Minibronchoalveolar (BAL) Culture Procedure, Quantitative

Principle

Ventilator-associated pneumonia (VAP) is defined as nosocomial pneumonia in mechanically ventilated patients that develops greater than 48 hours after initiation of mechanical ventilation. VAP is the second most common nosocomial infection and is a leading cause of death among critically ill patients requiring mechanical ventilation. The appearance of fever, pulmonary infiltrates, and purulent sputum is a frequent occurrence in these patients. Because of the high mortality rate, the rapid identification of patients requiring antibiotic therapy and the accurate selection of appropriate antibiotic therapy is paramount. The collection and culture of a bronchoalveolar lavage specimen may help in establishing a specific diagnosis of pneumonia in such critically ill patients. A quantitative culture technique is used to aid in distinguishing between airway colonization and significant infection.

Specimen

20-50 ml of lavage fluid is collected by the Respiratory Therapy Dept. using a Mini-bronchoalveolar lavage (BAL) catheter kit and brought directly to the Microbiology Dept. within 30 minutes. This specimen will be submitted in a sputum trap (leuki tube) and labeled to specify as either left or right lung. The correct test to be ordered is “Culture, Respiratory Quantitative BAL” which includes a Gram Stain. These specimens are difficult to obtain and should not be rejected unless the entire sample has leaked out into a non-sterile transport bag.

Materials

- Blood agar (BAP)
- Chocolate agar (CAP)
- MacConkey agar (MAC)
- Phenylethyl Alcohol Agar (PEA)
- Calibrated .01 ml loop

Specimen Processing

1. Vortex specimen for 15 seconds before processing. Inoculate the four plates listed above with a .01 ml calibrated loop, using the urine culture quantitation streak method. Incubate plates for 18-24 hours at 37º in the CO2 incubator.

2. Prepare a Gram Stain using the cytocentrifuge. When reporting the gram stain results, do not quantify microorganisms seen, list only cell types present. Also, be certain to report any intracellular bacteria observed.
Culture Reading

1. Cultures showing no growth at 18-24 hours are reincubated for an additional 24 hours. Release a preliminary report of “NO MICROORGANISMS ISOLATED.”

2. If there is no growth after the second incubation period, discard the plates and release a final report of “NO MICROORGANISMS ISOLATED.”

3. If the culture is growing, each colony on a plate represents 100 cfu/ml of the original specimen (1 col. x multiplication factor of 100 [.01 cal. loop] = 100 cfu/ml). Perform a colony count on each organism type isolated.

4. The following colony count ranges may be reported.

<table>
<thead>
<tr>
<th>Colony Count</th>
<th>CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10²</td>
</tr>
<tr>
<td>10</td>
<td>10³</td>
</tr>
<tr>
<td>100</td>
<td>10⁴</td>
</tr>
<tr>
<td>1000</td>
<td>10⁵</td>
</tr>
</tbody>
</table>

   Most clinical studies have reported that >10⁴ cfu/ml or >10,000 cfu/ml for BAL (and Bacterial Index, see below) provides the most accurate diagnosis of VAP.

   Example: 12 colonies is 12 x 100 or 1,200 cfu/ml which is 1.2 x 10³ cfu/ml; 135 colonies is 135 x 100 or 13,500 cfu/ml which is 1.35 x 10⁴ cfu/ml.

5. Determine the Bacterial Index (BI).

   The Bacterial Index is defined as the sum of the logarithm of the number of each organism isolated. For example: if 3 different bacteria were isolated with the following colony counts; 10² cfu/ml, 10³ cfu/ml, and 10⁴ cfu/ml. The BI would be 8 (2+3+3=8). Studies show that a BI of >5 is suggestive of VAP, especially when taken into account that a significant proportion of VAP are caused by multiple species of bacteria.

   Under “Culture Report” enter as: BACTERIAL INDEX = ____.

Culture Workup

1. On significant isolates that are >10 cfu/ml, perform an identification and sensitivity. These would include any significant pathogens that would normally be performed on any respiratory culture. Potential pathogens may include:

   S. aureus
   S. pneumoniae
   N. meningitidis
   beta-streptococcus, other than Group A
   beta-streptococcus, Group A
   yeast
   Enterococcus spp.
   Hemophilus species
Enterobacteriaceae  
_Pseudomonas aeruginosa_  
other non-fermenting Gram negative rods  
*M. catarrhalis*  
filamentous fungi


4. Gram negative rods and *Enterococcus* species:

   <10,000 cfu/ml—report quantitation and presumptive identification  
   >10,000 cfu/ml—report quantitation, identification and susceptibility.

5. Gram negative cocci—screen pure cultures for *Moraxella catarrhalis* and *Neisseria meningitidis*. If negative, report as non-pathogenic *Neisseria* species. In mixed cultures, screen suspected colonies if >1,000 cfu/ml.

6. Yeast, filamentous fungi, and isolates of *Nocardia* species—refer to procedure MIC.FUN.604 for further workup.

References


Bronchoalveolar Lavage and Bronchial Brush Cultures, Semi-quantitative, 1997, Laboratory Services Department, Health Alliance of Greater Cincinnati.

LaRocco, Mark, PhD, Quantitative Bronchoscopy Cultures in Intubated Patients, 1991, Memorial Hermann Laboratory Services Microbiology, Houston, Texas.

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