	• Sputum • Endotracheal aspirate (ETT) and Auger suction • Bronchial aspirates and Bronchoalveolar lavage (BAL)
Stability	If the sample is received in the laboratory and processed greater than 72 hours from collection: • Add specimen quality comment: "Delayed transport may adversely affect pathogen recovery"
Storage Requirements	Refrigerated

Criteria for rejection	1. Unlabeled/mislabeled specimen 2. Specimen container label does not match patient identification on requisition 3. Duplicate specimens obtained with same collection method within 24 hours 4. Leaking specimens 5. Improperly collected, labeled, transported, or handled bronchial aspirate, BAL specimens, lung aspirates and lung biopsy specimens should be processed. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO) and MacConkey agar (MAC)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Wooden sticks

EQUIPMENT

- Biosafety cabinet
- 35° ambient air and 35° CO_2 incubators
- Vitek 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential infectious materials or cultures.

- Ensure that appropriate hand hygiene practices be used.
- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to Test Manual for reagent quality control procedures

PROCEDURE INSTRUCTIONS:

Step	Action
Processing specimens for respiratory culture	
1	In the biosafety cabinet: • Use a sterile swab to inoculate BA, CHO and MAC from the specimen. Select the most purulent or most blood-tinged portion • Streak for isolated growth using a disposable inoculation needle • Prepare a smear by rolling the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements
2	Incubate all media: • Place BA and CHO in the CO_2 incubator • Place MAC in the O_2 incubator
3	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram Stain Procedure.
4	Ensure the quality of the specimen has been evaluated and is considered acceptable for culture. Refer to MIC20300-Gram stain resulting in LIS-Respiratory cultures. **NOTE:** Bronchial wash and bronchoalveolar lavage specimens are processed regardless of specimen quality

Probable Pathogens	
• *Streptococcus pyogenes* • *Streptococcus agalactiae* in newborn • *Neisseria gonorrhoeae* • *Nocardia* • *Burkholderia mallei/pseudomallei**+	• *Brucella* spp.*+ • Dimorphic fungi and Molds • *Cryptococcus neoformans/gattii* • *Bacillus anthracis**+ • *Yersinia pestis**+
Potential Pathogens	
• *Streptococcus pneumoniae* • *Haemophilus influenzae* • *Moraxella catarrhalis* • *Neisseria meningitidis* • *Pseudomonas aeruginosa* • *Stenotrophomonas maltophilia* • *Acinetobacter* spp. • *Burkholderia* spp.	• *Staphylococcus aureus* • β-hemolytic Strep B (adults), C or G • Enterobacteriaceae • *Corynebacterium* spp. • *Enterococcus* spp. • Coagulase-negative *Staphylococcus* • *Candida* spp.

*Risk group 3 organism. If suspected, refer to Policy B-0160: "Specimens Containing Suspected Risk Group 3 Pathogens" for Primary Specimen Handling Flow Chart
+All work should be performed in the BSC

INTERPRETATION OF RESULTS:

Step	Action
1	Ensure growth on culture media correlates with gram stain results. If discordant results are found between the gram stain and growth: • Re-examine smear and culture plates • Check for anaerobic growth • Re-incubate media to resolve • Consider re-smearing or re-planting specimen
2	• Observe BA and CHO plates at 24 hours and 48 hours • Observe MAC plate at 24 hours
3	Significant growth is defined as bacterial morphotypes that are: • Moderate to heavy growth in the second or greater quadrants • Colonies in the first quadrant of the plate provided there is little or no other normal respiratory flora and gram stain shows WBC
4	**Examine for and always report:**
	• *Streptococcus pyogenes* • *Streptococcus agalactiae* in newborns <=3 mon. • *Neisseria gonorrhoeae* • *Nocardia* spp. • *Bacillus anthracis* • *Burkholderia mallei/pseudomallei* • *Brucella* spp. • Fungi and Molds • *Cryptococcus neoformans/gattii* • *Yersinia pestis*
	Always report, but do not make an effort to find low numbers, unless seen in smear:
	• *Streptococcus pneumoniae* • *Haemophilus influenzae*
	Report if present in significant amounts, even if not predominant:
	• *Moraxella catarrhalis* • *Neisseria meningitidis*
	Report if present in significant amounts, even if not predominant for inpatients only:
	• *Pseudomonas aeruginosa* • *Stenotrophomonas maltophilia* • *Acinetobacter* spp. • *Burkholderia* spp. *This group of GNB can be colonizers, even in hospitalized patients
	Report if present in significant amounts AND if it is the predominant organism in the culture, particularly if smear suggests infection consistent with isolate:
	• *Staphylococcus aureus* • β-hemolytic strep group B (adults), C or G • *Corynebacterium* spp. • Single morphotype of Gram-negative bacilli • Fastidious Gram-negative bacilli
	Report as "Usual oropharyngeal flora": **Note: If *Enterococci* and/or coagulase-negative *Staphylococci* and/or *Candida* spp. are the only organisms present, list individually with minimal identification, if 90% pure culture**
	• Viridans *strept.* • Non-pathogenic *Neisseria* spp. • Anaerobes • *Haemophilus species* • *Eikenella* • *Capnocytophaga* • *Enterococci* • Yeasts • Coag-negative *Staph*

