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Bronchoscopic Diagnosis of Pneumonia

VICKIE S. BASELSKI[1]* AND RICHARD G. WUNDERINK[2]

Departments of Pathology[1] and Medicine,[2] University of Tennessee, Memphis, Tennessee 38163

* Corresponding author. Mailing address: Department of Pathology, University of Tennessee, Room 576, 800 Madison Ave., Memphis, TN 38163. Phone: (901) 545-7760. Fax: (901) 448-6979.

INTRODUCTION

The advent of flexible fiberoptic bronchoscopy (FOB) introduced a quantum improvement in the diagnosis of pulmonary disease, particularly neoplasia (127). The use of FOB rapidly expanded into other types of lung disease, but, fairly quickly, routine bacterial cultures of secretions obtained by FOB were demonstrated to offer insignificant advantages over noninvasive techniques (12). FOB was used to obtain specimens for recovery of pathogenic organisms, such as *Mycobacterium tuberculosis*, in otherwise smear-negative cases, but its role in diagnosing infectious diseases of the lung was generally limited. This situation prevailed until changes in the spectrum of pulmonary infections required more accurate diagnosis than noninvasive methods could provide. Modifications in bronchoscopic techniques in response to these changes have now made diagnosis of pneumonia an important indication for FOB.

The first important challenge in the diagnosis of pneumonia occurred as a result of the rapid increase in the population of immunocompromised patients. The dual explosions in organ transplantation and AIDS resulted in increased numbers of patients with life-threatening pneumonia potentially caused by a wide variety of opportunistic organisms. In response, the technique of bronchoalveolar lavage (BAL) was modified for use in the diagnosis of these infections (214). Segmental BAL had been performed with rigid bronchoscopies or Metras catheters prior to the development of the FOB (127). The major advantage of BAL was the ability to obtain specimens at the level of the alveoli, important in infections that have a minimal bronchial component, such as *Pneumocystis carinii* pneumonia (PCP). Since presence in respiratory secretions is indicative of disease for many of these opportunistic organisms, contamination of FOB specimens by oropharyngeal secretions and colonization did not interfere with the diagnostic accuracy.

The second expansion in the role of FOB for infectious pulmonary disease came as a result of the ongoing difficulty in the diagnosis of bacterial pneumonia, particularly in the intensive care unit. The major problem in the microbiologic diagnosis of bacterial pneumonia was contamination of specimens by organisms present in oropharyngeal, or even tracheal, secretions that were not causing disease. Other invasive methods, such as transtracheal aspiration, had been tried but were either inappropriate (e.g., in a setting of endotracheal intubation), poorly tolerated in severely ill patients, or also prone to contamination (27). Wimberly et al., therefore, tried a variety of modifications of a technique using a bronchial brush in an effort to obtain uncontaminated distal lower respiratory tract secretions (239). A distally plugged, telescoping, double-catheter design, since called a protected specimen brush (PSB), had the greatest accuracy with the least contamination. An important modification incorporated into the use of the PSB was quantitative culturing by serial dilution of the secretions obtained. This allowed exclusion of even low-level contamination by virtue of culture growth below a diagnostic threshold. With these modifications, use of bronchoscopic specimens to diagnose pneumonia has now become an important indication for FOB, second only to its use to diagnose suspected intrathoracic malignancy (178).

CLINICAL ASPECTS

To fully appreciate the role that the microbiology laboratory plays in the diagnosis of pneumonia using bronchoscopic specimens, the indications for FOB and therapeutic implications of the results obtained must be understood. The indications for use of FOB to diagnose pneumonia vary from physician to physician but fall generally into three categories.

Clinical Indications

Pneumonia in immunosuppressed patients. The least controversial indication for FOB to diagnose pneumonia is in the immunosuppressed patient with pulmonary infiltrates. The reason for the nearly universal reliance on bronchoscopic diagnosis in immunosuppressed patients is the wide variety of etiologic agents potentially responsible for pneumonia in these patients. While clinical patterns may narrow the differential diagnosis, organisms whose presence may vary the prognosis and antimicrobial therapy may have very similar clinical presentations. The abnormal host defenses often leave little room for a trial of empiric therapy. Aggressive diagnosis is therefore generally warranted.

An indication for FOB does vary slightly by the type of immunosuppression. Bronchoscopy has become the initial procedure for patients with AIDS or organ transplants and diffuse pulmonary infiltrates because of the high prevalence of opportunistic infection in them. Bronchoscopy is presently the standard of care for these patients, and its high diagnostic accuracy has nearly obviated the need for open lung biopsy (OLB). Recently, the pendulum has swung back somewhat toward noninvasive diagnosis in AIDS patients (26, 253) despite the lower diagnostic yield, principally as an attempt to decrease the reliance on bronchoscopic procedures.

In patients with neutropenia or with focal pulmonary infiltrates, bacterial pneumonia is a more common problem than opportunistic infections, and bronchoscopy is often reserved until a trial of empiric broad-spectrum antibiotics has failed to achieve clinical resolution.

VAP. By far the most controversial area of bronchoscopic diagnosis is its use for bacterial ventilator-associated pneumo-

nia (VAP) (142, 161). The inaccuracy of noninvasive diagnosis of VAP has clearly been demonstrated by a variety of studies. Critically ill, intubated patients become colonized in their tracheas with gram-negative bacilli soon after admission to the intensive care unit, but only a third or less develop pneumonia (104). Clinical parameters such as fever, leukocytosis, and purulent secretions have been demonstrated to be nonspecific and cannot separate pneumonia from colonization (5, 74). Even radiographic infiltrates are neither specific (241, 248) nor sensitive (25) for pneumonia in intubated patients. Individual physician accuracy in predicting pneumonia on the basis of clinical information and tracheal aspirate cultures ranged from 71 to 82% (73).

In contrast, diagnosis of VAP by bronchoscopic techniques is much more accurate than diagnosis based on endotracheal aspirates (ETAs), with both sensitivity and specificity being >85% (49). A variety of bronchoscopic techniques have been developed, each with certain advantages and disadvantages. The common denominator of all techniques is use of a method that will obtain distal secretions from the alveolar or respiratory bronchiole level while minimizing contamination by proximal secretions.

Controversy persists regarding whether diagnostic bronchoscopy should be performed on all patients suspected of VAP, particularly if the patient has already been started on empiric antibiotic therapy (161). The availability of broad-spectrum, highly effective antibiotics allows the treatment of many potential bacterial pathogens without the need to determine the exact etiology. Concern regarding the emergence of multidrug-resistant organisms associated with the increase in broad-spectrum antibiotic therapy has been growing (148). Because of this, use of bronchoscopy varies widely. Some centers use bronchoscopic diagnosis as the standard of care, whereas in other centers bronchoscopy is only rarely performed.

Use of bronchoscopy to diagnose nosocomial bacterial pneumonia in nonintubated patients is much less common. The need to pass the bronchoscope through the vocal cords and obtain specimens without the benefit of topical anesthetic agents makes the procedure more difficult technically and more uncomfortable for the patient. FOB may in fact induce acute respiratory failure and require subsequent endotracheal intubation in patients with severely compromised respiratory function. For nosocomial pneumonia, FOB is usually reserved for patients who fail to respond to empiric antibiotic therapy (78). In this situation, FOB is performed to diagnose unusual or nonbacterial infections or to exclude bronchogenic carcinoma or other endobronchial lesion as the cause of delayed resolution of disease.

CAP. The indications for bronchoscopy for community-acquired pneumonia (CAP) are poorly defined. In contrast to VAP, CAP is often characterized by the inability to detect any known etiologic agent. In more than 40% of the cases, the causative organism is not demonstrated despite an aggressive search. The percentage of cases with a defined etiology increases if FOB is used (19, 225), particularly if the procedure is performed prior to initiation of antibiotic therapy (102). However, the greatest increase in yield is principally organisms typically susceptible to empiric antibiotics. Unless penicillin-resistant pneumococci are common in the community, a more accurate diagnosis may not significantly change antibiotic therapy.

Recent findings may increase the indications for FOB in suspected CAP. The increase in human immunodeficiency virus (HIV)-infected patients and the resurgence of tuberculosis (34) increase the need to rule out atypical opportunistic or nonbacterial pneumonia. Even PCP has to be considered in patients without known causes of immunosuppression (39, 100). Increasingly, noninfectious disorders presenting as CAP are being described (2, 78).

Despite these considerations, the primary motivation to perform bronchoscopy in patients with suspected CAP remains the desire to exclude a neoplasm obstructing the bronchus (78). Typically, bronchoscopy is considered when resolution of the pneumonia is delayed beyond the expected norm (78, 117). The second most likely cause for failure to resolve is infection with an uncommon organism, especially when the presentation is that of chronic pneumonia rather than slow resolution of an acute pneumonia (117).

Therapeutic Implications

The implications of the results of bronchoscopic sampling vary somewhat according to the indication. In the immunosuppressed host, culture or smear positivity of bronchoscopic specimens generally indicates the need to treat. Positive viral studies are the major exception, particularly positivity for cytomegalovirus (CMV). The major dilemma occurs when cultures and smears are negative. Often only a BAL (±PSB) is performed on the initial bronchoscopy. If bilateral BAL (145) or transbronchial biopsies (TBB) (214, 215) are also performed, fewer false-negatives may result. If these were not performed initially, repeat bronchoscopy with these additional techniques may be indicated. In particular, lymphocytic interstitial pneumonitis and pulmonary alveolar proteinosis have been described in the AIDS population and can be demonstrated by TBB. As the use of induced sputum to diagnose PCP in the AIDS population increases, the proportion of BAL specimens with false-negative results may increase, and routine use of TBB on the initial bronchoscopy may be warranted.

In the past, OLB was an important tool to determine the cause of fever and persistent infiltrates in immunosuppressed patients (141). The clearest benefit for OLB in patients with negative bronchoscopy appears to be in the bone marrow transplant patient (208). The diagnostic yield of OLB in other types of immunosuppressed patients with negative bronchoscopic results, particularly that result in therapeutic changes, has not been adequately restudied since the development of improved bronchoscopic techniques.

As mentioned previously, visualization of the airway to rule out an obstructing tumor is often the goal of bronchoscopy in patients with CAP. Bronchoscopy in this setting is often performed days to weeks after the initiation of antibiotics. Therefore, while not diagnostic in itself, negative cultures and smears assure that diagnosis of an unusual or nonbacterial etiology has not been missed. In contrast, positive cultures would direct antibiotic therapy, typically with agents other than those traditionally used for CAP (77).

The therapeutic benefit of bronchoscopy in VAP is more controversial. The major debate revolves around two questions: the cost/benefit ratio compared with empiric antibiotic therapy (161) and the reliability of a negative culture of bronchoscopic samples (49).

Cost/benefit ratio. At first glance, bronchoscopy with quantitative cultures of PSB and/or BAL would appear to be more expensive in cost as well as patient risk than empiric antibiotic therapy based on culture of ETA. However, since the etiologic agent is not definitely identified, by nature empiric antibiotic therapy is broader (and usually more expensive) than specific therapy. Fagon et al. performed a very conservative cost analysis and concluded that, if patients with VAP were treated for at least 6 days, empiric therapy was more costly than treatment of only patients with positive PSB cultures (74).

They included only the actual price of antibiotics and did not include nursing and pharmacy time, administration fees, or the cost of monitoring antibiotic levels, the combination of which in most cases exceeds the cost of antibiotics (245).

Empiric administration of antibiotics is also not without risks. Fagon et al. (72) and Rello et al. (184) have demonstrated that prior antibiotic therapy is associated with a significantly higher mortality rate from VAP than the mortality of VAP in patients who had not received prior antibiotics. This excess mortality appears to be primarily related to selection for more virulent organisms, such as *Pseudomonas* spp., *Staphylococcus aureus*, and possibly *Acinetobacter* spp., as the etiology of VAP (184, 246). Therefore, treatment of suspected but undocumented pneumonia may actually predispose patients to more serious pneumonia. This increased risk is not limited to one patient but may increase the risk of colonization or infection by multidrug-resistant bacterial strains in patients throughout the intensive care unit and even the entire hospital (148).

For the patient, probably the most important risk of not performing bronchoscopy is that another site of infection may be missed. The major benefit of a negative bronchoscopy may in fact be to direct attention away from the lungs as the source of fever. The overwhelming majority of mechanically ventilated patients with negative bronchoscopic cultures have other sites of infection that can be identified via a simple diagnostic protocol (142). Patients with negative bronchoscopy cultures averaged more than two infectious and noninfectious sources of fever, and many of the infections required a therapeutic intervention in addition to antibiotics, such as chest tube placement for empyema (142). Delay in diagnosis or definitive treatment of the true site of infection may lead to prolonged antibiotic therapy, more antibiotic-associated complications, and induction of further organ dysfunction.

Reliability of negative culture results. The second major critical consideration is the reliability of negative cultures of bronchoscopy specimens. The specificity of quantitative cultures of bronchoscopy specimens has also been questioned. However, most of the studies that document false-positive results have been performed on patients without clinical evidence of pneumonia (224). In patients with clinical suspicion of pneumonia, the real indication for bronchoscopic sampling is either to exclude pneumonia or to document an organism different from that present in tracheal aspirate cultures. Overdiagnosis based on bronchoscopic findings will result only in antibiotic treatment of patients who would have been treated anyway if decisions were based solely on clinical criteria and ETA cultures.

