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

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 48 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. Swabs of sputa. 4. Duplicate specimens obtained with same collection method within 24 hours. 5. Specimen received greater than 72 hours after collection. 6. Leaking specimens. 7. Improperly collected, labeled, transported or handled bronchial aspirate (wash specimens), 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.

NOTE:

- **Refer lung biopsy specimens for culture to DynaLIFE.**

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
- Biosafety cabinet
- 35° ambient air and 35° CO_2 incubators
- Wooden sticks
- 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.

- 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. Universal precautions 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, inoculate Blood agar, Chocolate agar and MacConkey agar from the specimen. Select the most purulent or most blood-tinged portion. Make gram stain.
2	Streak for isolated growth using a disposable inoculation needle:  Streak out to cover the whole plate.
3	Place MAC plate in the O_2 incubator. Place BA and CHO plates in the CO_2 incubator.
4	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.
5	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.
6	Examine plates after 24 hour incubation. Record observations in the LIS.
7	Re-incubate CO_2 plates for an additional 24 hours. Discard O_2 plate.
8	At 48 hours, examine plates and record observations.

Probable Pathogens	Possible 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**+	• *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	Confirm gram stain has been read prior to reading culture plates. Ensure growth on culture media correlates with gram stain results. If discordant results are found: • Re-examine smear and culture plates. • Check for anaerobic growth. • Re-incubate culture to resolve. • May need to inoculate special selective media. • Consider re-smearing or re-planting specimen to exclude the possibility of error.	
2	Observe plates at 24 hours and 48 hours for growth.	
3	Significant growth is defined as bacterial morphotypes that are: • Moderate to heavy growth of an isolate in the second or greater quadrant of the plate. • Colonies in the first quadrant of the plate provided there is little or no other normal respiratory flora and gram stain shows WBC.	
4	**Use the following guidelines for reporting pathogens in lower respiratory culture**	
	Examine for and always report:	*Streptococcus pyogenes* *Streptococcus agalactiae* in newborns (<= 3 mon) *Neisseria gonorrhoeae* *Nocardia* spp. *Bacillus anthracis* *Burkholderia mallei/pseudomallei* *Brucella* spp. *Yersinia pestis* *Dimorphic fungi* *Cryptococcus neoformans/gattii* Molds
	Always report, but do not make an effort to find low numbers, unless seen in smear:	*Streptococcus pneumoniae* *Haemophilus influenzae*

4	Report if present in **significant** amounts, even if not predominant: **These organisms for inpatients only:**	*Moraxella catarrhalis* *Neisseria meningitidis* *Pseudomonas aeruginosa* *Stenotrophomonas maltophilia* *Acinetobacter* spp. *Burkholderia* spp. *This group of GNB can be colonizers, even in hospitalized patients. Refer to ASTM for need for susceptibility testing and comment to be added.
	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 Single morphotype of Gram-negative bacilli (especially *Klebsiella pneumoniae*) Fastidious Gram-negative bacilli *Corynebacterium* spp.
	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 *streptococci* Non-pathogenic *Neisseria* spp. Coagulase-negative *Staphylococci* Anaerobes *Haemophilus species* (not *H.influenzae*) *Eikenella* *Capnocytophaga* *Enterococci* Yeasts Insignificant numbers of *Staphylococcus aureus* and Gram-negative bacilli

***Enterococcus*:**

- Do not report unless in 90% pure culture, in which case, identify and perform susceptibility testing as per ASTM.
  Note: many Gram-positive cocci in the normal respiratory tract are PYR +, not just *Enterococci*.

***Staphylococcus*:**

- Identify and perform susceptibility testing as per ASTM on *Staphylococcus aureus* if present in **significant** amounts.
- Identify and perform susceptibility testing as per ASTM on coagulase-negative *Staphylococci* only if in 90% pure culture.

***Streptococcus* spp.**:

- Examine for large beta-hemolytic colonies and identify catalase - GPC in pairs and chains.
- Identify *Streptococcus pyogenes*, report any amount. Report susceptibility as per ASTM.
- Identify *Streptococcus agalactiae* in pediatric patients (<=3 months), report any amount. Report susceptibility as per ASTM.
- Identify other beta-hemolytic Streptococci in **significant** amounts only if they are predominant. Report susceptibility as per ASTM. Do not report small colony types of beta-hemolytic *Streptococcus* or group F *Streptococcus* as they are part of the upper respiratory microbiota.
- Identify alpha-hemolytic colonies with morphology consistent with *Streptococcus pneumoniae* with the optochin test or a Vitek 2 GP identification card. Report susceptibility as per ASTM.

