**MICRO.CULT.14.0 LOWER RESPIRATORY CULTURES**

**PRINCIPLE**

Infections of the lower respiratory tract are a major cause of morbidity and mortality. Diagnosis of these infections frequently is complicated by the contamination of specimens with upper respiratory tract secretions during collection. Because the upper respiratory tract may be colonized with potential pathogens not involved in the infection of the lower tract and may yield organisms capable of inhibiting the bacteria involved in lower tract pathology, the laboratory should ensure that an appropriate specimen is processed. The specimen must be microscopically examined to assess its quality and to look for organisms associated with an inflammatory cell response. Organisms known to cause lower respiratory tract infections include *Streptococcus pneumoniae, Haemophilus* *influenzae*, aerobic and facultative anaerobic gram negative bacilli, and yeasts. Infections caused by molds, *Chlamydia*, viruses, *Legionella*, mycobacteria, *Mycoplasma*, and *Pneumocystis carinii* require special testing and should not be expected to be recovered utilizing this routine lower respiratory culture.

**OWNERS**

Supervisor, Regional Microbiology

**SPECIMEN**

|  |  |
| --- | --- |
| Collection |  |
| Specimen types | Sputum [(expectorated or induced), first morning specimen is preferable as they are generally more productive]  Tracheal aspirate or suction (Luken’s trap)  **NOTE: Only one acceptable sputum or tracheal aspirate should be submitted per day**  Bronchial washings  Bronchial brushings  Bronchial biopsy  Bronchial alveolar lavage (BAL) |
| Collection containers | Sterile container  Luken’s trap |
| Storage requirements | Room temperature if < 2 hrs before processing. Refrigerate at 4°C if >2 hrs before processing. |

**MATERIALS**

1. Trypticase Soy agar with 5% Sheep Blood Agar (BAP), store at 2-8°C until use
2. MacConkey agar (MAC), store at 2-8°C until use
3. Chocolate II agar (CHOC), store at 2-8°C until use store at 2-8°C until use
4. Glass slide
5. Cotton tipped swab
6. Disposable loop

**QUALITY CONTROL**

1. BAP and MAC are exempt from QC beyond that performed by the manufacturer. Each new shipment is checked for appearance.
2. Each new lot or shipment of CHOC must have quality control performed on the agar plates.

**PROCEDURE**

|  |  |
| --- | --- |
| **Specimen Processing** | |
| **Step** | **Action** |
| 1 | Use a sterile transfer pipette, cotton tipped applicator swab or inoculating loop to inoculate BAP, MAC, CHOC. |

|  |  |
| --- | --- |
| **If** | **Then** |
| Specimen is a sputum, aspirate or suction | Choose the thick mucus secretions while avoiding the thin watery saliva for inoculation |
| Specimen is a bronchial biopsy | Grind the specimen in 0.5 ml sterile saline prior to inoculation |
| Specimen is bronchial brushing | Extract the material by vortexing the brush in 0.5 ml sterile saline |
| Specimen is bronchial washing | Vortex specimen well prior to inoculation |
| Specimen is BAL | Vortex specimen well prior to inoculation. Inoculate duplicate BAP, MAC and CHOC using a 1µl loop and a 10 µl loop. Mark each plate of the set to indicate the loop used for inoculation. |
| Specimen is from cystic fibrosis patient | Refer to separate procedure for the processing of specimens from Cystic Fibrosis patients |

|  |  |
| --- | --- |
| **Step** | **Action** |
| 2 | Streak plates for isolation. |
| 3 | Incubate plates in the 35°C CO2 incubator. |
| 4 | Prepare a gram stain. |

|  |  |
| --- | --- |
| **If** | **Then** |
| Specimen is a sputum, tracheal aspirate, or tracheal suction (Luken’s trap) | Choose the most viscous and/or bloody portion of the specimen, avoiding the loose watery saliva when making the Gram stain. |
| Specimen is bronch of any kind | Use the well vortexed specimen to prepare the Gram stain. |