Withholding or withdrawing antibiotics from a patient with clinical evidence of pneumonia but negative bronchoscopy cultures potentially exposes a patient with pneumonia to increased morbidity or mortality. Therefore, culture sensitivity is much more clinically important than specificity. Cook et al. reviewed the use of bronchoscopic diagnosis of VAP and could not document adverse consequences of withholding antibiotics in patients with negative bronchoscopic cultures (49). In the largest series reviewed, Fagon et al. found that pneumonia could definitely be excluded in 70% of patients with a nondiagnostic PSB culture and could not definitely be proven in any of the remainder (74). Other centers have found patients with negative PSB cultures but positive BAL cultures and vice versa (146). Therefore, while the sensitivity of culture appears to be high, false-negative cultures may occur.

The sensitivity is determined to a large extent by the threshold chosen to represent a "positive" quantitative culture. As the diagnostic threshold is raised, the sensitivity decreases and specificity concomitantly improves. A consensus of investigators in the area of bronchoscopic diagnosis of VAP has suggested that the appropriate diagnostic thresholds for PSB and BAL are $\geq 10^3$ and $\geq 10^4$ CFU/ml, respectively (144). The threshold for PSB was determined by comparison with quantitative cultures of OLB specimens (46), while the thresholds for the various types of BAL were based predominantly on clinical correlations.

These thresholds are clearly not absolute, and quantitative culture results within 1 $\log_{10}$ for PSB and 1 or 2 logs for BAL should be interpreted cautiously. Dreyfuss et al. have demonstrated that 30% of patients with a PSB culture of $>10^2$ CFU/ml, but $<10^3$ CFU/ml, ultimately developed colony counts diagnostic of pneumonia (63). Therefore, counts that are just below diagnostic thresholds may represent early pneumonia. An autopsy study by Rouby et al. tends to support this concept (189).

A false-negative culture can occur for a variety of technical reasons. Improper placement of the PSB or inadequate fluid return from the lavage procedure is a potential cause, particularly in patients with lower lobe infiltrates or collapsible airways. The major problem, however, in both published studies and clinical practice, is the use of antibiotics prior to the performance of bronchoscopy. Many clinicians initiate antibiotic therapy after the first occurrence of fever, often before a pulmonary source is suspected. The quantitative culture results may be affected by antibiotic present in the specimen, even if the organism is somewhat resistant. Recent (<48 h) institution of antibiotics appears to be more of a problem than a more prolonged course of therapy. Baughman et al. (23) and Fagon et al. (74) demonstrated that patients who develop new infiltrates and fever on antibiotics for >3 days still had a high diagnostic yield for bacterial pneumonia. In contrast, Montravers et al. (151) have demonstrated that PSB cultures repeated after 48 h were sterile in 93% of patients with previously diagnostic quantitative cultures. Fox et al. found similar results with repeat BAL cultures (81). Therefore, the initiation of antibiotic therapy to which the causative organism is susceptible may cause a false-negative result.

Conversely, patients who have antibiotic-resistant organisms or anatomic limitations to antibiotic penetration often have persistently positive bronchoscopic cultures, some above the diagnostic threshold, despite antibiotic administration (81, 151). Therefore, a negative result for a patient on empiric antibiotic coverage can be interpreted to indicate that the chosen antibiotic regimen is adequate and no adjustment is required. This situation is the predominant one when bronchoscopic diagnosis is used in patients with CAP.

No randomized, controlled study of patients with VAP comparing patient outcome after bronchoscopic diagnosis or empiric therapy has been done. Until those studies are performed, the cost-effectiveness of the two strategies can only be inferred and will be subject to both the underlying financial assumptions of the analysis (41) and the local expertise and antibiotic management strategy (142).

BRONCHOSCOPIC TECHNIQUES

Procedure

Technical aspects of the FOB procedure can have significant effects on the accuracy of subsequent culture results (144). The fiberoptic bronchoscope has one or more working channels through which medications and instruments are passed and to which suction is applied to retrieve specimens. Contamination of this working channel during passage of the bronchoscope

through the upper airway or trachea is the major limitation of bacteriologic diagnosis (12). Modifications of specimen retrieval, discussed below, and quantitative cultures are used to control for this contamination. However, poor technique during bronchoscopy can negate the benefit of these modifications.

Avoidance of suctioning through the working channel before retrieval of specimens for bacterial culture is critical. Technically difficult in the nonintubated patient, this is probably the major reason bronchoscopic diagnosis is still seldom used in the nonintubated patient with nosocomial pneumonia. Suctioning is performed principally to clear the distal tip of the bronchoscope of secretions to permit better visualization. While an endotracheal or tracheostomy tube avoids the need to suction in order to pass the bronchoscope through the nares, oropharynx, and vocal cords, the presence of large amounts of secretions in the trachea and proximal airways maintains the temptation to suction. Aggressive suctioning of the proximal airway with a separate suction catheter prior to beginning bronchoscopy may alleviate this problem. However, because visualization is adversely affected by avoidance of suctioning, precise localization for specimen retrieval may be difficult.

The channel of the bronchoscope may become contaminated by $>10^5$ CFU/ml despite avoidance of suctioning (221). In most bronchoscopies, suction is maintained on a side port of the working channel, drawing mainly room air from the proximal opening until it is occluded by the bronchoscopist. When the channel is occluded proximally, secretions are aspirated into the distal opening of the channel at the tip of the bronchoscope. High airway pressures are generated during cough or with positive-pressure mechanical ventilation. Since the channel of the bronchoscope is open to atmospheric pressure, it may represent a site for pressure release. The air flow generated from high airway pressure can then carry secretions with it into the bronchoscope channel, particularly with cough.

The use of topical anesthetic agents such as lidocaine may also lead to contamination of the specimen and, potentially, to suppression of growth of some bacteria (238). Since the concentration of lidocaine in specimens is below the minimal inhibitory threshold of most infectious agents (116), the major risk appears to be contamination by injection of lidocaine through the working channel with expulsion of secretions that had accumulated in the channel. This is a particular problem in nonintubated patients because of the need to pass the tube past the vocal cords. Aerosolization of lidocaine into the oropharynx and proximal airways provides adequate anesthesia in many, but not all, patients.

The fluid return on BAL varies greatly and may affect the validity of results, although the significance of this effect has not been fully studied. In order to sample alveolar lining fluid, at least 120 ml of lavage fluid should be instilled (127, 144). The percentage of fluid that can be aspirated back can vary considerably. In patients with emphysema, collapse of airways with the negative pressure needed to aspirate fluid may limit the amount of fluid retrieved. Return may also be poor in patients with lower lobe or posterior infiltrates because of the effect of gravity. Attempts to reposition the bronchoscope or patient may result in loss of the seal that prevents contamination of the specimen by proximal airway secretions. Conversely, a very small return may contain only diluted material from the bronchial rather than alveolar level and result in a false-negative result.

The last technical problem is proper localization of the area for sampling. Localization of an infiltrate on a posterior-anterior and lateral chest radiograph of a patient with lobar CAP is not difficult. However, many patients have either diffuse infiltrates or changes in a previously abnormal chest radiograph. In intubated patients, only a single portable anterior-posterior film is available, and determining the correct airway to sample may be very difficult.

In patients with CAP, sampling the airway containing purulent secretions should have a high diagnostic yield. In contrast, in intubated patients, sampling only areas of purulence seen endoscopically may be inadequate. Almost all intubated patients have purulent-looking secretions, and the secretions first seen may represent those aspirated from another site into gravity-dependent airways or from upper-airway secretions aspirated around the endotracheal tube.

Specimen Types

There are a variety of bronchoscopic specimens that may be sent for microbiologic analysis. The appropriate studies and the diagnostic accuracy vary by the specimen type.

Bronchial washings. Bronchial washings are the secretions aspirated back through the bronchoscope channel after instillation of saline into a major airway. The secretions obtained by this method do not represent material from the bronchiolar or alveolar level. In intubated patients, bronchial washings are no different than an ETA obtained with a suction catheter. In nonintubated patients, they may be contaminated by upper-airway secretions. Therefore, bronchial washings are not appropriate specimens for bacterial culture (12). The best potential use of bronchial washings is for diagnosing pneumonia caused by strictly pathogenic organisms, such as *M. tuberculosis* and endemic systemic fungi, particularly in patients for whom the BAL return volume is inadequate.

Bronchial brushings. Routine bronchial brushes are designed for exfoliative cytologic diagnosis of malignancies. The cytology brush is stiffer than a PSB in order to obtain cellular material from the airway wall. The incidence of mucosal hemorrhage is therefore slightly higher after this procedure. Because the usual bronchial brush is not protected from contamination during passage through the bronchoscope channel, it is inappropriate for bacterial cultures. In contrast, because cells are obtained from the airway walls, specimens from a cytology brush are appropriate and accurate for the diagnosis of cytopathic changes or viral inclusion bodies in airway cells.

PSB. PSB are collected with a brush within two telescoping catheters, the outer of which is occluded with a Carbowax plug. The Carbowax plug prevents secretions from entering the catheters during passage through the bronchoscope channel. Once the device has been passed through the bronchoscope channel, the inner catheter is advanced. The Carbowax plug is expelled into the airway lumen, where it is absorbed. Secretions pushed out of the lumen of the bronchoscope channel and secretions present in the colonized airway of intubated patients are bypassed when the inner catheter is advanced. The brush itself is then advanced past the tip of the inner catheter to obtain secretions from the distal bronchioles. In contrast to the cytology brush, the PSB bristles are more numerous and not as stiff in order to maximize the amount of secretions that will be obtained. However, the actual amount of secretions collected is small, only 0.001 to 0.01 ml (239). After the specimen is obtained, the sequence is reversed, with retraction of the brush into the inner catheter, and the inner catheter into the outer and removal of the PSB from the bronchoscope channel. The outer catheter is wiped clean with 70% alcohol and cut off distal to the inner catheter. The inner catheter is then advanced, wiped with alcohol, and cut off distal to the

brush. The brush is then advanced and cut off into 1 ml of diluent, which is submitted to the microbiology laboratory as soon as possible. Minor variations in technique include pulling out the bronchoscope with the PSB catheter still protruding distally in order to minimize contamination during retrieval and carrying the entire PSB to the microbiology laboratory rather than performing the initial dilution at the bedside (74). Since the PSB was developed for the diagnosis of bacterial pneumonia, its value is almost exclusively for that purpose. Therefore, only quantitative culture and possibly Gram stain (176) of the secretions obtained by PSB justify its use.

Single-sheathed catheter brushes (243) and telescoping plugged catheter tips (172), with or without distal plugs, are also available and have been used for the diagnosis of pneumonia. Single-sheathed devices were originally found to be less likely to remain uncontaminated in a model system (239), and neither has been subjected to the rigorous evaluation reported for the PSB (144).

BAL. The difference between BAL and bronchial washings is large despite a similar superficial appearance. BAL requires careful wedging of the tip of the bronchoscope into an airway lumen, isolating that airway from the rest of the central airways. A large volume of saline, generally greater than 140 ml, in several (three to four) aliquots (127, 144), is injected through the lumen. This large volume is designed to sample fluids and secretions in the distal respiratory bronchioles and alveoli. It is estimated that approximately 1 million alveoli (1% of the lung surface) are sampled, with approximately 1 ml of actual lung secretions returned in the total lavage fluid (127). The total volume returned varies with the amount instilled but is generally 10 to 100 ml. The initial aliquot of fluid is enriched for secretions found in the subsegmental bronchus and is usually discarded or used similarly to bronchial washings (55).

BAL has the double advantage of being appropriate for almost all microbiologic procedures and usually of adequate volume to perform the multiple tests. When adequate volume is instilled, BAL clearly samples at the alveolar level, which is important for infections such as PCP. For this reason, BAL is the mainstay of bronchoscopic diagnosis in the immunosuppressed host. Recent adaptations of the technique in which BAL is performed nonbronchoscopically by using a Metras catheter may further increase the availability of this specimen type (222).

Meduri et al. (143) introduced the protected BAL catheter in an effort to maintain the sensitivity of BAL while matching the reduced upper-airway contamination afforded by PSB. This technique involves the use of a distally plugged catheter with a distal inflatable balloon. The catheter is passed into the proximal airway lumen, and inflation of the balloon, rather than wedging of the bronchoscope, isolates the airway from contamination. The main advantage of protected BAL is increased utility for quantitative bacterial cultures in VAP, but it can be used for all of the tests for opportunistic pathogens usually performed on unprotected BAL.

TBB. TBB samples are obtained by passing a forceps through the working channel of the bronchoscope to obtain small samples of alveolar or peribronchial tissue. To obtain alveolar tissue, the forceps must be passed distal to the level visualized by the bronchoscope, and this procedure is therefore usually performed under fluoroscopic guidance. TBB specimens are principally examined by histologic techniques with special stains to exclude infection, but specimens can also be cultured if placed in sterile saline rather than formalin.