Fastidious Gram-negative bacilli (GNB): (slow or no growth on MacConkey agar)

- *Haemophilus influenzae*: coccobacilli, growth on CHO, no growth on BA except as satellitism, ALA -. Report susceptibility as per ASTM.
- *Pasteurella*: oxidase +, indole +. Represents normal mouth microbiota of animals. Report susceptibility as per ASTM.
- Do not identify most other fastidious Gram-negative bacilli such as *Eikenella*, unless they are predominant and present in large amounts, since they are part of the normal upper respiratory microbiota and rarely cause respiratory disease.

Gram-negative bacilli that grow well on MacConkey agar:

- Enteric Gram-negative bacilli (particularly *Klebsiella pneumoniae*): if there is only one morphology in **significant** amounts with no other pathogens in greater amounts, identify and perform susceptibility testing as per ASTM.
- For inpatients, regardless of the presence of other pathogens, examine for **significant** numbers of *Pseudomonas aeruginosa*, *Acinetobacter, Burkholderia* and *Stenotrophomonas* because they are typically resistant to multiple antimicrobials and are implicated in nosocomial infections. This group of GNB can be colonizers, even in hospitalized patients. Report susceptibility results as per ASTM.
- If more than one type of other Gram-negative bacillus is present in equal numbers, report as “Mixture of coliform organisms”.

Gram-negative diplococci:

- Examine colonies present in **significant** amounts that move when pushed. Report as *Moraxella catarrhalis* if oxidase + and disk test +. Report susceptibility as per ASTM.
- Examine CHO plate for any oxidase + colonies that do not grow or grow poorly on BA. Confirm as *Neisseria gonorrhoeae* or *Neisseria meningitidis* with API-NH and/or Vitek 2 NH card. Report any amount of *Neisseria gonorrhoeae*. Report **significant** amount of *Neisseria meningitidis.*

Gram-positive bacilli:

- Rule out *Nocardia* in any amount.
- Examine for large spore-forming Gram-positive bacilli. If present, perform wet prep for motility. Send **Cat A** non-motile *Bacillus* spp. to Prov. Lab for identification.
- *Corynebacterium* species: send to DynaLIFE for identification if direct Gram-stained smear is suggestive of infection with numerous WBC and Gram-positive bacilli and culture shows pure or predominant growth.
- Generally, do not work up other Gram-positive bacilli – they are unlikely to cause pneumonia.

Yeasts

- Rule out *Cryptococcus*. If Vitek YST card identifies isolate as *Cryptococcus* spp., report isolate as "Possible Cryptococcus spp., sent for further identification and susceptibility testing". Order REFE and refer isolate to DynaLIFE as per MIC10510.

Note: *Candida* organisms are not a cause of community acquired pneumonia and are most often contaminants of the procedure, except possibly in oncology or lung transplant patients or in neonates. Even in those cases, growth of any species of *Candida* in lower respiratory specimens does not correlate with disease. Yeasts are normal inhabitants of the mouth. Candida may be a cause of hospital acquired pneumonia.

Molds, fungi:

- Send to ProvLab for identification.

REPORTING RESULTS:

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 oropharyngeal flora	• Report **"Mixture of usual oropharyngeal flora"** • List quantitation.
Mix of enteric Gram-negative bacilli	• Report **"Mixture of coliform organisms"** • List quantitation.
Growth or mix of other non-pathogenic organisms	• Report **"Commensal flora"** • List quantitation.
Growth of pathogen(s) or possible pathogen(s)	• Report organism(s) identification under the isolates tab. • List quantitation. • Report susceptibility results as per ASTM. • Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to HPU1. • Refer to MIC35100 – Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Control. • Refer to L-0910-Laboratory: Critical Values for results that need to be phoned to ordering location.

CULTURE NOTES:

- Bronchoscopy specimens: The difference between a bronchoalveolar lavage (BAL) and a bronchial wash (BW) is not obvious from the appearance of the specimen. A BAL specimen is from distal respiratory bronchioles and alveoli. A BW samples the major airways (the same as an ETT).
- A positive culture with *Streptococcus pneumoniae* or *Haemophilus influenzae* generally indicates an infection, although carriage may lead to false-positive results.
- A positive culture with a predominant Gram-negative bacillus or *Staphylococcus aureus* generally indicates infection if the smear correlates with the culture.
- 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.
- 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.

LIMITATIONS:

1. 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.
2. 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.
3. 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.
4. 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*.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- 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, ASM Press, Washington, D.C.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven