KETTERING Health Network

Lower Respiratory Culture

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I. PRINCIPLE

Infections of the lower respiratory tract are the sixth leading cause of mortality in the United States, with 2,000,000 to 3,000,000 cases per year, resulting in 500,000 hospitalizations. Specimens from various respiratory sites are cultured to aid in the diagnosis of pneumonia, pharyngitis, sinusitis, tracheitis, and other respiratory illnesses. Careful evaluation of the Gram stain as well as the culture media is critical to assist in the differentiation between commensal flora of the upper respiratory tract and potentially pathogenic organisms.

II. SPECIMEN COLLECTION, TRANSPORT, AND HANDLING

A. **Specimens**
   1. For complete specimen requirements, refer to the Laboratory Collection Manual.
   2. For complete specimen processing requirements, refer to the Specimen Processing Procedure

B. **Transport and Handling**
   1. Store specimens at 2 to 8°C until samples can be transported or processed by the laboratory.

C. **Rejection Criteria**
   1. Sputums submitted for routine bacterial culture are screened for adequacy by Gram stain. See Gram stain procedure for rejection criteria.
   2. BAL specimens should never be rejected, since they are collected by an invasive procedure.

III. PROCEDURE

A. **Examination of Culture Plates**
   1. Observe plates after 18-24 hours incubation and again at 48 hours.
   2. Additional incubation may occur on request, which may allow detection of slow-growing, fastidious Gram-negative rods, such as *Bordetella bronchiseptica,* or *Pasteurella* spp. or molds such as *Aspergillus* spp.
   3. The original specimen Gram stain should be used as a guide to interpret the culture.
      a. Primary pathogen(s) grown in culture should be present in the original specimen Gram smear.
      b. If one or more isolates grow on culture that were not reported from the original Gram stain, the smear should be read a second time by a different person that the one who initially read the slide.
   4. Identify the organisms that meet the criteria below:
      a. Moderate to heavy growth of an isolate in the second or greater quadrant of the plate and are not considered to be part of the normal respiratory microflora.
      b. Colonies in the first quadrant of the plate provided there is little or no other normal respiratory microflora.
      c. If one or two probable pathogens are present in amounts equal to or greater than the normal flora-completely identify and perform susceptibilities as warranted.
      d. If greater than 2 probable pathogens are present, quantify and provide descriptive information based on colony morphology; no susceptibility testing performed.
      e. Note exceptions in sections below.
   5. Subculture isolates (if needed) to BAP and/or CHOC to obtain isolated colonies for accurate identification.
   6. Table 1 provides guidelines for definition of primary pathogens.

B. **Primary pathogens of lower respiratory tract infection**

1. ***Streptococcus* species**
   a. Examine for beta-hemolytic colonies and identify catalase-negative cocci in pairs and chains.
      1) Streptococcus Group A is **reported in any amount.**
      2) Examine for colonies with a small zone of hemolysis and identify group B streptococcus in pediatric patients, if present, in any amounts.
      3) Identify other beta-hemolytic streptococci in significant amounts only if they are predominant. Do **NOT** report small colony types of beta hemolytic streptococci or group F streptococci, as they are part of the upper respiratory microbiota.
   b. Examine alpha-hemolytic colonies for morphology consistent with *S. pneumoniae.*