Invaluable in the diagnosis of disorders such as neoplasms and sarcoidosis, TBB has a more limited role in the diagnosis of pneumonia. In AIDS patients with diffuse infiltrates, TBB may increase the diagnosis of PCP by 15% (214) and the rate of positive tuberculosis cultures marginally (112). TBB also offers an opportunity to document tissue invasion by opportunistic fungi and herpesviruses (125). For diagnosis of bacterial bronchopneumonia, sensitivity is compromised by sampling error (189), and specificity is compromised by the potential for low-level, upper-airway contamination (46). TBB is probably most important to document noninfectious etiologies.

Complications

The risk of FOB for diagnosis of pneumonia varies with the severity of the patient's disease. Some patients are clearly too ill to undergo bronchoscopy. In a critically ill patient with impending respiratory failure, performance of bronchoscopy may lead to a need for endotracheal intubation or to respiratory arrest. Certain procedures also increase the risk of complications, particularly in thrombocytopenic or mechanically ventilated patients (168).

The main complications are hypoxemia, bleeding, cardiac compromise, and pneumothorax. Hypoxemia is the major complication of BAL. However, in mechanically ventilated patients with the adult respiratory distress syndrome, only 5% of patients had arterial oxygen desaturation to <90% during bronchoscopy despite severe hypoxemia in many patients prebronchoscopy (211). Nonintubated patients with severe hypoxemia may have greater problems with hypoxemia and may require prophylactic intubation in order for the procedure to be performed safely.

Bleeding is principally a complication of brushing and TBB. The bleeding risk is particularly significant in patients with thrombocytopenia or a coagulopathy. While TBB samples can be obtained in the patients (168), the significantly higher risk should mandate that the information obtained be critical to optimal patient management.

Pneumothorax is also principally a complication of brushing and TBB, although it can occur after BAL alone in mechanically ventilated patients. In most patients, pneumothorax is the result of inadvertent distal sampling, particularly when such sampling is done without fluoroscopic guidance. In mechanically ventilated patients, pneumothorax probably results as commonly from barotrauma induced by the increased airway pressures associated with bronchoscopy.

Hemodynamic changes in nonintubated patients are often due to the sedative agents used as premedication. In intubated patients, induction of high positive-end expiratory pressures during bronchoscopy may cause bradycardia and hypotension (144). In some patients, hypotension may be the result of mediator release induced by the bronchoscopic procedure. While bacteremia does not appear to occur after PSB, release of tumor necrosis factor alpha has been documented in a normal patient undergoing BAL (209). Transbronchial spread of infection is also an extremely remote possibility (144).

OVERVIEW OF LABORATORY METHODS

The microbiologic analysis of bronchoscopic specimens is complicated by a number of variables relating to both the pathology of infectious lung disease and practical considerations of specimen handling. First, the etiologic spectrum is extensive, encompassing all major categories of microorganisms. Therefore, the laboratory must be prepared to identify a vast array of organisms by using a number of different procedures (16, 125). In addition, because many infections are polymicrobial, it is generally necessary to perform multiple analyses on the specimens submitted (125). Second, even when

TABLE 1. Relative frequency of etiologic agents of severe pneumonia in various clinical settings

Organism group	Relative frequency[a] in patients with:				
	HIV infection	Organ transplant	Hospital-acquired pneumonia	VAP	CAP
Gram-positive aerobes					
Staphylococcus aureus	++	++	+++	+++	++
Other *Staphylococcus* spp.	+	+	+	+	+
Streptococcus pneumoniae	+++	+	+	++	+++
Streptococcus, viridans group	+	+	+	+	+
Other	+	+	+	+	+
Gram-negative aerobes					
Pseudomonas aeruginosa	+	++	+++	+++	+
Acinetobacter spp.	+	+	++	++	+
Other nonfermenters	+	+	++	++	+
Enterobacter spp.	+	+	+++	+++	+
Klebsiella spp.	+	+	++	++	+
Other enteric bacilli	+	+	++	++	+
Moraxella catarrhalis	+	+	+	+	++
Haemophilus influenzae	++	+	+	++	+++
Other fastidious bacilli	+	+	+	+	+
Anaerobes	0	0	++	0	++
Legionella spp.	+	++	++	+	+++
Mycobacterium tuberculosis	+++	+	+	0	++
Mycobacterium avium complex	+++	++	?	?	+
Other *Mycobacterium* spp.	++	++	?	?	+
Nocardia spp.	++	++	?	?	+
Chlamydia pneumoniae	+	?	+	?	+++
Chlamydia psittaci	+	?	0	0	+
Mycoplasma pneumoniae	++	?	+	?	++
Other *Mycoplasma* spp.	+	?	?	?	?
Viruses					
Herpesvirus group	++	+++	0	+	0
Respiratory viruses	++	++	++	+	++
Fungi					
Cryptococcus neoformans	++	++	0	0	+
Candida spp.	++	++	0	0	0
Other yeasts	++	++	0	0	0
Histoplasma capsulatum	++	++	0	0	++
Blastomyces dermatitidis	+	+	0	0	++
Coccidioides immitis	++	+	0	0	++
Sporothrix schenkii	+	0	0	0	+
Aspergillus spp.	+	++	0	0	0
Zygomycetes	+	++	0	0	0
Other hyphomycetes	+	++	0	0	0
Protozoans					
Toxoplasma gondii	+	+	0	0	+
Leishmania spp.	+	?	0	0	0
Microspordia	+	?	0	0	0
Cryptosporidium parvum	+	?	0	0	0
Pneumocystis carinii	+++	++	0	0	+
Helminths					
Strongyloides stercoralis	+	+	0	0	0

[a] +++, very frequent; ++, occasional; +, rare; 0, not reported; ?, unknown.

careful bronchoscopic techniques are used, the analysis of specimens is confounded by the inevitable presence of colonizing organisms that may also be etiologic agents of disease in the respiratory tract of seriously ill patients (103). To differentiate colonization from infection, the laboratory must employ quantitative culture techniques (47). Third, because of the clinical need for timely institution of specific therapy, special emphasis is placed on the availability of tests with rapid turnaround times, especially direct microscopy. Rapid processing of specimens for culture is also desirable to prevent loss of viability of pathogens or overgrowth of contaminants in these unpreserved specimen types. Finally, the renewed emphasis on cost containment in the era of managed competition challenges the laboratory to accomplish all of these tasks in a cost-effective manner (203).

Microbiologic Spectrum

An overview of the microorganisms that may be etiologic agents of lower respiratory tract infection in adults in the clinical settings in which bronchoscopy is frequently performed is given in Table 1. The relative frequencies would vary somewhat in pediatric age groups. The organisms may be roughly categorized into two groups. A few are considered to be strict pathogens and include *Legionella* spp., *Mycoplasma pneumoniae*, *Chlamydia* spp., *Nocardia* spp., *M. tuberculosis*,

TABLE 2. General characteristics of bronchoscopic specimens

Anatomic site	Associated infection(s)	Bronchoscopic specimens	Specimen amt (ml)
Large bronchi	Bronchitis	Bronchial washings	10–20
		Bronchial brushings	0.01
Bronchioles	Bronchiolitis	PSB	0.01–0.001
Alveoli	Alveolitis	BAL	10–100
Parenchyma	Bronchopneumonia Interstitial pneumonitis	TBB	0.1 g

Francisella spp., *Bordetella* spp., systemic dimorphic fungi, seasonal respiratory viruses, protozoans, and helminths. While the presence of colonizing flora in respiratory specimens may pose technical difficulties for their detection, there is no difficulty in assessing the significance of a positive result. In contrast, assessment of the clinical significance of organisms capable of both colonizing upper airways and causing lower respiratory tract infection depends on both careful specimen selection and collection and critical interpretation of the findings.

To some extent, it is possible to predict which organisms are most etiologically probable in a given patient on the basis of a careful clinical assessment (76, 125). However, there is significant overlap in clinical features, generally necessitating that the laboratory process respiratory specimens simultaneously to detect several organism groups.

Of note is that, despite the number of organisms recognized as potential pathogens, in 30 to 60% of suspected cases of pneumonia no specific etiologic diagnosis is made even when an extensive battery of noninvasive tests has been performed (19, 75, 191). However, when FOB is incorporated into well-defined diagnostic protocols in immunocompromised (105, 125) or community (166, 205) settings, the percentage of cases with a defined etiology may rise to 80 to 90%. Since FOB also offers an opportunity to diagnose noninfectious etiologies that may be manifested similarly, the total diagnostic yield may be even greater.

Bronchoscopic Specimens

General characteristics. As previously discussed, FOB may produce several types of specimens for microbiologic analysis. The specimens are both qualitatively and quantitatively different and must be handled in a manner appropriate to each type (Table 2). Qualitatively, different anatomic sites, each of which may show evidence of infection, are sampled. Because of the need to pass through the upper respiratory tract, including the oro- or nasopharynx, or through endotracheal tubes to reach the lower respiratory tract, all samples are potentially subject to microbial contamination. However, since inflammatory secretions from lower levels mix with upper-level secretions, these specimens may yield a true pathogen. This continuum of sampling poses substantial interpretive difficulties in the analysis of bronchoscopic specimens and places special demands for processing on the laboratory.

Quantitatively, the samples also vary considerably. Samples collected by brushing or biopsy are limited in volume, and therefore the number of tests that may be performed on them is restricted. In contrast, washings or lavage is larger in volume and generally sufficient for multiple analyses.

Numbers submitted. During a single bronchoscopy procedure, multiple specimen types are generally obtained and indeed may provide complementary information (125, 142, 230). The specimen types vary to some extent with the clinical setting. For the HIV-infected patient, it is most common to obtain BAL specimens, with TBB providing little additional information (125) and PSB samples obtained only when suspicion of bacterial pneumonia is high (79, 249). For other immunocompromised hosts, BAL and PSB specimens are obtained, but TBB is particularly important to document tissue invasion by opportunistic fungi and herpesviruses (125, 147). Bilateral BAL may increase the sensitivity for detecting some pathogens, particularly *P. carinii* and CMV (145). In organ transplant patients, surveillance BAL and TBB may be used for early identification of infectious complications (187, 210).

In VAP, PSB and BAL samples are generally obtained from the affected subsegment of the lung (142), but the need to select a specific subsegment has been questioned (137). On occasion, in diffuse disease, bilateral sampling may provide additional information (142). In severe pneumonia in the nonventilated patient, again both PSB and BAL specimens may be obtained, with TBB used primarily to rule out noninfectious conditions. In all situations, follow-up bronchoscopy with procurement of additional specimens is dictated by clinical assessment of therapeutic failure or new-onset disease or if the original samples were nondiagnostic. Finally, as previously noted, ordinary bronchial washings provide little if any additional information when BAL samples have been obtained.

Guidelines for transport. For transport to the laboratory, ordinary brushings and washings are placed in sterile leak-proof containers. For PSB, it is recommended that the brush be aseptically severed into a measured volume (generally 1 ml) of sterile diluent, most commonly, nonbacteriostatic saline or lactated Ringer's solution (17). For BAL, which is a saline solution, transport in a sterile, leak-proof, nonadherent glass or polypropylene container is recommended to avoid loss of cells for cytologic assessment (91). The initial aliquot, which represents a bronchial fraction (55), should be either discarded or transported separately from the remaining pooled alveolar fractions. The only potential use of the sample would be for detection of strict pathogens if the alveolar fraction return was an inadequate volume for multiple analyses. A designated individual in the bronchoscopy area or laboratory should be responsible for aseptically dividing the alveolar sample into appropriate portions for cytologic, microbiologic, immunologic, and chemical analyses (128). Only microscopic and other microbiologic tests will be considered in this review, although the other analyses are an essential part of the overall utility of BAL specimens (128). For TBB, tissue pieces should be placed in a sterile container moistened with a small amount of nonbacteriostatic saline (48). However, some researchers advocate placement of the tissue pieces on a saline-soaked gauze to facilitate cell attachment in "touch preps" (94). Again, a designated individual in the bronchoscopy area should place tissue pieces into appropriate containers for frozen section, histopathologic, and microbiologic analyses.

Preanalytical variables. Several variables related to specimen collection and transport are important in obtaining accurate results on respiratory secretions, including bronchoscopic specimens. Excessive delays in transport which result in both overgrowth of contaminating or colonizing organisms and deterioration of more fastidious pathogens should be avoided. Quantitative culture of freshly collected sputa versus samples transported at room temperature over an approximately 4-h period showed selective decreases in *Streptococcus pneumoniae* and *Haemophilus influenzae* isolation rates and fewer morphotypes overall in delayed specimens but higher counts of some

other organisms, particularly gram-negative bacilli (150). Similarly, in an experimental canine model, *S. pneumoniae* recovery from bronchoscopic samples was substantially improved by "dogside" cultures compared with samples transported to a remote laboratory (153). Although no absolute guideline exists, it is generally accepted that 30 min is optimal (91) and 2 h at room temperature is the outside limit for transport and holding of respiratory specimens before they are processed for bacterial pathogens, excluding anaerobes (15). Refrigeration to prolong transport time may be used, but its effect on bacterial culture results, particularly quantitative culture, has not been evaluated. For recovery of anaerobes, the role of bronchoscopy is controversial, but if attempted from PSB or protected BAL specimens, samples should be transported in an anaerobic device and processed within 30 min (15). For other organism types (*Mycobacterium* spp. or fungi), refrigeration for longer periods is acceptable, but some more fastidious organisms (viruses, mycoplasmas, and chlamydiae) require transfer into specific transport media for holding.

A second variable of concern is the potential for inhibition of some microorganisms by the solutions used in bronchoscopy and specimen transport. Lidocaine and other topical anesthetics may be inhibitory (238) but generally not at the concentrations encountered in respiratory secretions when the agents are applied by nebulization (116). However, the almost universal use of saline for the BAL procedure and for PSB transport may be of more concern. *S. pneumoniae* and *H. influenzae* may show a population decrease of 45 to 97% over a 60-min period when suspended in saline or lactated Ringer's solution at room temperature (183), and it has been suggested that saline is toxic to *Legionella* spp. (94). These observations further emphasize the need for expedient transport and processing.

Finally, as previously discussed, it is clear that prior antibiotic therapy may influence the accuracy of results obtained on cultures of respiratory secretions. In transtracheal aspirates, prior antibiotic use dramatically decreased recovery of *S. pneumoniae* while shifting the predominant isolates to gram-negative bacilli, the significance of which was difficult to ascertain (9). Similarly, in CAP, prior antibiotic therapy is significantly associated with undetermined etiology (75). Using bronchoscopy to diagnose VAP, a setting in which prior antibiotic therapy is common, both sensitivity (146, 223) and specificity (46, 72, 224) are compromised, with the effects most pronounced in PSB specimens (146). Reduced sensitivity presumably results from antibiotic inhibition of pathogens, while reduced specificity probably results from increased airway colonization. However, one recent study using follow-up PSB to assess treatment in nosocomial pneumonia suggests that significant growth may actually be more indicative of emerging pathogens resistant to the initial antibiotic regimen (151). Although problematic, the conclusion is that samples should be obtained before antibiotic use if at all possible, and results should be interpreted cautiously when antibiotics have been given (144).

Specimen Handling

Once bronchoscopic specimens are received in the laboratory, they should be handled according to clearly defined guidelines. Guidelines should address specimen preparation, staining techniques and smear interpretation, use of non-culture-dependent tests, culture techniques, and culture interpretation.

For nonprotected bronchial washings and brushings received in fluid, the protocol should ensure that a portion is submitted for cytologic examination if requested. In microbiology, those specimens are primarily useful only for mycobacteria, fungi, and a few other special groups of organisms (*Legionella* spp. or possibly respiratory viruses) and should be processed in accordance with laboratory protocols for these agents.

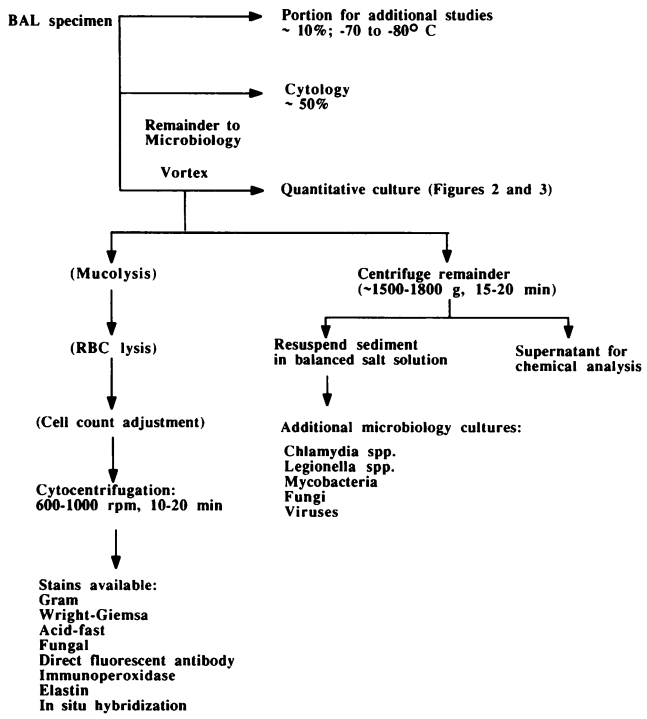

FIG. 1. BAL processing guidelines for microscopy, culture, and other analyses. Parentheses denote variable steps. RBC, erythrocytes. Reprinted from reference 17 with permission of the publisher.

PSB received in diluent should be vortexed manually or mechanically prior to inoculation (138). In general, only quantitative culture for routine bacterial pathogens is indicated or even possible since the amount of material received is so limited. However, a role for diagnosis of anaerobic infection has been suggested (30). The sensitivity of direct microscopy, primarily Gram stains, is generally low (97, 146), unless smears are prepared directly from an additional brush specimen (218) or aseptically prior to placement in the transport diluent (176).

BAL samples are the most versatile of all bronchoscopic specimen types and, as such, require more elaborate handling (Fig. 1). The pooled alveolar aliquots can be used for microscopy and culture for most organism groups. The minimal acceptable volume for comprehensive microbiologic studies is approximately 10 ml. After the specimen is vortexed, quantitative culture is first performed directly from the fluid. For other analyses, the remaining fluid is concentrated by centrifugation. Although the optimal relative centrifugal force and time have not been determined, 1,500 to 1,800 $\times$ *g* for 15 to 20 min appears adequate (17, 108). Some authors also advocate straining the specimen through sterile gauze before centrifugation (108). Samples that are extremely mucoid may be processed with a mucolytic agent such as dithiothreitol to facilitate handling (109). The sediment is resuspended in an adequate volume of diluent to perform other requested tests.

For preparation of smears from BAL for staining, cytocentrifugation of an adjusted cell suspension is the preferred

method (108). This technique, involving low-speed centrifugation of cells in suspension with simultaneous absorption of fluid onto a filter pad, results in a discrete 6-mm-diameter monolayer of organisms and host cells with well-preserved morphologic features. This technique has been shown to increase the sensitivity of Gram stains of a variety of body fluids, including respiratory secretions (200), stains for *P. carinii* in respiratory secretions (89), and acid-fast stains for mycobacteria (195). The volume of BAL specimens available generally permits the preparation of several smears for a variety of chromophore and immunohistochemical stains.

TBB samples, which are obtained at some risk to the patient and often in settings in which prior samples have failed to yield a diagnosis, should be accorded special attention. A protocol similar to that recommended for transthoracic biopsies should be established (48). In the laboratory, tissue may be used to aseptically prepare touch preps for a variety of rapid stains (94). Subsequently, the tissue should be homogenized in diluent for culture inoculation and additional stains. Homogenates may be prepared by a number of acceptable procedures, including sterile mortar and pestle, disposable or reusable sterile tissue grinders, or Stomacher processors (Tekmar, Inc., Cincinnati, Ohio).

Direct Microscopy

Direct microscopy may be viewed as three distinct categories: stains for cytologic assessment, stains for general organism categories, and stains for specific organism types.

Cytologic assessment. Histopathologic analysis of TBB and cytopathologic analysis of washings, brushings, and BAL specimens to detect noninfectious conditions, to document cellular changes associated with viral infection, or to document fungal invasion are extremely important components of laboratory testing. However, the microbiology laboratory's role is primarily in assessment of specimen quality. For washings or brushings, a Gram stain report of relative numbers of inflammatory cells and squamous or bronchial epithelial cell types is probably sufficient to indicate degree of oropharyngeal contamination.

For BAL, it is recommended that a total cell count be performed to assess specimen adequacy and a differential count be performed to assess cellularity (128). The differential should be expressed by a standard format, probably as percentages of both total cells and inflammatory cells (17). For quality assessment, the percentage of squamous and bronchial epithelial cells may be used to predict heavy upper respiratory contamination. A level of >1% of the total cells has been suggested, but not universally accepted, as a rejection criterion (110, 190, 197). At the least, this criterion can be used to guide the subsequent extent of culture workup. For most infectious etiologies, an increased percentage of neutrophils or lymphocytes will be noted (129). The recommended staining method for quality assessment is a modified Giemsa stain (e.g., Dif-Quik; Scientific Products, McGaw Park, Ill.). This stain offers a number of advantages over a Gram stain, including better host cell morphology, improved detection of bacteria (particularly gram-negative or intracellular ones), and detection of some unusual protozoan and fungal pathogens (e.g., *Histoplasma*, *Pneumocystis*, *Toxoplasma*, and *Leishmania* spp.) (129).

Another cytologic marker that may be detected in bronchoscopic specimens is elastin fibers. These fibers originate from parenchymal destruction associated with necrotizing pneumonia, generally of gram-negative bacillary origin. Detection may be accomplished by using a simple KOH method or by specific stains and has been performed on sputa (201), tracheal aspirates (197), and BAL specimens (180). In the latter, fibers were noted in 47% of infected individuals with VAP compared with only 8% of controls. The major problem with the procedure is the occasional presence of fibers due to adult respiratory distress syndrome alone.

Organism detection. For the detection of general organism groups, a variety of readily available staining procedures may be employed. For bacteria, the Gram stain is the most frequently employed procedure, providing rapid morphologic information essential for selection of initial antibiotic therapy. Since it is a generally accepted tenet that approximately 10^5 CFU/ml are required for reliable microscopic detection, it follows that the finding of large numbers of a specific morphotype would correlate with infection. Indeed, for PSB (146, 172, 176, 218, 243) and BAL (109, 146, 180), although the Gram stain sensitivities reported have been variable, specificities have been 90% or better.

The determination of percentage of intracellular organisms in alveolar phagocytic cells has also been reported to be useful with BAL specimens from ventilated patients. Although varying cutoff points have been used to define a positive result (2 to 25%), sensitivities have ranged from 73 to 100% and specificities have been generally >90% in the diagnosis of pneumonia (44, 45, 146, 180). However, one recent study has suggested that prior antibiotic therapy may dramatically reduce the sensitivity (61). One should keep in mind that many organisms, especially encapsulated ones, exist primarily extracellularly, so it seems prudent to also consider these morphotypes significant. In one study, 100% sensitivity was reported for BAL in diagnosing VAP by the evaluation of intracellular organisms, extracellular forms, and elastin fibers (180).

Finally, the antibody-coated bacterium (ACB) test has been applied to FOB aspirates from nonventilated patients in an attempt to differentiate infected from colonized patients (243). In this study, a sensitivity of 73% and a specificity of 98% were achieved by using a procedure similar to that used for detecting ACB in urine. Three of four pneumonia patients with false-negative PSB cultures had positive ACB results, leading the authors to suggest that the ACB test may allow recognition of infection when factors such as previous antibiotic therapy cause false-negative cultures. The ACB test has not been evaluated with other bronchoscopic specimen types. However, when the test was performed on ETAs from intubated patients, variable sensitivities (48 to 73%) but excellent specificities (98 to 100%) were noted (123, 247); however, the test did not perform better than Gram stains alone (123).

For detection of other organism groups, several additional stains should be used, particularly in the evaluation of BAL specimens from immunocompromised patients (94, 125). For *Mycobacterium* spp., both auramine-rhodamine- and carbol-fuchsin-based acid-fast stains should be performed since some species other than *M. tuberculosis* may not be detected by the former (129). For *Nocardia* spp., a modified acid-fast stain should be used to verify Gram stain findings (43). Fungi and *P. carinii* may be detected by a variety of cell wall stains; methenamine silver (129) and calcofluor white (18) are particularly useful.

Specific stains. For specific detection of a number of organisms including *Legionella* spp., herpes simplex virus (HSV), CMV, respiratory viruses, and *P. carinii*, commercially available direct fluorescent-antibody (DFA) stains may be used (94, 125). For HSV and CMV, in situ hybridization has also been used (129). The application of these techniques will be discussed below.

TABLE 3. Interpretation of quantitative culture results from lower respiratory tract secretions

Specimen type	Quantity collected	Dilution factor	Diagnostic threshold (CFU/ml)
Sputum, bronchoscopic aspirates, ETAs	Generally several ml	1	10^5–10^6
PSB	0.01–0.001 ml in 1 ml of diluent	1/100–1/1,000	10^3
BAL	1 ml in 10–100 ml of effluent	1/10–1/100	10^4

Non-Culture-Dependent Methods

Other than direct microscopy, relatively few non-culture-dependent methods have been applied to bronchoscopic specimens and, when used, have been primarily with BAL specimens. Latex agglutination for pneumococcal antigen has been used (166, 205), and in immunocompromised patients a variety of formats have been used to detect *Cryptococcus* (21), *Histoplasma* (234), *Candida* (157), and *Aspergillus* (6) antigens. These tests have been moderately sensitive, occasionally positive when direct smears were negative, and generally quite specific for disease caused by these organisms. A few enzyme or other immunoassay procedures for RNA viruses are available, but the specimens of choice are nasopharyngeal or tracheal aspirates rather than bronchoscopic specimens (235). Finally, an enzyme immunoassay for the lipid A component of endotoxin has been used experimentally to diagnose gram-negative pneumonia (38) but has not been verified as clinically useful in human disease. These antigen tests should be considered adjuncts to, but not replacements for, culture.

Nucleic acid hybridization tests have been relatively insensitive compared with culture in a variety of situations, but nucleic acid amplification techniques such as the PCR offer great promise for improved detection of strict pathogens, including those from respiratory sources (219). The exquisite sensitivity of these tests should allow them to be used for routine, first-line testing of noninvasive specimens, with bronchoscopic specimens providing a useful secondary specimen type.

Culture Procedures

For most organism types, culture remains the definitive diagnostic method. Media and incubation conditions should be appropriate for cultivation of the organism group being sought (15). A variety of specialized, selective media may be used to detect specific organism groups (e.g., *Mycobacterium*, *Legionella*, *Mycoplasma*, and *Nocardia* spp.), and appropriate cell cultures may be inoculated to detect *Chlamydia* spp. and viruses.

Basis for quantitative cultures. Due to the inevitable oropharyngeal bacterial contamination that occurs in the collection of all bronchoscopic samples, quantitative culture techniques have been advocated to differentiate oropharyngeal contaminants present at low counts from higher-count infecting organisms (17, 47, 125, 144). The basis for the quantitative techniques recommended is shown in Table 3. In fact, the concept of quantitative bacteriology to improve diagnosis of lower respiratory tract infection is not new. Almost three decades ago, Monroe and colleagues (150, 174) demonstrated that organisms associated with pneumonia could be found in liquefied sputum in concentrations of $\geq 10^7$ CFU/ml. Importantly, pathogens rapidly decreased in concentration in response to appropriate therapy, and detection of emerging superinfections with organisms in high counts was possible. Bartlett and Finegold (14) reported similar findings in a comparison of quantitative culture of washed, liquefied sputum and transtracheal aspirates in which potential pathogens were generally recovered from both in a mean concentration of $\geq 10^6$ CFU/ml, with oropharyngeal contaminants present at a mean of $<10^4$ CFU/ml. Jordan et al. (106) extended these findings to tracheobronchial secretions obtained by FOB in which only low-level contaminants ($<10^4$ CFU/ml) were found in normal volunteers and suggested that a threshold for significance of $\geq 10^5$ CFU/ml was appropriate. More recently, Salata et al. (197) reported higher mean counts (10^6 CFU/ml) in ETAs from infected intubated patients than in those from colonized patients (10^4 CFU/ml). Finally, Marquette et al. (135) have reported that, for ETAs, 10^6 CFU/ml provides a reasonable diagnostic threshold (sensitivity, 82%; specificity, 83%) for pneumonia in mechanically ventilated patients.

Taken together, these studies suggest that pathogens are present in lower respiratory tract inflammatory secretions at a concentration of at least 10^5 to 10^6 CFU/ml, and contaminants generally are present at $<10^4$ CFU/ml. The diagnostic thresholds proposed for PSB and BAL are an extension of this concept. Thus, 10^3 CFU/ml for PSB, which collects 0.001 to 0.01 ml of secretions (a 1/1,000 to 1/100 dilution when placed in 1 ml of diluent), actually represents 10^5 to 10^6 CFU/ml of secretions. Similarly, 10^4 CFU/ml for BAL, which collects 1 ml of secretions in 10 to 100 ml of effluent, represents 10^5 to 10^6 CFU/ml.

Numerous studies have evaluated the utility of quantitative cultures of PSB and BAL in the diagnosis of bacterial pneumonia in a variety of clinical settings. These studies are summarized in Tables 4 and 5. Although there is considerable variability in methods used, in both clinical definitions and technical aspects, most studies show a moderate to high sensitivity and specificity when quantitative techniques are used. In addition, in normal volunteers, both PSB and BAL specimens generally yield growth at concentrations less than the established thresholds (116), particularly when recommended precautions to avoid contamination are taken (167). Further, when PSB and BAL results are compared with simultaneous culture of tissue, there is reasonably good concordance (>70%) of isolates obtained (46, 85, 189, 190).

Two important questions regarding the utility of quantitative cultures remain unanswered. First, is quantitative culture really better than ordinary semiquantitative culture? This question is salient since culture results reported as "moderate to numerous" (3 to 4+) generally indicate high colony counts ($\geq 10^5$ to 10^6 CFU/ml) and results reported as "rare to few" (1 to 2+) generally represent lower counts (10^2 to 10^3 CFU/ml) (186). The issue has not been specifically addressed for bronchoscopic samples. However, early studies using sputum showed poor correlation of semiquantitative reporting with actual numbers of organisms present (60, 115, 150, 237); in particular, pathogen counts were underestimated. It is likely that dilution plating facilitates detection by dispersing organism clumps, reducing effects of inhibitors (including antibiotics), and pre-

TABLE 4. Quantitative culture of PSB in the diagnosis of bacterial pneumonia[a]

Patient group[b]	Reference	Pneumonia definition[c]	Threshold (CFU/ml)	No. positive/no. with pneumonia (% sensitivity)	No. negative/no. without pneumonia (% specificity)
Normal	Halperin et al., 1982 (96)	None	Growth	NA[d]	14/52 (27)
	Kirkpatrick and Bass, 1989 (116)	None	10^3	NA	8/8 (100)
VAP	Chastre et al., 1984 (46)	Histopathology	10^3	6/6 (100)	12/20 (60)
	Villers et al., 1985 (229)	Laboratory data	Growth	7/7 (100)	10/10 (100)
	Baughman et al., 1987 (23)	Final diagnosis	10^2	8/8 (100)	12/13 (92)
	Torres et al., 1988 (222)	Final diagnosis	10^3	12/18 (66)	7/7 (100)
	Fagon et al., 1988 (74)	Final diagnosis	10^3	34/34 (100)	72/76 (95)
	Chastre et al., 1988 (44)	Final diagnosis	10^3	5/5 (100)	13/13 (100)
	Chastre et al., 1989 (45)	Final diagnosis	10^3	12/14 (86)	47/47 (100)
	Lambert et al., 1989 (123)	Final diagnosis	10^3	16/18 (89)	4/4 (100)
	Torres et al., 1989 (223)	Final diagnosis	10^3	19/34 (56)	6/7 (86)
	DeCastro et al., 1991 (56)	Final diagnosis	10^3	41/49 (84)	35/36 (97)
	Meduri et al., 1991 (143)	Final diagnosis	10^3	5/13 (38)	28/33 (85)
	Pham et al., 1991 (172)	Laboratory data	10^3	11/17 (65)	57/61 (94)
	Meduri et al., 1992 (146)	Final diagnosis	10^3	3/9 (33)	14/14 (100)
	Violan et al., 1993 (230)	Final diagnosis	10^3	16/25 (64)	20/20 (100)
	Marquette et al., 1993 (135)	Final diagnosis	10^3	14/22 (64)	22/23 (96)
SP	Teague et al., 1981 (218)	Final diagnosis	10^4	18/18 (100)	28/32 (88)
CAP	Wimberly et al., 1982 (240)	Final diagnosis	10^3	45/53 (85)	12/12 (100)
SP	Pollack et al., 1993 (176)	Final diagnosis	10^3	72/75 (96)	33/35 (94)
CAP	Jimenez et al., 1993 (102)	Final diagnosis	10^3	28/40 (70)	NA
HIV	Ferrer et al., 1992 (79)	Final diagnosis	10^3	8/15 (53)	50/67 (75)
IC	Xaubet et al., 1989 (249)	Final diagnosis	10^3	25/25 (100)	78/88 (89)

[a] Taken from reference 17 with permission of the publisher.
[b] Normal, volunteers without pneumonia; SP, severe pneumonia; HIV, HIV infected; IC, immunocompromised.
[c] Histopathology, evidence of inflammation in tissue; laboratory data, other culture sources or serology positive; final diagnosis, assessment based on clinical criteria, response to therapy, additional laboratory data, and/or histopathology.
[d] NA, not available.

venting overgrowth of more fastidious pathogens by more rapidly growing contaminants.

Second, if one accepts that quantitative culture is useful in differentiating contaminants from pathogens, which is the best specimen type to use? Unfortunately, there is not a consensus on this question. A number of studies have suggested that in experimental (104) and human (146, 230) VAP BAL provides the most accurate reflection of etiology. However, other investigators have found PSB to be equally useful (223) or preferred (44). In one study of CAP, PSB and BAL results were equivalent (102), but other comparative studies are not available. The conflicting findings are most likely related to differences in patient population, exposure to antibiotics, specimen collection methods, and laboratory techniques. Finally, the recent resurrection of the concept of quantitative culture of noninvasive specimen types (i.e., ETAs in VAP) further clouds the issues and poses the question of whether bronchoscopy is even necessary (135, 197). The result of this uncertainty is that the decision relies on the preference of the clinicians involved, with many taking a conservative approach and submitting multiple specimen types. Indeed, sensitivity may be higher when more than one type is processed (230).

A final issue relates to the need for quantitative culture of tissue samples obtained by TBB or OLB. It has been suggested that quantitation with a threshold of 10^4 CFU/g may be used to discriminate bronchial contaminants from invading organisms in clinical investigations (17, 46). However, this procedure has not been evaluated for ordinary clinical use.

Quantitative culture methods. For quantitative culture of PSB and BAL specimens, two approaches have been employed. In the serial dilution method (Fig. 2), the most common scheme is the preparation of two 100-fold dilutions with counts obtained from measured 0.1-ml amounts spread on agar plate surfaces (240). Counts are made from the dilution containing the greatest number of colonies without confluence or overcrowding, generally up to several hundred (52). Results are given as actual CFU per milliliter. The advantages of this method are the availability of several dilutions from which to select the "best" plate for counting and the ability to accurately count organisms within a wide range.

Alternatively, and more practically, a "calibrated loop" method (Fig. 3) may be used (143, 220, 240). The method is similar to that employed for urine cultures and involves the selection of one or two measured amounts of sample for plating that allow discrimination at the proposed breakpoints of 10^3 CFU/ml for PSB and 10^4 to 10^5 CFU/ml for BAL specimens. Higuchi et al. (97) have determined that a 0.1-ml sample provides optimal results for PSB, and it follows that 0.001 or 0.01 ml would be suitable for BAL. Results with this method are most commonly given as $\log_{10}$ ranges (17).

Quantitative culture interpretation. For either approach, each morphotype present should be individually quantitated and reported. The subsequent extent of identification and susceptibility testing can be determined on the basis of the quantitation, with isolates in counts below the thresholds accorded less effort.

It should be appreciated that results near the thresholds should be interpreted cautiously. Many technical factors, including medium and adequacy of incubation (52) and antibiotic or other toxic components (144, 173), may influence results. The reliability of PSB sampling has also been recently evaluated (136, 221). Two groups have concluded that, although in vitro repeatability is excellent and in vivo qualitative recovery is 100%, quantitative results are more variable. In 14 to 17% of patients, results of replicate samples fell on both sides of the 10^3-CFU/ml threshold, and results varied by more than 1 $\log_{10}$ in 59 to 67% of samples. This variability is presumably related to both irregular distribution of organisms

TABLE 5. Quantitative culture of BAL in the diagnosis of bacterial pneumonia[a]

Patient group[b]	Reference	Pneumonia definition[c]	BAL method (vol, ml)[d]	Threshold (CFU/ml)	No. positive/no. with pneumonia (% sensitivity)	No. negative/no. without pneumonia (% specificity)
Normal	Kirkpatrick and Bass, 1989 (116)		B (60)	10^4	NA[e]	8/8 (100)
	Pang et al., 1989 (167)		B (90–150)	10^4	NA	10/10 (100)
VAP	Chastre et al., 1988 (44)	Final diagnosis	B (100)	10^4	4/5 (80)	9/13 (69)
	Torres et al., 1989 (223)	Final diagnosis	B (150)	10^3	19/34 (56)	NA (71)
	Gaussorgues et al., 1989 (85)	Histopathology	NB (NA)	Growth	8/9 (89)	3/4 (75)
	Rouby et al., 1989 (190)	Histopathology	PNB (20)	Growth	28/40 (70)	20/29 (69)
	Guerra and Baughman, 1990 (93)	Final diagnosis	B (120–240)	10^4	15/17 (88)	24/24 (100)
	Pugin et al., 1991 (180)	Final diagnosis	B (100)	BI > 5[f]	NA (93)	NA (100)
			NB (100)		NA (73)	NA (96)
	Rouby et al., 1992 (189)	Histopathology	PNB (20)	Growth	24/30 (80)	19/29 (66)
	Meduri et al., 1992 (146)	Final diagnosis	PB (150)	10^4	9/9 (100)	32/33 (97)
	Violan et al., 1993 (230)	Final diagnosis	B (150)	10^5	19/25 (76)	20/20 (100)
SP	Meduri et al., 1991 (145)	Final diagnosis	B (150)	10^4	12/13 (92)	32/33 (97)
SP	Thorpe et al., 1987 (220)	Final diagnosis	B (240)	10^4	15/15 (100)	73/77 (95)
CAP	Jimenez et al., 1993 (102)	Final diagnosis	B (180)	10^3	31/40 (78)	12/14 (86)
IC	Kahn and Jones, 1987 (109)	Final diagnosis	B (150)	10^5[g]	16/18 (89)	57/57 (100)

[a] Taken from reference 17 with permission of the publisher.
[b] Normal, volunteers without pneumonia; SP, severe pneumonia; IC, immunocompromised.
[c] Histopathology, evidence of inflammation in tissue; final diagnosis, assessment based on clinical criteria, response to therapy, additional laboratory data, and/or histopathology.
[d] B, bronchoscopic; NB, nonbronchoscopic; P, protected.
[e] NA, not available.
[f] BI, bacterial index (sum of logarithmic concentrations of species).
[g] Samples with ≤1% squamous epithelial cells.

in secretions and the very small volume actually sampled by PSB. The reproducibility of BAL has not been similarly evaluated. However, systematic investigation of variables affecting quantitative culture of liquefied sputum failed to reveal significant differences relating to loop, counting, or replication error (115), although use of a 0.001-ml calibrated loop for urine culture has shown a ±50% error rate (1).

