The Standardization of Criteria for Processing and Interpreting Laboratory Specimens in Patients With Suspected Ventilator-Associated Pneumonia*

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The microbiologic analysis of respiratory specimens is a key component of both the clinical investigation of ventilator-associated pneumonia (VAP) and patient care. Although there is a substantial body of literature on the use of a variety of lower respiratory tract specimens, there is little standardization of the approaches to processing and interpreting results from the various specimens. The purpose of this communication is to delineate specific recommendations for the microbiologic analysis of specimens originating from the lower respiratory tract, as developed by the Laboratory Analysis Group of the Consensus Conference.

**MICROBIOLOGIC PROCESSING OF RESPIRATORY SPECIMENS**

**General Concepts**

In reviewing the literature on microbiologic processing of respiratory specimens for the diagnosis of VAP, several basic principles emerge that hold true for all specimen types and bear stating at the outset.

1. **Etiologic agents in pneumonia are generally present in high concentrations in lung secretions (≥10⁶-10⁸ cfu/ml).** Colonizing flora from upper airway sources are generally present in lower concentrations in good-quality specimens. Quantitation is therefore useful to discriminate infecting from colonizing organisms.

2. **In general, collection of samples protected from upper airway colonization will improve specificity.**

3. **Direct microscopy is an essential component of clinical investigation and initial patient management.** Analysis should include semiquantitation of both intra- and extracellular organisms, inflammatory cells, and squamous epithelial cells indicative of heavy oropharyngeal contamination.

4. **Antibiotic therapy may markedly influence results.** Specimens should be obtained prior to therapy, if possible; if not, this information should be available.

5. **Specimens should be transported to the laboratory in a timely fashion.** Transport within 30 min is ideal and 1 to 2 h maximal. Longer time intervals may significantly influence results.

6. **Specimens should be transported in a medium that maintains organisms in a viable state with little proportional change.**

7. **Although the microbiologic spectrum of VAP is extensive, the most commonly encountered bacteria fall into several main groups that are easily isolated and identified (eg, enterics, nonfermenters, streptococci, staphylococci, Hemophilus). However, a variety of special procedures should be available when unusual or epidemiologically defined organisms are suspected or when results of initial tests fail to demonstrate a pathogen (eg, Legionella, Chlamydia, viruses).**

8. **Few data are available that evaluate results between different infected patient groups (eg, geriatrics, chronic underlying diseases, otherwise healthy trauma) or according to time frame (eg, prospective screening, early onset, autopsy). However, the clinical situation may very well influence test choice and interpretation.**

9. **Communication, cooperation, and coordination among the microbiologist, pathologist, and pulmonologist are essential to ensure that adequate specimens are submitted, appropriate tests performed, and accurate results obtained.** To facilitate processing and rapid reporting, the laboratory should be notified directly when a procedure to obtain specimens is scheduled.

**Processing of Tracheal Aspirates**

**Background:** Tracheal aspirates (TA) obtained by suctioning represent specimens that are easily obtainable but notoriously nonspecific in the diagnosis of pneumonia. When compared to culture results of open-lung biopsy (OLB) or results from other specimen types such as protected specimen brushings (PSB),** cultural analyses of TA specimens show moderate to high sensitivity but generally low specificity. Studies performed in intubated patients are summarized in Table 1. These results are not surprising in view of the facts that most VAPs derive from aspiration of the upper airway flora and that colonization in intubated patients is very frequently with a variety of Gram-negative and other organisms generally considered pathogens.**

The net result of these findings is the frequent exposure of patients to antimicrobial therapy aimed at pathogens not necessarily present in the lower respiratory tract. Attempts to improve specificity through the use of quantitative cultures to detect organisms present at a level of 10⁶ to 10⁸ cfu/ml or greater have yielded mixed results, improving specificity when performed on blind bronchial suctioning** but showing equivalent results for tracheal aspirates.**