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: "**No Growth after 1 Day. Further report to follow"**
No growth after 2 days	**FINAL:** • Report: "**No Growth after 2 Days"**
Mix of enteric Gram-negative bacilli	• Report **"Mixture of coliform organisms"** • List quantitation
Growth or mix of other non-pathogenic organisms	• Report **"Commensal flora" or "Commensal skin flora"** • List quantitation
Growth of potential pathogen(s)	• Report organisms(s) identification • List quantitation
Growth of pathogen(s)	• Report organisms(s) identification • List quantitation • Report susceptibility results as per ASTM

NOTE:

- Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to OCPHO (HPU1)
- Refer to LQM70620-Laboratory Critical Results List-Microbiology for results that need to be phoned to ordering location
- Refer to MIC36100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Prevention and Control
- Refer to MIC36200-Referral of Category A Specimens to APL for sending category A isolates to APL
- Refer to MIC36300-Referral of Category B Specimens to APL for sending isolates to APL
- Refer to MIC36400-Referral of Category B Specimens to DL for sending isolates to *Dyna*LIFE

LIMITATIONS:

1. A positive culture with *Streptococcus pneumoniae* or *Haemophilus influenzae* generally indicates an infection, although carriage may lead to false-positive results.
2. A positive culture with a predominant Gram-negative bacillus or *Staphylococcus aureus* generally indicates infection if the smear correlates with the culture.
3. A negative culture does not rule out a respiratory tract infection. The primary pathogen is frequently not recovered either because patients have already been started on antimicrobial therapy or because they have an infection with another type of organism (virus, parasite, fungus, mycoplasmas, or mycobacterium) that will not be recovered by bacterial culture.

4. There is controversy regarding the need to culture for CAP (community acquired pneumonia), but there is agreement of the benefits of culture for ventilator associated and nosocomial pneumonia.
5. A delay in processing of more than 1-2 hours may result in loss of recovery of fastidious pathogens such as *Streptococcus pneumoniae* and the overgrowth of oronasal microbiota.
6. False-negative cultures can result from improper collection, delayed transport, contamination of the specimen with normal oral microbiota, low organism levels or from prior antimicrobial therapy.
7. False-positive cultures can result from contamination of the specimen by normal respiratory flora and its subsequent growth on culture and over interpretation by the laboratory.
8. Immunocompromised patients with progressive pneumonia are more likely to have infection due to *Legionella* or a nonbacterial cause of infection. Lower respiratory tract specimens (such as BAL) should be collected early in the course of the infection in order to optimize the recovery of unusual pneumonia pathogens, including *Legionella, Pneumocystis jirovecii*, viruses, fungi, *Mycoplasma* and *Mycobacteria*.

CROSS-REFERENCES:

- LQM70620-Laboratory Critical Results List-Microbiology
- MIC20115-Gram Stain Procedure
- MIC20300-Gram stain resulting in LIS-Respiratory cultures
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36200-Referral of Category A Specimens to APL
- MIC36300-Referral of Category B Specimens to APL
- MIC36400-Referral of Category B Specimens to DL

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4th ed.) Washington, D.C.: ASM Press
2. Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. (2015). *Manual of Clinical Microbiology, 11th edition*. Washington, D.C: ASM Press

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	28 May 18	Initial Release	L. Steven
2.0	05 Mar 21	Procedure reviewed and added to NTHSSA policy template	L. Steven