The conclusion is that one must not be procrustean (242) by strictly interpreting quantitative bronchoscopic culture results. Rather, as with all tests, one must consider the clinical circumstances. In fact, Dreyfuss et al. (63) recently showed that, in 35% of instances in which an initial PSB culture yielded 10^2 to 10^3 CFU/ml and suspicion of pneumonia persisted, a repeat sample identified $>10^3$ CFU of the same organism per ml. Likewise, many false-positive results fall within 1 $\log_{10}$ of the threshold (72, 79, 109). Finally, significant infections other than pneumonia (e.g., bronchitis) may yield values above the threshold (176, 189, 220).

SPECIFIC ETIOLOGIC AGENTS

Depending on the specific etiologic agent, bronchoscopy may play varying roles in the diagnostic process. In some cases, bronchoscopy serves as a first-line procedure. However, in many other cases, bronchoscopy is secondary and follows empiric therapy failure or inability to establish an etiology by noninvasive means or to confirm another laboratory finding. In all cases, however, the specific staining, culture, and other methods applied to bronchoscopic samples are dictated by the etiologic agents being sought in a given clinical situation.

Bacterial Pathogens

Aerobic bacteria. For the diagnosis of pneumonia caused by common aerobic or facultative bacteria, microscopic analysis using Gram staining and quantitative culture of PSB and BAL samples has emerged as a primary diagnostic method in mechanically ventilated patients (17, 47). In this group of patients, clinical judgment is notoriously inaccurate (73), and ETAs have generally been considered to be sensitive but nonspecific in identifying pathogens (17). Recent studies have suggested that accuracy may be improved by applying quantitative culture techniques, using a 10^6-CFU/ml threshold, with sensitivity actually higher than for PSB (82 versus 64%) and specificity only slightly lower (135). Coupled with the observation that ETAs can be microscopically screened and rejected if >10 squamous epithelial cells per low-power field or no bacteria are seen (152), the role of bronchoscopy may shift to a secondary one if these results are verified. In particular, these results must be reconciled with the earlier observation that mean counts of 10^6 CFU/ml were common in long-term tracheostomized patients (13) and with the recent observation that such counts may be reached in patients on antibiotics but without pneumonia (224). In severe pneumonia in nonventilated patients, immunocompetent or immunocompromised, if bronchoscopy is done, Gram stains and quantitative cultures of PSB or BAL specimens should also be performed. However, first-line testing still generally uses noninvasive specimens (expectorated or induced sputum) despite the recognized inadequacies of these specimens (162). In all cases, blood culture should be an adjunctive procedure. Particularly in VAP, a positive blood culture may arise from an extrapulmonary source (46), but the clinical significance of a positive culture is still great. The organisms recovered as significant agents follow the pattern of expected pathogens as shown in Table 1, the actual frequencies showing great variability on the basis of population differences (184). The laboratory should be alert, however, to the possibility of finding unusual, significant agents such as *Rhodococcus equi* (69), *Bordetella pertussis* (160), or *Francisella tularensis* (212).

Anaerobes. A role for anaerobes in lower respiratory tract infections was clearly established by Bartlett during an anaerobe renaissance period in the 1970s, largely by using the techniques of transtracheal or percutaneous aspiration (10, 11). The percentage of samples with anaerobes varies with the

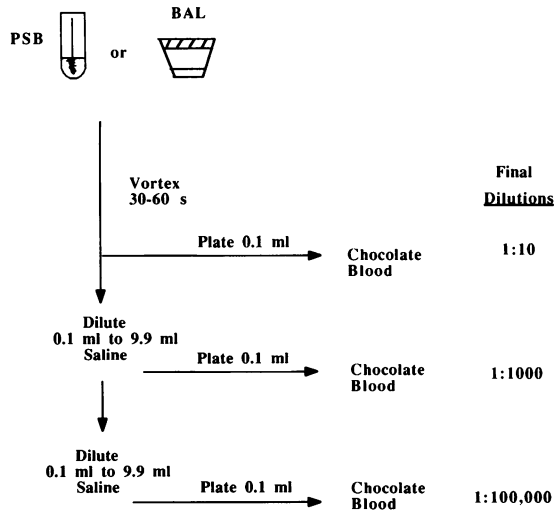

FIG. 2. Quantitative culture: serial dilution method. Quantitate each morphotype present and express as CFU per milliliter. Reprinted from reference 17 with permission of the publisher.

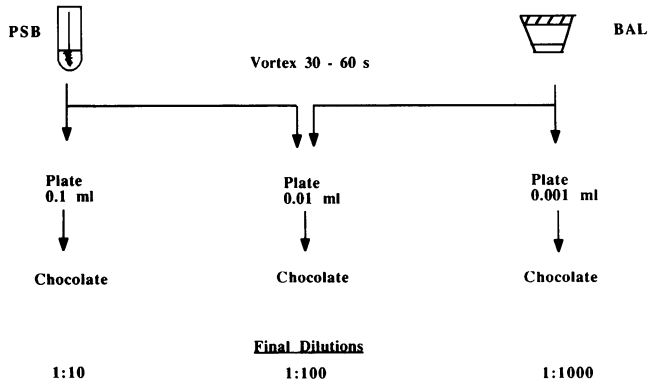

FIG. 3. Quantitative culture: calibrated loop method. Quantitate each morphotype present and express as $\log_{10}$ colony count ranges. Reprinted from reference 17 with permission of the publisher.

situation, ranging from 85 to 94% in pulmonary abscess, 62 to 100% in aspiration pneumonia, 22 to 33% in CAP, and 35% in hospital-acquired pneumonia (11). In most cases, aerobes are also isolated. However, documentation of anaerobic involvement in VAP is lacking, despite efforts to recover anaerobes from bronchoscopic samples obtained (17). This finding is compatible with a failure to recover significant numbers of anaerobes in tracheal aspirates from long-term tracheostomized patients on mechanical ventilation (13).

The main problem with bronchoscopic diagnosis of anaerobic infections is the same as for aerobes; namely, differentiation of contaminants from pathogens. Quantitative culture methods have been reported useful in nonventilated patients, using sputa (14) and PSB (176). However, direct comparison of anaerobes recovered from transtracheal aspirates (30) or transthoracic aspirates (80) with PSB has shown only 20 to 60% sensitivity, as well as finding isolates in PSB not recovered from the reference sample. Whether the disagreement stems from issues related to PSB sampling error or inadequate anaerobic handling is unclear. In addition, the role of FOB in the diagnosis of lung abscess has been questioned (206). Therefore, at this point, the role of bronchoscopy in the diagnosis of anaerobic infections is not firmly established, and additional well-designed studies are needed.

***Legionella* spp.** *Legionella* spp. have been recognized as important pulmonary pathogens in a variety of clinical settings, including CAP (185) and hospital-acquired pneumonia (120), and in transplant patients (3). Although isolation from a noninvasive specimen would be preferred, bronchoscopy is frequently employed in these patients since many do not produce sputum. In any respiratory secretion, increased polymorphonuclear cells in the absence of a recognizable morphotype may suggest legionellosis, and weakly acid-fast bacilli may be *Legionella micdadei* (3).

BAL has been reported to provide a useful specimen for rapid diagnosis by DFA staining with confirmation by selective culture (101, 119). For DFA, a polyclonal reagent which is genus specific may be preferred to an *L. pneumophila*-specific reagent since other species are clinically important (66). For culture, the notion that saline may be inhibitory to *Legionella* spp. is bothersome (94) and has prompted some investigators to incorporate a water lavage aliquot in their BAL protocols (67). The exact requirements for culture of *Legionella* spp. in BAL are not established, but most authors perform both direct plating and plating of HCl-KCl-treated portions onto selective and nonselective media (67, 119). Direct hybridization (Gen Probe, Inc., San Diego, Calif.) has received only limited evaluation, with bronchoscopic specimens of low sensitivity (50%) and specificity (67%) reported (65). However, a recent evaluation of a commercially available PCR test for environmental *Legionella* spp. (Perkin-Elmer Cetus, Norwalk, Conn.) using seeded and clinical BAL specimens has suggested that this may be a valuable future assay (114). At present, a combination of DFA with culture probably provides the best diagnostic approach, using serology as an adjunctive test when DFA and culture are negative or of questionable significance.

***Nocardia* spp.** *Nocardia* spp. have been increasingly recognized as important pulmonary pathogens in immunocompromised patients, particularly solid organ transplant groups (7, 43). Although isolation from a noninvasive specimen is significant, BAL has been reported to be useful in many cases (7, 84). Microscopically, the organism is readily recognized in a high percentage of cases as delicate, branching, gram-positive, beaded filaments that are acid fast, using a weak acid decolorizer modification (43). For culture, charcoal-yeast extract medium has emerged as particularly useful for primary isolation (84).

Mycoplasmas. Experience with bronchoscopic specimens for the diagnosis of pneumonia caused by mycoplasmas is very limited, due at least in part to the technical difficulties associated with culture of the organism and lack of an alternate direct specimen method (113, 134). In patients with *M. pneumoniae*, sputum is rarely produced, and the most commonly used specimens are from the upper respiratory tract (e.g., throat or nasopharynx) (113). However, in both CAP (124) and pneumonia in immunocompromised patients (171), *M. pneumoniae* has been isolated from BAL specimens. In the latter group, other mycoplasma species have also been isolated. No studies comparing upper respiratory swabs with BAL are available. It may also be that newer non-culture-dependent techniques employing nucleic acid hybridization (with or without amplification) or antigen detection will be sensitive alternatives (134). However, at present, serologic confirmation is a necessary adjunctive test for *M. pneumoniae*.

***Chlamydia* spp.** *Chlamydia pneumoniae* has been recently recognized as an important cause of pneumonia in a variety of clinical settings (92), and *C. psittaci* has long been appreciated

as a significant zoonotic respiratory pathogen (165). As for *M. pneumoniae*, upper respiratory swabs have been the specimens of choice for detection of these organisms (92). However, *C. pneumoniae* has been successfully isolated from BAL specimens of HIV-infected patients (8) and in a hospital-acquired setting (192). Culture is difficult, requiring special transport medium, suitable cell cultures, and specific confirmation reagents. Therefore, serologic confirmation is an important adjunct to culture. For other respiratory secretions, genus-specific DFA stains and enzyme immunoassay tests have been reported useful (165, 202), but these techniques have not been extensively evaluated with BAL samples. Most recently, Gaydos et al. (86) have reported the finding of 12 PCR-positive results in 132 (11%) culture-negative BAL samples from immunocompromised patients, suggesting that PCR may provide a more sensitive approach.

Mycobacterial Infections

M. tuberculosis. With the resurgence of tuberculosis, including drug-resistant strains, rapid and sensitive laboratory detection of *M. tuberculosis* has become an important focus of control efforts (219). Isolation from noninvasive respiratory specimens provides a critical first-line approach, with culture sensitivities as high as 70 to 90% compared with recovery from bronchoscopic samples in both HIV- and non-HIV-infected patients (112, 122, 149). However, not all investigators have reported sensitivities this high (20), and rapid diagnosis by acid-fast staining has considerably lower sensitivity even in HIV-infected patients (118). Therefore, bronchoscopy has emerged as an important secondary procedure in several situations, including in patients with negative sputum smears or cultures in whom clinical suspicion is high and patients with an atypical presentation, as in miliary disease or when neoplasm is in the differential diagnosis (54, 232, 236). Which bronchoscopic specimen provides the best information is unclear. The results of studies evaluating bronchoscopy for diagnosis are shown in Table 6. Bronchial washings and BAL appear similarly sensitive but additive (20, 33, 57), and TBB adds some incremental information (112, 122, 149). Therefore, it is a common and probably justifiable practice to process multiple specimens obtained from a single bronchoscopy procedure. At which point in the diagnostic protocol to perform bronchoscopy is also unclear. Since newer culture techniques provide data more rapidly than conventional cultures, and given that the smear-negative patient is probably a low infection control risk, it may be more cost-effective to wait a week or so before performing bronchoscopy (156). However, this is an institution-specific decision.

Procedurally, the microbiology laboratory should follow all recommendations for rapid isolation and identification, including timely acid-fast staining, use of selective agar and broth media (e.g., BACTEC or SeptiChek), rapid identification by *p*-nitro-α-acetylaminol-β-hydroxypropionhenone inhibition (BACTEC), probes (Gen-Probe), or high-performance liquid chromatography, and rapid susceptibility testing (BACTEC) (219). It is also clear that a sensitive nucleic acid amplification test would gain widespread acceptance for this organism.