Microscopic analysis may be of potential use in the empiric selection of antimicrobial therapy. Salata et al** reported that specimens from intubated patients with pneumonia showed higher semiquantitative grading of neutrophils and bacteria, including intracellular organisms. All specimens included in
**Table 1 — Tracheal Aspirate Culture in the Diagnosis of VAP**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Comparative Cultures*</th>
<th>Lab Method†</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OLB SQ</td>
<td>9/11 (82)</td>
<td>10/37 (27)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Other SQ</td>
<td>11/10 (59)</td>
<td>NA NA</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Other SQ</td>
<td>7/6 (70)</td>
<td>0/11 (0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PSB SQ</td>
<td>8/9 (89)</td>
<td>12/13 (92)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>PSB SQ</td>
<td>16/16 (100)</td>
<td>6/6 (33)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Other Q (≥10&lt;sup&gt;4&lt;/sup&gt;)</td>
<td>18/21 (90)</td>
<td>13/22 (59)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>PSB SQ</td>
<td>18/18 (100)</td>
<td>2/7 (29)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>PSB SQ</td>
<td>32/34 (94)</td>
<td>7/7 (14)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>PSB Q (≥10&lt;sup&gt;4&lt;/sup&gt;)</td>
<td>30/31 (97)</td>
<td>16/16 (100)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>BAL SQ</td>
<td>11/15 (73)</td>
<td>12/25 (48)</td>
<td></td>
</tr>
</tbody>
</table>

*OLB, open-lung biopsy; PSB, protected specimen brush; BAL, bronchoalveolar lavage; other, blood, lung aspirates, autopsy, clinical diagnosis; SQ, semiquantitative (routine plating with indication of relative numbers); Q, quantitative (plating of several serial dilutions).

†Numbers in parentheses are thresholds in cfu/ml.

The study were screened for quality similar to sputa, showing ≥10 epithelial cells per low-power field. The suggestion was made that serial examination of specimens for increases in grading representing increasing organism load might provide a means for early diagnosis and treatment aimed at predominant morphotypes. As a corollary, it is expected that a negative smear would identify patients at low risk of pneumonia.

Salata et al<sup>6</sup> additionally reported that the demonstration of elastin fibers originating from parenchymal destruction in KOH preparations<sup>40</sup> was highly specific (91 percent) for infection with Gram-negative bacilli (10 of 11 cases) or *Staphylococcus aureus* (1 case). False-positives occurred in the setting of ARDS. Sensitivity was low, but positivity frequently preceded development of infiltrates (mean 1.8 ± 1.3 days).

The antibody-coated bacteria (ACB) test is another microscopic procedure that has been applied to tracheal aspirates from intubated patients.<sup>44</sup> The results from one study<sup>44</sup> were similar to those reported for sputa from patients not receiving ventilation,<sup>18</sup> with low sensitivity in detection of pneumonia (45 to 73 percent) but excellent specificity (98 to 100 percent). In another study, however, specificity was only 50 percent when compared to quantitative PSB culture.<sup>8</sup> It has also been suggested that a positive test may predict a subsequent infection, may be positive when quantitative culture results are low due to adverse specimen conditions, and may show antibiotic effectiveness by conversion to a negative test after treatment.

**Controversy:** A role for tracheal aspirate analysis in clinical investigation or patient management has not been firmly established.

**Recommendations:** Although analysis of TA is a routine practice in many institutions and is still incorporated into many investigational protocols, it should not serve as a basis for definitive diagnosis of pneumonia. When specimens are analyzed, they should be transported to the laboratory and processed for microscopy and culture as soon as possible. Direct microscopic analysis should include squamous epithelial cell (SEC) screening as number per low-power field, semiquantitative grading of polymorphonuclear leukocyte (PMN) by a standard format, and presence or absence of elastin fibers. The TA culture results should be reported semiquantitatively.

**Areas of Future Investigation:** The diagnostic performance of serial TA analyses, including semiquantitative stains, quantitative cultures, and ACB testing should be verified. In addition, the appropriateness of SEC screening as a criterion for specimen rejection should be evaluated.

**Processing of Protected Specimen Brushings**

**Background:** The most widely evaluated specimen type for the microbiologic diagnosis of bacterial VAP is PSB obtained by the technique of Wimberly et al.<sup>7</sup> Quantitative culture of PSB has shown oropharyngeal flora to be present at concentrations <10<sup>4</sup> cfu/ml of brush eluant<sup>17</sup> and pathogens at >10<sup>4</sup> cfu/ml of brush eluant in acute pneumonia.<sup>10,19</sup> Since PSB samples approximately 0.001 ml of secretions,<sup>18</sup> a 10<sup>4</sup> concentration represents 10<sup>6</sup> cfu/ml of secretions, which corresponds well with quantitation of pathogens in sputa<sup>30</sup> and tracheal aspirates.<sup>4</sup> In the setting of VAP, repeated studies have similarly shown the utility of quantitative culture of PSB for diagnosis.<sup>24,37,38,41-47</sup> These studies are summarized in Table 2.