2. **Fastidious Gram-negative rods (these microorganisms grow slowly or not at all on MAC). Perform spot tests on significant numbers to differentiate them from normal respiratory microbiota.**
   a. *H. influenzae* organisms are coccobacilli that grow on CHOC but not on BAP. Note:Satelliting growth may be seen around staphylococci on a BAP.
      1) Confirm identification and perform beta-lactamase test for penicillin susceptibility for *H. influenzae* isolates.
   b. Rarely seen organisms that may require consultation with the lab for growth and detection:
      1) CONSULT LAB IF SUSPECTED:  *Francisella tularensis* organisms are coccobacilli that can grow on CHOC but do not grow on BAP, even with staphylococci for satelliting. They are weakly catalase positive and oxidase and urease negative. They are beta-lactamase positive.
      2) REQUIRES SPECIALIZED MEDIA and CONSULT WITH LAB IF SUSPECTED: *Legionella* spp.
      3) *Bordetella* non-pertussis spp. grow on BAP are catalase and urease positive. They may be visible only after 48 h. *B. bronchiseptica* may be part of the normal microbiota.
      4) *Pasteurella* organisms are indole and oxidase positive and represent normal mouth microbiota of animals.
      5) CONSULT LAB IF SUSPECTED:  *Yersinia pestis* in any amount. It presents as a non-lactose-fermenting rod on MAC, but it may appear as pinpoint colonies on BAP at 24 h of incubation
3. **Gram-negative diplococci**
   a. Examine colonies present in significant amounts that move when pushed. Confirm as *Moraxella catarrhalis.* Since more than 90% of *M. catarrhalis* organisms are beta-lactamase positive, testing is not helpful to treatment.
   b. Examine CHOC for any oxidase-positive colonies that do not grow or grow poorly on BAP. Confirm identification of *Neisseria gonorrhoeae* and *N. meningitidis* (only if pure culture as this organism is commonly seen as usual oral microbiota). Do not perform AST.

NOTE: CONSULT LAB FOR SPECIAL REQUESTS TO LOOK FOR N. GONORRHEA

4. **Gram-negative rods that grow well on MAC**
   a. Criteria: If one or two probable pathogens are present in amounts equal to or greater than the normal flora-completely identify and perform susceptibilities as warranted. If greater than 2 probable pathogens are present, quantify and provide descriptive information based on colony morphology, spot tests (e.g., indole, oxidase, odor, and morphology on MAC, and colony pigment); no susceptibility testing performed.
   b. Commonly seen organisms:
      1) *Klebsiella pneumoniae*:
      2) *Pseudomonas aeruginosa, Acinetobacter, Burkholderia,* and *Stenotrophomonas* may be resistant to multiple antimicrobials and can be implicated in nosocomial epidemics.
5. **Staphylococci**
   a. Identify *S. aureus* if present in significant amounts.
      1) Perform AST if the Gram stain shows predominant cocci in clusters associated with WBCs and no other pathogen in significant amounts.
      2) Identify coagulase-negative staphylococci, without species identification or AST, only if they are in 90% pure culture. Otherwise include in respiratory flora.
6. **Do not report *Enterococcus* unless the culture is 90% pure.**
7. **Gram-positive rods – ID by Gram Stain**
   a. Rule out *Nocardia* in *any* amount and *Rhodococcus equi* (mucoid and urease positive) from immunocompromised patients
   b. Limit identification of *Corynebacterium,* to those present in *numerous and predominant* amounts and when either of the following is true.
      1) The organism is rapid urea positive. (*Corynebacterium pseudodiphtheriticum* is urea positive.)
      2) The specimen is from an intubated patient from an ICU.
   c. Generally, do not pursue other Gram-positive rods, as they are unlikely to cause pneumonia.
8. **Identify Molds, unless the organism is consistent with a laboratory or environmental contaminant (e.g., *Penicillium*)**
   a. Biphasic fungi *(Histoplasma capsulatum* and *Coccidioides immitis)* can be isolated from plates held for 48 h, even as the yeast-phase colony morphology. Use caution in examination of older cultures.
   b. Give plate to a mycologist. The mycology tech will continue with identification
      1) Refer to fungal culture procedure for details regarding fungal workup
9. **Rule out *Cryptococcus* and do not identify other yeasts further.**
   a. *Candida* organisms are not a cause of pneumonia, except possibly in oncology (e.g., leukemia) or lung transplant patients or in neonates. Even in those cases, growth of *Candida* in lower respiratory specimens, regardless of species, does not correlate with disease. Yeasts are normal inhabitants of the mouth.