Mycobacteria other than *M. tuberculosis*. Although a few other mycobacterial species are generally considered highly significant (e.g., *M. kansasii*), the clinical significance of isolation of most others from respiratory secretions may be difficult to ascertain. Species such as the *M. avium* complex from HIV-infected patients (132) or the *M. fortuitum-M. chelonae* complex from intensive care unit patients (35) may colonize the upper respiratory tract in the absence of disease, although colonization may be a predictor of subsequent dissemination by the *M. avium* complex (132). Criteria used to determine the clinical significance of isolation from respiratory secretions have included compatible clinical findings, isolation from a sterile site, and histopathologic detection (193).

As for *M. tuberculosis*, a variety of bronchoscopic specimens have yielded mycobacteria other than *M. tuberculosis*, and it is unclear which is preferred (110, 139, 215, 233). However, BAL with TBB, if clinically feasible, probably gives the maximum diagnostic yield. It is equally desirable to use rapid isolation and identification methods for mycobacteria other than *M. tuberculosis*. In fact, in one study, implementation of the BACTEC system for *Mycobacterium* isolation dramatically increased the yield from bronchoscopy specimens (193), although the clinical significance of increased isolation rates was questioned.

Fungal Infections

Systemic fungi. Isolation of a systemic, dimorphic fungus from any respiratory secretion is clinically significant. Therefore, if feasible, it is logical to first evaluate noninvasive specimens and then follow with bronchoscopy if clinical suspicion persists in the face of negative results. However, in the few studies comparing sputum with bronchoscopy specimens, sputum appears to have lower sensitivity for detecting fungi in culture (37, 179) or smear (88) than bronchoscopy, although in many cases tissue diagnosis is ultimately required. This is particularly true in patients with a single pulmonary nodule (179). Recent studies employing BAL predominantly in immunocompromised patients have yielded sensitivities of 85 to 100% (20, 37, 133, 234), suggesting this may be the preferred bronchoscopic specimen type. Studies evaluating bronchoscopy for diagnosis of serious fungal infections are summarized in Table 7.

For any respiratory secretion, it is important for the microbiologist to be familiar with the distinctive microscopic morphologies of the tissue forms and report results accordingly (95). However, since microscopy is not 100% sensitive or specific, isolation should be performed with appropriate selective media and rapid identification should be performed with methods such as exoantigen or nucleic acid probe testing. In immunocompromised patients, dissemination is common, and extrapulmonary samples are also frequently submitted.

Opportunistic fungi. Opportunistic filamentous fungi and yeasts present a number of interpretive problems in immunocompromised hosts when bronchoscopic samples are used to establish an infection (254). For filamentous fungi, particularly *Aspergillus* spp., the major problem with bronchoscopic samples is lack of sensitivity (111, 196, 249). Fungi are isolated in only 0 to 50% of cases, and the final diagnosis is usually made from an OLB or at autopsy. Asymptomatic colonization is uncommon (139, 215) and probably represents a risk for invasion if the patient is neutropenic (251). Recently, both an *Aspergillus* antigen (6) test and a PCR test (217) have shown promise for use with BAL samples in increasing sensitivity.

In contrast, for *Candida* spp. and other yeasts, excluding *Cryptococcus* spp., the major problem with bronchoscopic samples is lack of specificity. Asymptomatic colonization is frequent, and up to 25% of samples will show *Candida* spp. on smear or culture (139, 196, 233). Microscopic specificity is improved when one considers only the finding of large numbers of yeasts and pseudohyphae to be significant (130, 157), but again, final diagnosis generally depends on the demonstration of organisms in tissue. An antigen test for *Candida* spp.

TABLE 6. Bronchoscopy in the diagnosis of tuberculosis

Patient group[a]	% Sensitivity of specimens for culture (smear)[b]					Reference
	Pre-Spu	BW/BB	BAL	TBB	Post-Spu	
SN	59 (0)	95 (34)	—[c]	41 (—)	71 (21)	54
SN	67 (0)	4 (13)	—	20 (30)	35 (9)	232
SN	—	68 (42)	—	—(50)	—	236
HIV	—	80 (—)	—	50 (—)	—	33
R/O TB	—	53 (—)	88 (18)	—	46 (—)	57
IC	—	—	80 (—)	—	—	249
HIV	88 (61)	—	57 (0)	43 (14)	—	122
R/O TB	51 (34)	—	92 (68)	—	—	20
HIV (SN)	89 (0)	—	62 (23)	52 (2)	88 (41)	112
Non-HIV (SN)	93 (0)	—	66 (16)	44 (11)	62 (37)	
HIV	77 (23)	95 (26)	95 (4)	50 (14)	—	149
Non-HIV	75 (22)	92 (13)	73 (0)	100 (25)	—	

[a] SN, sputum smear negative; HIV, HIV infected; R/O TB, rule out tuberculosis; IC, immunocompromised.
[b] Pre-Spu, prebronchoscopy sputum; BW/BB, bronchial washings or bronchial brushings; post-Spu, postbronchoscopy sputum.
[c] —, sample type not evaluated.

has shown promise for use with BAL samples (130). Quantitative culture techniques have not been investigated.

Cryptococcus neoformans is generally considered more significant than other yeasts, and bronchoscopy has been useful for detecting this organism (21, 37, 83). BAL appears to be the most useful specimen type for smears and culture, and latex agglutination has been reported useful (21).

P. carinii

The advent of the AIDS epidemic and the early recognition of the importance of *P. carinii* as a common pulmonary pathogen led to the rapid evaluation and acceptance of FOB for definitive diagnosis (33, 110, 139, 164, 175, 214, 233). In a recent review of 17 studies, BAL was noted to have an overall sensitivity of 82% compared with 83% for TBB, but only 53% for ordinary brushings or washings (24), and to have the greatest yield when a combination of specimen types was considered. With the inherent risks of TBB, BAL specimens have emerged as the specimens of choice, with a bilateral lavage procedure having somewhat greater sensitivity than a unilateral one (145). More recently, induced sputum has been reported to provide a useful noninvasive alternative approach to bronchoscopy, but sensitivities have varied widely from 15 to 100% (26, 29, 58, 121, 175, 216, 253). The variability has been related to several factors, including the institution where performed, patient selection process, experience of the operators, sample preparation method, and staining method (24). Finally, it has been suggested that in selected HIV patients institution of empiric therapy with bronchoscopy delayed for use only in nonresponders may be the most cost-effective approach (226). Therefore, selection of the exact diagnostic approach depends on the experience of each institution, but in all cases bronchoscopy plays a primary or secondary role (163).

Staining techniques applied to detection of *P. carinii* in bronchoscopic specimens have also varied widely. The most commonly used stains include Gomori's methenamine silver, Gram-Wiegert, toluidine blue O, and calcofluor white for cyst detection and a modified Wright-Giemsa (Dif-Quik) for detection of trophozoites and intracystic bodies (24, 140). Recently, several companies have marketed fluorescent monoclonal antibody stains that have proven sensitive and specific for diagnosis (126). The diagnostic accuracy of the various cyst stains have been quite comparable for BAL samples, with sensitivity of Dif-Quik being perhaps slightly lower (22, 24, 28, 126, 216). However, in practice, many institutions prefer to use a combination of stains or multiple slides to ensure maximal sensitivity (42). In contrast, for induced sputa with lower numbers of organisms generally present, maximal sensitivity is achieved by using the more expensive fluorescent-antibody reagents (22, 121, 126, 216).

The practice of routine prophylaxis for PCP in HIV-infected patients has introduced new concerns regarding accuracy of diagnostic techniques. Ng et al. (159) demonstrated a lack of effect of prophylactic aerosolized pentamidine on the diagnostic yield of Dif-Quik staining of induced sputa or BAL samples when comparing patients with or without such therapy. Further, no differences in numbers of clumps or morphology of organisms was observed. In contrast, Jules-Elysee (107) demonstrated a significant difference in organism detection with Gram-Wiegert and toluidine blue O stains in specimens from patients receiving (62%) or not receiving (100%) aerosolized pentamidine prophylaxis. They also noted fewer organism clumps in positive samples. One possible explanation may be the effect of antimicrobial agents on cyst morphology, resulting in poorly staining, degenerate forms (24). We have noted this effect of pentamidine and trimethoprim-sulfamethoxasole on cyst morphology with calcofluor white but not Giemsa staining. To overcome these effects, differential upper-lobe lavage provides a higher diagnostic yield, with greater numbers of organisms present than standard middle- or lower-lobe lavage in pentamidine-treated patients (182). Presumably, this is related to lower concentrations of antimicrobial agents and more organisms in the upper lobes.

Viral Infections

Common respiratory viruses. A role for bronchoscopy in detection of common, seasonal respiratory viruses (e.g., respiratory syncytial virus, parainfluenza viruses, and influenza viruses) is not well established. As these viruses initially infect upper respiratory epithelial surfaces, and only secondarily involve the lung in a subset of patients, the specimens of choice for diagnosis by culture or antigen detection are nasopharyngeal and tracheal swabs or aspirates (235). However, in a seriously ill patient with suspected nonbacterial pneumonia who undergoes bronchoscopy, these viruses may be detected in the specimens obtained (70, 235). Particularly in an epidemic period, the laboratory should be prepared to process bronchoscopic samples for detection of these viruses by culture, DFA,

TABLE 7. Bronchoscopy in the diagnosis of serious fungal infections

Patient group[a]	Fungi (no.)[b]	% Sensitivity of specimens for culture (smear)				Reference
		Sputum	BW/BB[c]	BAL	TBB	
Non-HIV	B (1), Cr (1) H (4), A (6)	73 (55)	75 (75)	—[d]	—	88
IC	A (4), Cr (1)	—	50 (—)	83 (—)	0 (—)	215
HIV	Cr (6), Co (1), H (1)	—	—	71 (—)	87 (—)	33
HIV	Cr (8)	—	— (63)	— (83)	— (75)	83
IC	NS (4)	—	—	75 (—)	—	139
IC	A (9), Ca (4)	—	—	31 (23)	—	196
IC	NS (18)	—	22 (—)	30 (56)	50 (—)	110
IC	NS (12)	—	—	58 (—)	—	249
Non-HIV	H (27)	71 (—)	81 (—)	—	—	179
IC	Cr (2), Co (1)	—	—	100 (0)	— (0)	233
HIV and non-HIV	Co (7), Cr (3), A (2)	—	—	58 (41)	—	204
Culture positive	H (25), Cr (11) B (4), Co (1)	5 (0)	—	85 (66)	—	20
HIV	Cr (12)	50 (—)	—	100 (—)	—	37
IC	Cr (15)	—	—	87 (33)	—	21
Culture positive	H (27)	—	—	89 (67)	—	234
HIV	Co (2)	—	—	100 (0)	—	133

[a] HIV, HIV infected; IC, immunocompromised; culture positive, culture positive at any time from various respiratory specimens.
[b] B, *Blastomyces dermititidis*; Cr, *Cryptococcus neoformans*; H, *Histoplasma capsulatum*; A, *Aspergillus* spp.; Co, *Coccidioides immitis*; Ca, *Candida* spp.; NS, not specified.
[c] BW/BB, bronchial washings or bronchial brushings.
[d] —, sample type not evaluated.

or antigen detection. Recognition of characteristic cytopathologic features in BAL cells has also been useful for respiratory syncytial virus (170), and ciliacytophthoria may be seen with all respiratory viruses (130). Occasionally, other viruses that cause pulmonary disease may be encountered in bronchoscopic specimens (252). Anecdotally, both adenovirus and measles virus have been detected in bronchoscopic samples cytopathologically or on culture, so the laboratory should be alert to their possible occurrence.

Latent viruses. The interpretation of laboratory tests for latent viruses of the herpes group remains one of the most problematic areas in the management of immunocompromised patients. This is particularly true for detection of CMV and, to a lesser extent, HSV in bronchoscopic specimens. Since immunosuppression of cell-mediated immunity allows reactivation of latent infection, the presence of virus may represent asymptomatic excretion or be the result of clinical disease. Rarely, primary infection may occur. As for colonizing bacteria, the distinction is difficult. Nevertheless, pneumonia can occur, and CMV has been frequently implicated in patients with HIV infection (231) and after organ transplantation (4). The permissive growth of CMV but not HSV in alveolar macrophages probably contributes to the high rates observed (62).

(i) CMV. Histopathologically, CMV pneumonitis is manifest in a spectrum from a mild, focal, interstitial process to severe, diffuse, alveolar damage (231). Because CMV may be unevenly or sparsely distributed, techniques that sample large lung areas are most useful in diagnosis. Therefore, BAL has emerged as a common first-line approach (4, 33, 82, 110, 207, 214, 215), the sensitivity of which may be increased by bilateral sampling (145). Since CMV frequently coexists with other pathogens, BAL offers the added advantage of the opportunity to detect other pathogens. However, on occasion, the diagnosis is not made until OLB or autopsy tissue is obtained.

Methods which may be applied to BAL or tissue for detection of CMV include cytopathology, DFA, in situ hybridization, and culture. Of these methods, culture generally has the greatest sensitivity (50, 98, 169, 207, 215, 233). Culture techniques have varied, with some authors using uncentrifuged BAL, some using cellular sediments, and some using supernatants. In one study, the best overall sensitivity was achieved with both cells and supernatant in separate cultures (207). The highest culture sensitivities have been reported when centrifugation cultures in shell vials followed by monoclonal antibody detection of early viral antigen were used (51, 71, 90, 139, 244).