In isolates recovered, PSB has shown good concordance with isolates identified by reference methods. Protected specimen brushing identified 100 percent of isolates (7 of 7) identified by blood, pleural fluid, or lung tissue culture or by serology, with only two additional contaminants recovered.<sup>4</sup> Similarly, PSB identified 79 percent of organisms (15 of 19) isolated from lung tissue,<sup>43</sup> with only one additional contaminant.

The major shortcoming of PSB relates to the small sample volume obtained. This results in low sensitivity of direct stains<sup>44</sup> unless performed on slides prepared directly from a brush.<sup>43,44</sup> In addition, multiple brushes or other specimens must be obtained to perform multiple studies for additional pathogens. Finally, at least some variations in sensitivity probably relate to the small sample volume,<sup>43,44</sup> and it is suggested that quantitation in the 10<sup>4</sup>-10<sup>5</sup> range be viewed as possibly significant.<sup>49</sup> An additional shortcoming of PSB relates to the impact of prior antimicrobial therapy on results. Both sensitivity<sup>47,49</sup> and specificity<sup>47,49</sup> can be decreased as a result of antimicrobial exposure, thereby making interpretation difficult.

One final issue of concern is the almost uniform use of Ringer's or physiologic saline solutions for the transport of PSB. More fastidious organisms (i.e., *Haemophilus influenzae*, *Streptococcus pneumoniae*) show a population decrease of 45 to 97 percent when suspended for 60 min in these solutions.<sup>45</sup> Anaerobes are unlikely to survive in such an oxygen-exposed environment, and it has been suggested that saline solution may be inhibitory to Legionella.<sup>50</sup> The use of saline solutions may therefore partially explain why anaerobes are infrequently isolated in VAP, although PSB is considered a potentially useful specimen.<sup>39</sup> Furthermore, in one study of PSB done in a routine laboratory setting with delayed transport,<sup>4</sup> the diagnostic value was set tenfold lower than the 10<sup>4</sup> threshold used in other studies in which processing was carried out within 15 min. These facts emphasize the need for expedient transport and processing.
Quantitative cultures should be performed. Two methods are available. A serial dilution method can be performed, as previously described.\textsuperscript{14,15} This approach is outlined in Figure 1. Results should be reported as cfu/ml for each morphotype. The advantages of this method include the ability to choose the most statistically appropriate dilution for counting and the ability to accurately count organisms in all count ranges. Alternatively, a calibrated loop method can be used. This approach, which is similar to that used for quantitative urine culture, is outlined in Figure 2. Results should be reported as log\textsuperscript{10} colony count ranges for each morphotype. This method is less laborious but also less accurate. However, it may be preferred in multicenter evaluations involving laboratories unable to perform a serial dilution procedure.

\textbf{Areas of Future Investigation:} The use of alternative, nontoxic holding media for transport should be investigated. An appropriate transport medium for anaerobes and the role of PSB in identifying anaerobes in VAP should also be determined. A role for direct microscopy in diagnosis should be verified and the optimal method of smear preparation identified. Finally, new methods for obtaining similar specimens should be compared to the standard brush currently in use.

\textbf{Processing of Bronchoalveolar Lavage}

\textbf{Background:} Bronchoalveolar lavage (BAL) has been estimated to sample approximately 1 million alveoli (about 1 percent of the lung), with the returned fluid representing a 100-fold dilution of the epithelial lining fluid.\textsuperscript{16} For concordance with PSB quantitation, therefore, a count of 10\textsuperscript{5} cfu/ml represents 10\textsuperscript{6} cfu/ml of undiluted fluid. In fact, studies in patients without ventilation have shown 10\textsuperscript{6} to 10\textsuperscript{7} cfu/ml to be an appropriate value to differentiate contamination from infection.\textsuperscript{17,20,24} Unfortunately, results in patients receiving ventilation have been more variable.\textsuperscript{1,10,12,20,27} These results are summarized in Table 3. Because the studies vary in techniques for collection as well as patient population, conclusions are difficult.

In isolates recovered, nonbronchoscopic BAL has shown good concordance with recovery from lung tissues in humans: 74 percent (32 of 43) in one study\textsuperscript{26} and 93 percent (13 of 14) in a second.\textsuperscript{27} In addition, BAL isolates in a baboon model showed better concordance with lung tissue isolates (74 percent) than those recovered from either PSB (41

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Quantitative culture: serial dilution method. Quantitate each morphotype present and express as cfu/ml.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Quantitative culture: calibrated loop method. Quantitate each morphotype present and express as log\textsuperscript{10} colony count ranges.}
\end{figure}

or, alternatively, the use of a transport medium that is noninhibitory. A few investigators have used various broths, which may be an alternative.\textsuperscript{15,25}

\textbf{Controversy:} A number of technical aspects are not standardized, including the choice of transport media and conditions, and the utility and method of preparation for direct microscopy. Clinical issues that are unclear include the role of anaerobes in VAP and the comparative roles of PSB and BAL in routine diagnosis.

\textbf{Recommendations:} For culture analysis, the brush should be aseptically severed into 1 ml transport medium and processed as soon as possible. At the present time, sterile nonbacteriostatic saline solution or Ringer's lactate solution may be used but are not optimal transport media because of possible organism toxicity.

Direct microscopy may be performed, but the optimal method for smear preparation has not been established. Methods used include aseptic preparation directly from the brush and cytocentrifugation of the suspended material. Cell types (eg, PMN, SEC, macrophages), and bacterial morphotypes should both be semiquantitatively reported in a standard fashion.
Table 2 — Results of Quantitative Cultures of PSB in VAP

<table>
<thead>
<tr>
<th>Reference</th>
<th>Pneumonia Definition</th>
<th>Transport Diluent*</th>
<th>Lab Method</th>
<th>Threshold, cfu/ml</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Histopathologic</td>
<td>B</td>
<td>Q</td>
<td>10^6</td>
<td>6/6 (100)</td>
<td>12/20 (60)</td>
</tr>
<tr>
<td>3</td>
<td>Confirmatory lab data†</td>
<td>B</td>
<td>SQ</td>
<td>Growth</td>
<td>7/7 (100)</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td>4</td>
<td>Final diagnosis‡</td>
<td>S</td>
<td>Q</td>
<td>10^6</td>
<td>8/8 (100)</td>
<td>12/13 (92)</td>
</tr>
<tr>
<td>7</td>
<td>Final diagnosis</td>
<td>R</td>
<td>Q</td>
<td>10^6</td>
<td>12/18 (66)</td>
<td>7/7 (100)</td>
</tr>
<tr>
<td>22</td>
<td>Final diagnosis</td>
<td>R</td>
<td>Q</td>
<td>10^6</td>
<td>34/34 (100)</td>
<td>72/76 (100)</td>
</tr>
<tr>
<td>23</td>
<td>Final diagnosis</td>
<td>S</td>
<td>Q</td>
<td>10^6</td>
<td>5/5 (100)</td>
<td>13/13 (100)</td>
</tr>
<tr>
<td>24</td>
<td>Final diagnosis</td>
<td>NA</td>
<td>Q</td>
<td>10^6</td>
<td>12/14 (86)</td>
<td>47/47 (100)</td>
</tr>
<tr>
<td>5</td>
<td>Final diagnosis</td>
<td>B</td>
<td>QS</td>
<td>10^6</td>
<td>16/18 (89)</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td>8</td>
<td>Final diagnosis</td>
<td>R</td>
<td>Q</td>
<td>10^6</td>
<td>19/34 (56)</td>
<td>6/7 (86)</td>
</tr>
<tr>
<td>25</td>
<td>Final diagnosis</td>
<td>NA</td>
<td>QS</td>
<td>10^6</td>
<td>41/49 (84)</td>
<td>35/36 (97)</td>
</tr>
<tr>
<td>26</td>
<td>Final diagnosis‡</td>
<td>S</td>
<td>Q</td>
<td>10^6</td>
<td>5/13 (38)</td>
<td>28/33 (85)</td>
</tr>
<tr>
<td>27</td>
<td>Confirmatory lab data</td>
<td>S</td>
<td>Q</td>
<td>10^6</td>
<td>11/17 (65)</td>
<td>57/61 (94)</td>
</tr>
</tbody>
</table>

*S, saline or balanced salt solution; R, lactated Ringers solution; B, broth; NA, not available; Q, quantitative, serial dilution method; QS, quantitative, calibrated loop method; SQ, semiquantitative, routine plating with indication of relative numbers.
†Blood, lung aspirate, or biopsy cultures; or serology.
‡Final diagnosis based on clinical criteria, response to therapy, additional lab data, or pathologic data when available.
§Includes some patients who were not receiving ventilation.

percent) or needle aspiration (56 percent). All three techniques showed similar false-positive isolations.6

Quantitative analysis of BAL, therefore, appears to be potentially useful in the diagnosis of VAP, and additional studies are warranted. In addition, BAL has a number of advantages over PSB, primarily the yield of a larger specimen volume able to accommodate multiple test requests, including a variety of microscopic procedures. In addition, previous antibiotic exposure seems to have less effect on results.6-24

Direct microscopic analysis of BAL has proven to be a particularly useful rapid procedure for the diagnosis of pneumonia, including VAP. Samples can be screened for SEC as an index of high oropharyngeal contamination, with >1 percent shown in one study to accurately predict contamination that might confound quantitative interpretation.23 It is of note that higher rates of excessive SEC are found when bronchial and alveolar lavage returns are pooled.24 Samples can also be screened for elastin fibers,24 which indicate parenchymal destruction associated with Gram-negative bacillary pneumonia similar to TA.4 For the detection of bacteria, the Gram stain is the most commonly employed procedure and provides information that is useful in selecting empiric antimicrobial therapy. Sensitivity and specificity have been reported to be 73 and 100 percent respectively in patients without ventilation23 and 100 percent and 88 to 100 percent in patients receiving mechanical ventilation.26 However, a Wright-Giemsa type stain (eg, Diff-Quik, Baxter Healthcare Corp, McGaw Park, Ill) is a useful adjunctive procedure for detecting bacteria, especially intracellular organisms (ICO).19

Percentage of ICO in alveolar phagocytic cells (PMN and macrophages) has also been reported to have clinical significance.16,20,31-33 Although various cutoffs ranging from 2 percent20 to 25 percent24 have been used to define a positive result, it is clear that the presence of ICO is a good predictor of VAP. However, one should keep in mind that many encapsulated microorganisms exist primarily extracellularly, so it seems prudent to at least semiquantitatively assess both intra- and extracellular bacteria. In fact, extracellular bacteria are increased in number in VAP20 and optimal microscopic sensitivity (100 percent) was achieved in one study of VAP by evaluating ICO, extracellular bacteria, and elastin fibers.20

Table 3 — Results of Quantitative Cultures of BAL in VAP

<table>
<thead>
<tr>
<th>Reference</th>
<th>Pneumonia Definition*</th>
<th>BAL Method† (Vol)</th>
<th>Lab Method</th>
<th>Threshold, cfu/ml</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Final diagnosis</td>
<td>B (100 ml)</td>
<td>Q</td>
<td>10^6 cfu/ml</td>
<td>4/5 (80)</td>
<td>9/13 (69)</td>
</tr>
<tr>
<td>3</td>
<td>Final diagnosis</td>
<td>B (150 ml)</td>
<td>Q‡</td>
<td>10^5 cfu/ml</td>
<td>19/34 (56)</td>
<td>NA (71)</td>
</tr>
<tr>
<td>35</td>
<td>Final diagnosis</td>
<td>B (120-240 ml)</td>
<td>QS</td>
<td>10^6 cfu/ml</td>
<td>15/17 (88)</td>
<td>24/24 (100)</td>
</tr>
<tr>
<td>10</td>
<td>Final diagnosis</td>
<td>B (100 ml)</td>
<td>QS</td>
<td>BI=5</td>
<td>NA (63)</td>
<td>NA (100)</td>
</tr>
<tr>
<td>37</td>
<td>Histopathologic</td>
<td>NB (100 ml)</td>
<td>NA</td>
<td>9/9 (90)</td>
<td>3/4 (75)</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Histopathologic</td>
<td>NB (NA)</td>
<td>SQ</td>
<td>Growth</td>
<td>34/30 (80)</td>
<td>19/29 (69)</td>
</tr>
<tr>
<td>26</td>
<td>Final diagnosis</td>
<td>PB (150 ml)</td>
<td>Q</td>
<td>10^6 cfu/ml</td>
<td>9/9 (100)</td>
<td>15/15(100)</td>
</tr>
</tbody>
</table>

*Final diagnosis based on clinical criteria, response to therapy, additional lab data, or pathologic diagnosis when available.
†B, bronchoscopic; NB, nonbronchoscopic; P, protected; vol, volume instilled; Q, quantitative, plating of several serial dilutions; QS, semiquantitative, routine plating with indication of relative numbers; and BI, bacterial index (sum of logarithmic concentrations of species).
‡Also used an initial tenfold concentration by centrifugation.
§Includes some patients not receiving mechanical ventilation.
One final point of discussion involves the processing methods employed in preparing samples for microscopy. There are a number of differences among investigators, including pooled or unpooled bronchial and alveolar fractions, centrifugation and cytocentrifugation speed and time, and method of reporting cell differential, which could result in significant interlaboratory variability. The guidelines published by the BAL Cooperative Group and the American Thoracic Society can serve as a basis for future standardization.

Controversy: As for PSB, the issue of effect of transport and holding conditions on results from a saline solution suspension is not clear. In addition, the methods for processing for direct microscopy are quite variable, as are the methods for expressing cell count and differential. Finally, the comparability of BAL to PSB in the setting of VAP has not been firmly established.

Recommendations for Specimen Handling: The first aliquot (bronchial fraction) should be processed as a separate specimen for stains and cultures for special groups of organisms only (eg, mycobacteria, fungi, Legionella). Other aliquots (alveolar fractions) should be pooled and used for microscopy and quantitative culture. Nonadherent glass or polypropylene containers should be used for transport, and samples should be delivered to the laboratory as soon as possible. The specimen should be aseptically divided into appropriate portions for microscopic, microbiologic, immunologic, and chemical analyses. The minimum acceptable volume for microscopic and microbiologic analysis of alveolar fractions is 5 ml each. Specimens for most nonmicrobiologic studies should be held at 4° to 8°C. For microbiologic or cell marker studies, samples should be at room temperature.

Recommendations for Microscopic Analysis: A total cell count should be performed on an unfractionated, uncentrifuged specimen using a hemocytometer counting chamber. The average of two counts is reported (excluding erythrocytes) as number of cells per milliliter of BAL fluid.

Bronchoalveolar lavage processing for other microscopic analyses may include an initial centrifugation step and should include cytocentrifugation of an adjusted suspension to obtain good-quality slides for staining. If a cytocentrifuge is not available, direct smears of a concentrated sediment may be prepared. Smears should be fixed in a manner appropriate to the stains to be performed. Figure 3 outlines a proposed processing guideline.

A Wright-Giemsa type stain of cytocentrifuged smears should be made in duplicate to obtain a differential count based on 300 cells (average of 100 consecutive cells counted 3 times under high-power magnification) excluding erythrocytes. Additionally, a Papanicolaou stain of smears or cellulose membrane filters should be performed. The report should include percentages of macrophages, neutrophils, eosinophils, lymphocytes, bronchial epithelial cells (if present), and SEC (if present). Due to a lack of consensus on the most appropriate reporting method, it is recommended that results be expressed both as percentage of total cells and percentage of inflammatory cells. The report should also include the presence of atypical, malignant, or other cells; intracytoplasmic microorganisms (percentage of macrophages and neutrophils); extracellular microorganisms (bacteria, fungi, protozoa); intracellular viral inclusions and viral cytopathic changes; and noncellular material. Other highly recommended cytologic stains include PAS and iron stains. A Gram stain should be performed, with the report semiquantitatively expressed in a standard fashion to include host cell types (PMN, macrophages, lymphocytes, SEC) and both intra- and extracellular microbial morphotypes. Additional slides can be used for a variety of special stains and

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**Figure 3.** BAL processing guidelines for microscopy, culture, and other analyses. Parentheses denote variable steps.
unstained slides prepared for future studies. Additional stains may include acid-fast (Kinyoun or auramine-rhodamine; modified acid-fast for Nocardia); fungal (Gomori methenamine silver, calcofluor white or periodic acid-Schiff); direct fluorescent antibody (e.g., Legionella, herpes simplex virus, cytomegalovirus, respiratory viruses); immunoperoxidase (viruses, kappa and lambda light chains, B and T cell markers, nonlymphoma malignancy markers); acridine orange (for ICO); iron; antibody-coated bacteria; and elastin (KOH mount or cytochemical).

**Recommendations for Microbiologic Analysis:** Quantitative cultures should be performed. As for PSB, two methods are available. The serial dilution method is outlined in Figure 1 and calibrated loop method in Figure 2. The remaining specimen should be concentrated by centrifugation and used for additional cultures to detect other unusual or epidemiologically implicated organisms, such as Legionella, mycobacteria, fungi, viruses, mycoplasmas, or Chlamydia.

**Areas of Future Investigation:** The use of alternative transport media designed to stabilize microorganisms (e.g., borate, Ames, or Stuart's) should be investigated. The roles of protected BAL and PSB in detecting anaerobes should be compared using an appropriate transport device. With regard to microscopic analysis, the appropriate thresholds for interpretation of microscopic findings should be validated using a standardized processing and reporting approach (e.g., percentage of SEC representing contamination, percentage of ICO representing infection). In addition, the clinical usefulness of other staining methods should be investigated (e.g., acridine orange, ACB). Finally, the diagnostic value of any new bronchoscopic or nonbronchoscopic technique to obtain BAL should be compared to a standard method.

**Processing of Lung Tissue**

**Background:** Lung tissue obtained by transthoracic biopsy provides a definitive diagnosis in VAP. Several recent studies suggest that this procedure may be tolerated by VAP patients and is indicated when results of tests on other respiratory specimens or blood fail to yield a diagnosis for which appropriate therapeutic response is seen. In immediate postmortem specimens from patients with histologically verified pneumonia, bacteria are present at levels >10⁶ cfu/g tissue, and colonizing flora from bronchial secretions are present at generally <10⁵ cfu/g. Whether this similarly holds true for living patients has not been determined. In addition, culture results from autopsy tissue other than that obtained in the immediate postmortem period have been highly variable compared to premortem findings and should not serve as a basis for definitive diagnosis.

Since a highly invasive procedure is required to obtain tissue, the laboratory should focus special attention on the analyses performed. Testing should therefore be comprehensive, including a wide variety of both stains and cultures. Close communication among the clinician, pathologist, and microbiologist is essential to ensure appropriate specimen handling.

**Recommendations:** A proposed specimen handling guideline is shown in Figure 4. Tissues obtained in the operating room (OR) should be aseptically divided by the pathologist or other designated individual for frozen section analysis and histopathologic and microbiologic examinations. The microbiologic portion should be placed in a sterile cup moistened with a small amount of nonbacteriostatic saline solution, and transported to microbiology as soon as possible.

Direct microscopy should be performed immediately to provide rapid information. Two methods are used to prepare smears: (1) touch preps may be used for a few stains (e.g., fluorescent antibody, Gram); and (2) homogenates are generally used for most stains (e.g., Gram, acid-fast, fungal).

 Cultures should be performed on aseptically prepared homogenates. Broth is preferred for the homogenizing liquid to avoid the potentially deleterious effects of saline solution. Homogenates may be prepared by a number of acceptable methods, including sterile mortar and pestle, stomacher (Tekmar, Inc.; Cincinnati, OH), and disposable or reusable sterile tissue grinders.

Quantitative cultures should be performed in clinical investigations using a serial dilution procedure. This method is outlined in Figure 5. At a minimum, cultures should include appropriate media for aerobes and anaerobes.

Samples should be held at 4°C to 8°C at least 1 week for additional requests if indicated and then stored indefinitely at −70°C to −80°C for future studies. It should be noted that

![Figure 4. Laboratory processing guidelines for open lung biopsy specimen.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21659/...)

![Figure 5. Quantitative lung-tissue culture. Quantitate each morphotype present and express as cfu/ml.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21659/...)

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**Processing and Interpreting Laboratory Specimens (Basselet et al)**

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similar procedures can be used in handling other lung tissue samples (e.g., transbronchial biopsies).

**Areas of Future Investigation:** For microscopic analysis of lung tissue, the use of alternate stains for the detection of bacteria and the significance of ICO in tissue should be investigated. Regarding culture analysis, the proposed threshold for interpretation of quantitative culture results should be validated in settings other than the immediate postmortem period and the effect of antibiotic therapy on results evaluated.

**Histologic Diagnosis of Pneumonia**

**Background**

Open-lung biopsy, the definitive pulmonary diagnostic procedure, is rarely indicated in the management of patients with clinical pneumonia who are receiving ventilation. The reliability of bronchoscopic techniques has relegated the use of those patients who fail to improve and have negative findings or who need the most rapid and specific diagnosis. Two recent reports utilizing OLB in patients with late ARDS have shown that the proliferative phase of diffuse alveolar damage can give rise to fever and leukocytosis. In one study, this phase of late ARDS could not be distinguished clinically from pneumonia.