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Gram Stain the prepared slide. |
| 2 | Read the stained gram stain. |

|  |  |
| --- | --- |
| **If** | **Then** |
| Specimen is a sputum, endotracheal or tracheal aspirate | Examine 20 to 40 fields under low power and use the assessment criteria for specimen quality listed below to determine if culture will be performed |
| Specimen is other than sputum | Report gram stain per criteria under Reporting Results |

|  |  |
| --- | --- |
| **Assessment Criteria for Specimen Quality** | |
| **If** | **Then** |
| Under low power examination, GS shows < 10 squamous epithelial cells | Report gram stain per criteria under Reporting Results |
| **Sputum**:  Under low power examination, GS shows ≥ 10 squamous epithelial cells  **Exception:** If the number of Polys is 10 times the number of epithelial cells AND there is Moderate to Many of a single morphotype of bacteria, refer to the < 10 squamous epithelial cells procedure). | Report ‘The Gram stain result demonstrates oral contamination (≥ 10 squamous epithelial cells/LPF). Specimen recollection is suggested. The specimen is unsatisfactory for routine culture.’  SQ Code = UNSPU  Cancel and credit culture request. (SQ code = SR1)  Call rejection to submitting location and request recollection. Record call in the LIS (Regional)/Rejection Log (SVEV). |
| **Tracheal Aspirate**:  Under low power examination, GS from adults shows ≥ 10 squamous epithelial cells OR no organisms are seen. | Report ‘The Gram stain result demonstrates oral contamination (≥ 10 squamous epithelial cells/LPF). Specimen recollection is suggested. The specimen is unsatisfactory for routine culture.’  SQ Code = UNTRA  Cancel and credit culture request. (SQ code = SR2)  Call rejection to submitting location and request recollection. Record call in the LIS (Regional)/Rejection Log (SVEV). |

|  |  |
| --- | --- |
| **Step** | **Action** |
| 3 | Following overnight incubation, evaluate the plates for the presence of pathogens according to the guidelines for ID and susceptibility found in Table I. Review the smear results to aid culture workup. |

|  |  |
| --- | --- |
| 4 | Note the presence of contaminating normal oropharyngeal flora which includes Coagulase negative staphylococci, *Streptococcus viridans group, Corynebacteria* species, Gamma hemolytic *streptococcus,* commensal *Neisseria* species, *Neisseria meningitidis Moraxella, Haemophilus* species (other than *H.* *influenzae),* diphtheroids, *Rothia, Actinobacillus, Eikenella, Capnocytophaga, Candida* sp, group F streptococci, enterococci and anaerobes. |

**TABLE 1. GUIDELINES FOR IDENTIFICATION AND SUSCEPTIBILITY TESTING OF POTENTIAL PATHOGENS FROM LOWER RESPIRATORY CULTURES**

|  |  |
| --- | --- |
| **Action** | **Organism** |
| Examine for and always report | *Streptococcus pyogenes* Group B beta strep in pediatric patients *Neisseria gonorrhoeae* Nocardia *Cryptococcus neoformans* Molds MRSA1 / AST *Pseudomonas aeruginosa* *Rhodococcus equi* in immunocompromised patients |
| Always report but do not make effort to find low numbers unless predominant in smear | Streptococcus pneumoniae / AST Haemophilus influenza / Beta lactamase |
| Report if moderate to many (>104 CFU/mL if quantitative) even if not predominant | *Moraxella catarrhalis Neisseria meningitidis Stenotrophomonas maltophila* Acinetobacter / AST Burkholderia |
| Report if moderate to many (>104 CFU/mL if quantitative) and predominant organism | *Staphylococcus* *aureus1* / AST Beta strep B (adults), C or G Single morphotype of GNR2 / AST Fastidious GNR / Beta lactamase *Corynebacterium* spp. *Rhodocuccus equi3* |
| Report SQ: <Quant>, ENGNR “ENTERIC GRAM NEGATIVE RODS” QMPS: NF MESS3 | One or more morphology of Gram-negative rods2 (not already reported) that grow on MAC and are oxidase negative, and either indole positive, lactose positive, or spreading. |
| Report SQ: <Quant>, NLFGN “GLUCOSE NONFERMENTING GRAM NEGATIVE RODS” QMPS: NF MESS3 | One or more morphotype of Gram-negative rods (not already reported) that are oxidase positive and grow on MAC. |
| Report SQ: NFUR “GROWTH CONSISTENT WITH UPPER RESPIRATORY TRACT FLORA” QMPS: FR NORF  If in addition to another organism(s):  SQ: ADFUR QMPS: NF NORF  If none of these organisms are seen report: SQ: XNFUR QMPS: FR NORT | Viridans streptococci and/or nonpathogenic *Neisseria*; diphtheroids;  coagulase-negative staphylococci; *Rothia*, group F streptococcus;  *Haemophilus* species (not *H. influenzae*);  *Eikenella*; *Actinobacillus*; *Capnocytophaga; Moraxella*; enterococci;  yeasts; and insignificant numbers of *S. aureus* organisms, Gram-negative rods, and *N. meningitidis*. |