The specificity of BAL culture varies greatly with the population being studied. In solid organ transplant patients (105), a positive culture is viewed as more clinically significant than in most other immunosuppressed groups (194). In HIV-infected patients, specificity as low as 6% has been reported (244).

Direct staining techniques employing single or pooled monoclonal antibodies against a variety of early or late antigens in infected alveolar mononuclear cells have relatively high sensitivity as well (50, 51, 68, 169), with some variability dependent on the exact method used. False-positive results may occur, but specificity is improved when only samples with many specifically stained cells are considered. Variable cutoff points have been used, including >10 staining cells in one study (169) and >0.5% of the total cells in another (68). Nonspecific staining of cellular debris has also been stated as a problem, with a high degree of experience required to obtain consistent results (110). Results using in situ hybridization have been quite similar to those with DFA (90, 98).

Cytopathologic or histopathologic evidence of CMV nuclear or cytoplasmic inclusions has consistently yielded the highest specificity, virtually 100% in all studies (51, 68, 71, 98, 207, 244). However, sensitivity is uniformly low, generally only 50% or less.

Because the tests have variable performance characteristics, the most common approach is one that employs several techniques simultaneously and includes a careful clinical assessment in interpreting results. Serology may be a useful adjunctive test to detect primary infection, and simultaneous detection of CMV in leukocytes by antigen detection or culture methods provides strong supportive evidence for an etiologic

role in pneumonia (64). Nucleic acid amplification techniques with their exquisite sensitivity probably add little to diagnosis, until targets that differentiate latency and asymptomatic infection from disease are identified (155). However, in heart-lung transplant patients, PCR positivity may precede culture or histopathologic evidence of CMV and represents a clinically significant finding (36).

(ii) HSV. Analogous to CMV, HSV may be present in respiratory secretions of immunocompromised patients (181) and in the oropharynx of seriously ill patients with pneumonia (227) in the absence of lower respiratory tract disease. However, pneumonia may arise from contiguous spread or, less commonly, by hematogenous spread to the lungs. Rarely, primary disease may occur. As a consequence, in bronchoscopic samples, HSV may be a contaminant or a pathogen. Experience with diagnostic techniques is more limited than for CMV, but it may be presumed that they yield similar results, with culture, DFA, and in situ hybridization being relatively sensitive but nonspecific and histology or cytology being relatively insensitive but highly specific.

(iii) Other latent viruses. Other latent viruses have been incriminated in pulmonary infection. HIV may itself cause pathology, and HIV and Epstein-Barr virus have been associated with lymphocytic interstitial pneumonitis (4, 231, 235). However, a role for bronchoscopy in documenting these diseases by means other than cytopathology or histology has not been established.

Protozoans and Helminths

Bronchoscopic samples have on occasion provided serendipitous diagnoses for a number of unusual parasitic diseases in immunocompromised hosts. The diagnoses depend on the awareness and ability of the microbiologist to recognize these agents. Since pneumonia is usually a manifestation of a disseminated infection, other laboratory studies (including stool exams and/or serology) are also indicated.

Toxoplasma gondii. BAL has been particularly useful in the diagnosis of pulmonary toxoplasmosis (31, 59, 99, 177). A Wright-Giemsa-type stain, including the Dif-Quik stain, has been used to detect both intracellular and extracellular tachyzoites. However, organisms are easily overlooked, and ×1,000 oil immersion magnification is generally required for examination. Unfortunately, no specific stains or alternative non-culture-dependent methods that would be of use in detecting these protozoans are commercially available for in vitro diagnosis. Since most disease results from reactivation of organisms dormant in cysts under the condition of immunosuppression, serology may be useful to screen for patients at risk.

Other protozoans. Several other opportunistic protozoans have been demonstrated in bronchoscopic specimens. Intracellular *Leishmania* amastigotes have been observed in alveolar macrophages of BAL specimens from HIV-infected patients residing in areas of endemicity (188). *Cryptosporidium* cysts have been detected in a variety of respiratory samples (including bronchoscopic samples) by using acid-fast or specific DFA stains, generally in patients with concomitant intestinal disease (53). Finally, with a special chromotrope stain, microsporidia (specifically, *Encephalicytozoan hellum*) have been noted in large numbers in a BAL sample from an HIV-infected patient with disseminated disease (199). Uvitex 2B (Ciba-Geigy, Basel, Switzerland) may be a useful rapid stain for these organisms and fungi (228).

Strongyloides stercoralis. *S. stercoralis* filariform larvae may be present in respiratory secretions of immunocompromised patients with the hyperinfection syndrome. Larvae are readily apparent on low-power examination of almost any stained material and have been seen in large numbers in BAL samples (87, 213). Stool examination to detect preexisting infection is indicated to identify patients at risk for developing this syndrome.

Other helminths. Other helminths have rarely been reported in respiratory samples, and their possible isolation from bronchoscopic specimens from areas where such agents are endemic clearly exists. For example, *Paragonimus westermani* ova have been noted in sputa obtained from southeast Asians suspected of having tuberculosis (250). Therefore, it is essential that the microbiologist be alert to the possibility of finding other unusual agents.

ORGANIZATION OF SERVICES

The variety of specimen types generated by bronchoscopy, the diversity of etiologic agents encountered, and the requirement for availability of many different test procedures make it essential for the laboratory to organize services for efficient delivery of health care. Cooperation, communication, and coordination are key elements of this process. Cooperation between pulmonary and other physicians performing bronchoscopy and the microbiologists and pathologists performing testing is important to define expectations for results. There should be agreement on areas such as test menu, turnaround times, and reporting mechanisms. Communication should be a bidirectional process, with ordering physicians clearly specifying test requests and the laboratory clearly defining guidelines for collection and transport. The former can be accomplished by using a specific order form (203), and the latter can be done through a current laboratory handbook. Finally, coordination of all activities pertaining to bronchoscopic specimen handling is critical to ensure that all appropriate tests are performed in a timely manner. One effective means to coordinate these activities is to devise guidelines for testing that are specific to the patient group under evaluation. Thus, guidelines might be developed for the general categories of (i) immunocompromised patients, (ii) patients with VAP, and (iii) nonventilated patients with severe nonresolving CAP or hospital-acquired pneumonia. The most common analyses performed in these settings are shown in Table 8. It should also be kept in mind that bronchoscopy provides only one facet of the pulmonary diagnostic evaluation, and the complete protocol would include a history, a physical exam, screening laboratory tests, chest radiography, serology, and microbiologic analysis of other specimen types (131, 143).

Immunocompromised Patients

The indications for performing multiple tests on bronchoscopy specimens, particularly BAL, are most clear in immunocompromised patients, both HIV-infected (94, 154) and organ transplant groups (105). In these groups, infections with multiple pathogens are common and clinical manifestations are indistinctive. Therefore, agents detected in a comprehensive protocol generally guide therapy (67). In some institutions, sputum induction yields high sensitivity for *P. carinii* and other agents (125, 158) and may precede bronchoscopy. Further, when initial samples fail to demonstrate a pathogen and the patient's condition is not improving, repeat bronchoscopy or transthoracic biopsy may be indicated if survival prolongation is a possibility.

In this setting, microscopy is of primary importance and should be completed as soon as possible, with alertness to the

TABLE 8. Common tests on bronchoscopic specimens from various patient groups

Sample	Tests	Diagnostic role in given patient group[a]		
		IC	VAP	CAP/HAP
Bronchial washings/brushings	Acid-fast stain and culture	P	O	P
	Fungal stain and culture	P	O	P
	Legionella stain and culture	P	S	P
PSB	Gram stain and quantitative culture	P	P	P
BAL	Cytopathology	P	S	P
	Cell count and differential	P	P	P
	Gram stain and quantitative culture	P	P	P
	Acid-fast stain and culture	P	S	P
	Fungal stain and culture	P	S	P
	Pneumocystis stains	P	O	O
	CMV/HSV DFA or in situ hybridization and culture	P	O	O
	Chlamydia pneumoniae culture	S	S	P
	Mycoplasma culture	S	S	P
	Respiratory virus DFA and culture	S	S	P
TBB	Histopathology with special stains	P	S	P
	Gram stain and culture	P	S	S
	Acid-fast stain and culture	P	S	S
	Fungal stain and culture	P	S	S
	Viral stains and cultures	P	S	S
	Legionella stain and culture	P	S	S

[a] IC, immunocompromised; CAP/HAP, severe CAP or hospital-acquired pneumonia in nonventilated patients. Diagnostic role: P, primary; S, secondary; O, not generally indicated.

possibility of unusual findings. A role for other rapid techniques has not been firmly established. In addition to bronchoscopic specimens, blood cultures are important to document sepsis and clarify the significance of isolation of problematic organisms such as CMV and *M. avium* complex from the respiratory tract.

VAP

Guidelines for analysis of bronchoscopic specimens from patients with pneumonia arising as a complication of mechanical ventilation have recently been published from an international consensus conference (17). Quantitative culture of PSB with a threshold of 10^3 CFU/ml or BAL at 10^4 CFU/ml is accepted as a probable indication of etiology. For rapid diagnosis of bacterial pneumonia, Gram stains and cytologic assessment for intracellular organisms and predominant extracellular morphotypes are important. If stains and quantitative culture fail to demonstrate a common bacterial etiology, testing for a variety of other organisms may be indicated on the basis of clinical or epidemiologic suspicion. Blood culture to document sepsis and culture of other sites to rule out extrapulmonary foci are important in the total diagnostic protocol (142). Although many clinicians continue to rely on stains and routine culture of ETAs, this approach is not recommended to establish a definitive diagnosis (17). Recent results suggesting that quantitative culture of ETAs provides an accurate alternative to bronchoscopy are interesting and may alter the previous recommendations (135, 197).

Severe CAP or Hospital-Acquired Pneumonia

Although historically CAP and hospital-acquired pneumonia have been considered separately, in fact, both present similarly and may be caused by a wide overlapping range of organisms (198). Thus, a specific microbiologic diagnosis is desirable to optimize therapy. Although a role for routine bronchoscopy in this setting has not been well established, in the seriously ill hospitalized patient requiring mechanical ventilation or for whom an expected response to empiric therapy does not occur, bronchoscopy is probably indicated (162). This is particularly true if sputum analysis and blood culture have failed to demonstrate an etiology. Unfortunately, in most cases, antecedent therapy is likely and may reduce diagnostic sensitivity (75, 166). If bronchoscopy is performed, testing should target a variety of nonopportunistic pathogens and should include cytologic and histologic evaluation to rule out noninfectious etiologies. In addition, serology is important, albeit retrospective, for many "atypical" agents (75). Therefore, an attempt should be made to obtain an acute serum sample at the time of onset.

FUTURE CONCERNS

Despite the existence of a substantial body of literature on the role of bronchoscopy in the diagnosis of pneumonia, there remain a number of areas requiring additional studies. For a given disease or clinical situation, it is not clear which is the "best" specimen for testing. In many cases, this is dependent upon the experience and skill of the bronchoscopist and is an institution-specific choice. In some cases, optimal sensitivity is achieved by processing multiple specimen types. In addition, it is desirable to first test noninvasive specimen types, but their sensitivity and specificity have not yet been established for all etiologic agents. Finally, most studies of bronchoscopy to diagnose pneumonia have been done in adult groups, and extension to younger age groups is necessary (255).

It is also not clear which is the best test to use for each agent. Although there are well-established roles for direct microscopy to provide rapid information and for culture to provide definitive information, exact methods used generally rely on the experience and preference of the microbiologist. Rapid non-culture-dependent tests would be useful for detecting the

more problematic agents, but a role for such tests is not yet clearly established. As novel, expensive therapeutic modalities are developed, the need for rapid, accurate tests will clearly increase. Finally, with the real possibility of finding new agents of pneumonia, the laboratory must be willing to expand the diagnostic armamentarium accordingly.

Perhaps the greatest challenge to the microbiologist, however, is the need to accomplish all of the recommended diagnostic maneuvers in a cost-effective manner. Not surprisingly, few studies that assess the cost-effectiveness of performing comprehensive analyses on bronchoscopic specimens are available. Fagon and colleagues (74) have estimated that quantitative culture of PSB in VAP is more cost-effective than treatment of all patients with a clinical suspicion of pneumonia for 6 days or more. However, similar analyses in other patient groups do not exist. Therefore, cost-effectiveness is primarily presumed from the assumptions that an accurate diagnosis promotes rational use of therapy, allows discontinuation of unnecessary agents, ultimately reduces emergence of drug resistance, and increases patient survival (67, 184, 225).

Given that pneumonia and influenza have consistently ranked sixth as leading causes of death (40), the importance of accurate and timely diagnosis cannot be overemphasized. For nosocomial pneumonia, hospital costs exceed reimbursement in 94% of cases, thus adding an obvious economic incentive to the task of diagnosis (32). To facilitate the diagnostic process, bronchoscopy has become a common tool. Therefore, the microbiology laboratory must make every effort to ensure that the specimens obtained are handled in a manner appropriate to patient care needs, including the implementation of special techniques. To ensure that the tests are being appropriately ordered and used, it is strongly recommended that quality assurance monitors be established for diagnostic yield, diagnostic accuracy, and appropriate utilization.

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