C. **Reporting Results**

1. Report all pathogens and susceptibility tests performed using Table 1 as a guide. Always list pathogen first.
2. When normal oral flora is growing, it should be semi-quantitated in the report using: rare, light, moderate, and heavy using the mnemonic “OPFP” (oropharyngeal flora present)

Number of Colonies	Code
0-20 colonies	Rare
20 or more colonies in the 1st and 2nd quadrants	Light
Growth out to the 3rd quadrant	Moderate
Growth out to the 4th quadrant	Heavy

3. When normal oral flora is absent, note that in the report as ”OPFA” (oropharyngeal flora absent) or “OPFATD” (oropharyngeal flora absent to date)
4. Report “No growth to Date” if there is no growth on any plates on day 1 and “No Growth” if there is no growth after two days of incubation.

Table 1:

Action	Organism(s)
Examine for and always report.  , Do not work up at lab bench. Seal and submit to LRN laboratory for identification.	1. *Streptococcus pyogenes* 2. Group B streptococci in pediatric population 3. *Francisella tularensis*  4. *Yersinia pestis*  5. N*eisseria gonorrhoeae* 6. *Nocardia* 7. *Bacillus anthacis*  8. *Cryptococcus neoformans* 9. Molds, not considered saprophytic contaminants
Always report, but do not make an effort to find low numbers, unless they are seen in the smear.	1. *Streptococcus pneumoniae*, report AST. 2. *Haemophilus influenzae*, report beta-lactamase
Report if present in *significant*[a] amounts	1. *Moraxella catarrhalis* Report the following on inpatients only (report AST per criteria above): 2. *Pseudomonas aeruginosa,* 3. *Stenotrophomonas maltophilia* 4. *Acinetobacter* 5. *Burkholderia*
Report if present in *significant*[a] amounts **and** is the predominant organism in the culture, particularly if smear suggests infection with morphology consistent with isolate.	1. *Staphylococcus aureus*, report AST 2. Beta-hemolytic streptococcus B (adults), C, or G, 3. Single morphotype of Gram-negative rod (especially *Klebsiella pneumoniae*), report AST 4. Fastidious Gram-negative rods, usually report beta-lactamase 5. *Corynebacterium* if urea positive or from intensive care unit. 6. *Rhodococcus equi* in immunocompromised patients
Report as “oropharyngeal flora present.”	Viridans streptococci and/or nonpathogenic *Neisseria*; diphtheroids, coagulase-negative staphylococci; *Rothia*, group F streptococcus; anaerobes; *Haemophilus* species (not *H. influenzae*); *Eikenella*; *Actinobacillus*; Capnocytophaga*; Moraxella*; enterococci; yeasts; and insignificant numbers of *S. aureus* organisms, Gram-negative rods, and *N. meningitidis*.

a Significant is defined as moderate-heavy growth on the plate or $>10^4$ in a bronchial alveolar lavage specimen; small amounts of a bacterial species in the culture that are consistent with an etiologic agent seen in the Gram stain associated with inflammatory cells; or colonies in the first quadrant of the plate, only if there is little or no other microbiota on the plate (i.e., 90% pure) and the smear suggests inflammation.

I. INTERPREATION

A. A positive culture with *S. pneumoniae* or *H. influenzae* generally indicates infection with that organism, although carriage of these organisms may lead to false-positive results.

B. A positive culture with a predominant Gram-negative rod or *S. aureus* generally indicates infection with that agent if the smear suggests an infectious process involving the corresponding morphology.

C. A negative bacterial culture does not rule out lower respiratory infection. The primary pathogen is frequently not recovered from patients with pneumonia, either because they have already been started on antimicrobial therapy when samples are taken or because they have infection with another type of organism (e.g., virus, parasite, fungus, mycoplasma, or mycobacterium) that will not be recovered by bacterial culture.

II. LIMITATIONS

A. Some primary pathogens of pneumonia do not grow in routine bacterial culture.

B. False-negative bacterial cultures usually result from improper collection, prior antimicrobial therapy, and/or delayed transport of lower respiratory specimens to the laboratory.

C. False-positive results are usually caused by contamination of the specimen by normal respiratory flora and its subsequent growth on culture and overinterpretation by the laboratory. Contamination of a lower respiratory sample may also occur by leakage during transport.

III. REFERENCES

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