Open-lung biopsy in patients with respiratory failure receiving mechanical ventilation has been demonstrated to be a relatively safe procedure. In over 160 published cases, no mortality was reported; reported morbidity was only 4 to 19 percent, related mainly to air leak into the pleural space. Biopsy analysis provided a specific and accurate diagnosis that affected treatment decisions for the majority of patients. The chief advantage of obtaining OLB specimens in critically ill patients is to obtain a specimen large enough for a variety of laboratory studies in addition to the routine hematoxylin-eosin stained sections. The optimal site for biopsy is somewhat controversial in patients with diffuse lung disease. It is unclear whether or not the easily accessible and resectable tip of the lingula of the left lung is representative of the pathologic condition in diffuse lung disease. Because of the circuitous route this upper lobe division takes over the heart, some have noted a nonspecific increase in inflammation, scarring, and vascular thickening. As a result, some authorities have suggested that the lingula as well as the right middle lobe be avoided as OLB sites.

**Recommendations**

**Processing of Open-Lung Biopsy Specimens:** A strict protocol for proper handling of diagnostic OLB specimens should be instituted. The clinician notifies the pathologist of an impending OLB at least 30 min before the tissue is removed and provides pertinent clinical, laboratory, and radiographic information. Upon excision, the tissue removed is placed on a saline solution-moistened sponge in a sterile container and handed directly to the pathologist who is in the operating room and is responsible for dividing the tissue appropriately. Immediately after receiving the tissue and while still in the sterile environment of the operating room, the pathologist submits a small piece of the grossly abnormal tissue for microbiologic analyses, as described previously.

The specimen is then transferred to the surgical pathology laboratory for gross examination and histopathologic evaluation. New sharp blades or razors should be used and the tissue cut in sawing motions without excessive pressure to avoid artifactual atelectasis. First of all, a frozen section evaluation of a representative part of the abnormal lung tissue is performed in all cases but most importantly in acutely ill patients, where a rapid diagnosis is required for initiation of appropriate therapy. Frozen sections can also be used to assess need for special stains if infection is suspected. An additional reason for doing frozen sections is to assess adequacy of the tissue obtained and to determine whether or not it is representative of the disease process and its correlation with clinical and radiologic findings.

Tissue from the grossly abnormal portion of the lung biopsy specimens is sliced into small and very thin cubes and immersed in glutaraldehyde fixative for electron microscopic evaluation as needed. The remaining tissue can then be fixed in formalin for routine processing. If the tissue is large enough, a piece should be snap-frozen in liquid nitrogen and stored at −70°C for further immunofluorescence studies or tissue analysis. For critically ill patients, a rapid technique for fixation and processing of the lung tissue for permanent sections may be used.

**Interpretation of Histologic Findings:** The interpretation of lung biopsy slides can be facilitated by an organized systematic approach to their examination, as described by Katzenstein and Askin. Strict criteria are used in evaluating the OLB for presence of pneumonia or diffuse alveolar damage. The presumptive histopathologic criterion for the diagnosis of pneumonia includes the presence of neutrophilic infiltration in the region of the terminal bronchioles surrounded by alveoli, which are partially filled with neutrophils, fibrinous exudates, and cellular debris. The etiologic diagnosis is provided following the completion of special stains for organisms and microbial cultures.

Absence of the above findings in the clinical setting of diffuse pulmonary infiltrates may suggest the development of diffuse alveolar damage (DAD). Histologic criteria favoring the diagnosis of DAD vary, depending both on the time interval between injury and biopsy and on the extent and localization of the lung injury.

During the first week, intra-alveolar proteinaceous exudate with hyaline membrane formation represents the hallmark of DAD. Secondary findings include interstitial edema; inflammatory cell infiltrates consisting predominantly of lymphocytes, plasma cells, and macrophages; and an alveolar lining cell hyperplasia with cytologic atypia. In the second week, the hallmark is interstitial and intra-alveolar, showing loosely aggregated fibroblasts and poorly formed fibrosis and fragments of hyaline membrane with scattered mononuclear inflammatory cells.

Based on the relative absence of intra-alveolar and broncholar neutrophils in both the frozen and permanent sections in addition to negative special stains for the presence of microorganisms, the diagnosis of pneumonia can be excluded, and diagnosis of DAD is suggested.

Although data are limited, similar histopathologic interpretive criteria may be applied to transbronchial biopsy specimens.

**Areas of Future Investigation**

The role of transbronchial biopsy in the diagnosis of VAP
should be investigated and alternative approaches to the acquisition of lung tissue for diagnostic studies developed.

REFERENCES

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