1 *Staphylococcus aureus*: All suspect S. aureus should be tested (subculture if pure colonies are not present) using PBP2a to identify MRSA. If PBP2a is positive and isolate is S. aureus, report MRSA and perform AST. If PBP2a is negative and isolate is confirmed S. aureus report SQ: <Quant>, MSSAP “MSSA PRESENT, PART OF RESPIRATORY FLORA;” QMPS: FR MSSA with Quantity.

2Mucoid lactose fermenting isolates suspect of *Klebsiella* should be screened for resistance. If patient is in ICU, has history of CRE, or in a LTAC (Kindred North, SVSE, etc.) all present Enterobacteriaceae should be screened for resistance. Any CRE, ESBL, or MDR present should be identified and AST reported.

*3Rhodococcus equi* is mucoid GPR, does not grow on MAC. Can be identified by MALDI-TOF (not claimed) and confirmed by Rapid CB.

4All beta hemolytic group A strep should be examined for and reported, all beta strep group B on pediatric patients should be reported. Beta strep B in adults, C and G should be reported when mod/many and predominant. Beta strep group F is considered normal flora.

|  |  |
| --- | --- |
| **If** | **Then** |
| There is no growth on the plates on day 1 | Reincubate plates for an additional day and issue a preliminary report. |
| There is only normal oropharyngeal flora present on day 1 | Reincubate plates for an additional day and issue a preliminary report. |
| There are pathogens present on day 1 | Proceed according to Table I and issue a preliminary report. Also report the presence or absence of normal oropharyngeal flora with appropriate quantitation. See step 5 |

|  |  |
| --- | --- |
| **If** | **Then** |
| There is no growth after 48 hours | Discard plates and issue a final report. |
| There is only normal oropharyngeal flora present after 48 hours | Discard plates and issue a final report. |
| There are pathogens present after 48 hours. | Report the pathogen’s identification and susceptibility, if indicated. Also report the presence or absence of normal oropharyngeal flora with appropriate quantitation. |

|  |  |
| --- | --- |
| **Step** | **Action** |
| 5 | \*PLATE QUANTITATION  SQ QLS  RARE RARE = 1-5 colonies  FEW LIGHT = 1st quad only - non confluent  MOD MOD = confluent 1st quad or growth in 2nd quad  MANY HEAVY = growth in 3rd or 4th quad  (if more than 1 culture plate, depending on the organism, quantitate plate with heaviest growth) |
| 6 | Perform identification and susceptibility (as indicated) according to procedures found in procedure manual and flowcharts. |
| 7 | Save representative original plates and all purity plates for 1 week before discarding. |

**REPORTING RESULTS**

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Issue gram stain report. Report presence or absence of squamous epithelial cells, polys, and organisms seen on every smear. Report other findings only when observed (i.e. RBCs, columnar epithelial cells, etc.) |
| 2 | Issue culture report. See above for lower respiratory culture codes. |

**PROCEDURAL NOTES**

1. If a beaded gram-positive rod suggestive of *Nocardia* is seen on direct smear and fungal culture is not ordered, add BCYE to routine set up to aid recovery.
2. Do not repeat susceptibility testing on isolates tested within 4 days unless physician requests repeat testing.
3. For BAL specimens, report the larger quantitation observed. Inoculation errors may result in a large variance from one set of plates to the next with the same specimen.
4. Occasionally, there will be scenarios with culture results that do not fit the procedural guidelines exactly. Consultation with the Supervisor or Lead Technologist may be warranted.

**REFERENCES**

1. *Clinical Microbiology Procedures Handbook*. 4th Edition. American Society for Microbiology, 2016.
2. Murray, Patrick R., ed. *Manual of Clinical Microbiology*, American Society for Microbiology, p 46-47, 1999.
3. Sharp, S. E., A. Robinson, M. Saubolle, M. Santa Cruz, K. Carroll, and V. Baselski. 2004. *Cumitech 7B, Lower Respiratory Tract Infections.* Coordinating ed., S. E. Sharp. ASM Press, Washington, D.C.
4. Sodeman, T.M., “Microbiology of the Respiratory Tract”, *Laboratory Medicine*, 96-102, 1983.