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Diagnostic test for etiologic agents of community-acquired pneumonia

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This article deals with methods and indications for detecting the etiologic agent of pulmonary infections. Its discussion is restricted to diagnostic tests in immunocompetent adults. The article offers an overview of the topic, a description of conventional microbiology methods, and a discussion of detection methods for specific microbes, with emphasis on microbes that are the most important or most frequent.

Studies to detect the etiologic agent of pneumonia have undergone major shifts in technology and emphasis during the past 100 years. The basic technique that is available to most physicians and considered standard when any diagnostic tests are done are gram stain and culture on agar media using expectorated sputum involving methods that were described by Robert Koch in the 19th century. Serologic testing has been available for many pathogens for many decades, but clinicians rarely have found it useful except for epidemiologic reviews. Many serologic tests are not reliable, and the information usually becomes available after all of the therapeutic decisions have been made. Modern molecular methods are becoming more available but have been relatively slow in development or adaption for pulmonary pathogens. Polymerase chain reaction (PCR) for detection of specific pathogens is rapid and sensitive, but often fraught with technical difficulties. Its use is rational only for detection of microbes that are not found in healthy hosts, such as viruses, *Legionella* spp, and *Mycobacterium tuberculosis*. An additional limitation in the application of modern molecular techniques for many bacterial pathogens is the need for culture to determine the results of sensitivity tests.

Divergent opinions have evolved concerning the clinical usefulness of diagnostic tests, making this topic one of the more controversial areas of

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pulmonary infections. Paradoxically, studies to detect the etiologic agent of pneumonia were well emphasized in the pre-penicillin era, a time when clinicians had few treatments to offer. An example of such a study is Heffron's book on the pneumococcus, which Robert Austrian [1] has called the greatest book ever written about a single microbe. It was published in 1937. Probably the zenith for microbial detection occurred in the late 1930s, when expectorated sputum studies were sophisticated for the time. The Quellung reaction plus mouse inoculation was used for the detection of *Streptococcus pneumoniae*, serotyping was standard practice, and Bullowa [2] reported his use of more than 1400 transthoracic aspirations to detect an etiologic agent. The rationale for this aggressive approach in part was related to the need for microbial detection and serotyping of *S pneumoniae* at a time when type-specific antiserum was the only available treatment. This treatment was supplanted by penicillin when it became available in the late 1940s, and bacteriologic studies of pneumonia never have regained this quality, emphasis, or respect.

In the 1970s, two technological developments seemed to have the potential to reverse the steady decline in quality and interest in microbiology of pulmonary infections. The flexible bronchoscope brought about the potential for direct sampling from the infected site with minimal patient discomfort, but it was expensive, required an experienced technician, was unrealistic for generalized use, and turned out to be no better than expectorated sputum because of instrument contamination during passage through the upper airways [3]. Subsequent studies have shown that this technique can be a valid method for quality microbiology if quantitative cultures are done using bronchoalveolar lavage (BAL) or protected brush catheter specimens that are collected before antibiotic treatment [4]. Quantitative cultures of expectorated sputum or endotracheal aspirates probably also work. The second technologic advance of the 1970s was the use of transtracheal aspirates to bypass the contaminating flora of the upper airways to obtain an uncontaminated specimen from the lower airways. Multiple studies showed the accuracy of this method in patients who did not have chronic lower airway disease, but the technique fell into disfavor in the late 1970s as a result of the need for experienced technicians, side effects of the procedure, and, possibly, the inability to bill a third party for it.

The current status of microbiology studies is relatively dismal. Most outpatients with pneumonia, a group that accounts for about 75% of community-acquired pneumonia (CAP) cases, rarely undergo any studies for an etiologic agent. Most hospitalized patients with pneumonia have blood cultures, and 5% to 12% of them yield a likely etiologic agent; *S pneumoniae* accounts for 65% of bacteremic pneumonia cases. If an additional study is done, it is gram stain and culture of expectorated sputum, which is done on a minority of patients using methods that are highly variable in quality and that have an overall low yield. A possible or probable pathogen is identified in only about 10% to 15% of hospitalized patients with pneumonia. Many

clinical trials of antibiotics or prospective studies of CAP in which microbiologic studies are driven by the protocol are comprehensive in microbiology studies, including unconventional tests such as PCR, urine antigen assays, and acute and convalescent serologic studies. Even in these reports, it is rare to identify a likely etiologic agent in more than 50% of patients [5–9]. The conclusion from these experiences is that many pulmonary pathogens have not been identified yet or the available microbiologic techniques are not sensitive enough. In contrast, during the pre-penicillin era, a likely pulmonary pathogen was identified in more than 80% of cases.

There are multiple reasons for the decline in microbiology, and in many ways, they reflect the state of modern medicine. The practical issues that compound standard microbiology methods include the facts that many patients have no expectorated sputum, many patients have undergone previous antibiotic treatment (often precluding any meaningful culture for common pathogens), and policies that mandate rapid institution of treatment may shift emphasis from specimen collection to antibiotic delivery. A second concern is the general decline in quality control: The art of specimen collection with rapid processing is often difficult to achieve because of the loss of laboratories on hospital wards as a result of Clinical Laboratory Improvement Act regulations, outsourcing of microbiology studies by many hospitals, and the breakdown of communication between clinicians and microbiologists. There are also arguments about medical need, as some investigators argue that empiric antibiotic treatment works well, well-done microbiology studies are difficult to perform, the tests are expensive, and the detection of a pathogen does not permit exclusion of copathogens that may require treatment. It commonly is argued that microbiology studies never have been clearly shown to alter outcome in terms of morbidity, mortality, length of stay, or cost. It also may be argued that the studies to show such results have never been done.

Microbiology studies in patients with CAP are evolving. The techniques that were used 50 years ago no longer seem to work well and are viewed as labor-intensive and unrealistic; new technology with molecular methods is being developed slowly, the Food and Drug Administration (FDA) has approved PCR detection for only two pulmonary pathogens: *M tuberculosis* and *Legionella pneumophila*. One of the main issues confronting clinicians is how much they want or need these tests. This article attempts to classify pathogens into three categories: those that clinicians need to know about, those that clinicians would like to know about, and those that clinicians cannot know about.

Microbial pathogens with a compelling need for detection

For the following agents, there is general acceptance of a compelling need for detection because of the requirement for specialized therapy or epidemiologic intervention:

- Severe acute respiratory syndrome (SARS)
- Influenza, but especially avian influenza
- Agents of bioterrorism such as anthrax, plague, and tularemia
- *Legionella* spp, because of the high-mortality rates and potentially important epidemiologic implications
- Community-acquired methicillin-resistant *Staphylococcus aureus*, which is a relatively rare cause of pneumonia, but has mortality rates that exceed 50% (even in young, previously healthy adults) and requires treatment with antimicrobial drugs that generally are not used for empiric therapy [10]

Microbes that clinicians would like to know about

These microbes especially apply to hospitalized patients with pneumococcal pneumonia, in whom sensitivity tests may be important because of the relatively high-mortality rates (15%–20%), even with appropriate antibiotic treatment of patients with pneumococcal bacteremia [11]. *Pseudomonas aeruginosa* is a relatively rare pathogen, but may be encountered in patients with chronic lung disease and has great variations in sensitivity to antibiotics. The same situation applies to coliforms (Enterobacteriaceae), which probably account for no more than 1% to 2% of CAP cases, but are relatively easy to recover from expectorated sputum on MacConkey agar; negative cultures usually exclude their presence. *Haemophilus influenzae* is the second most common bacterial pathogen, but it is also a common contaminant and almost always is treated with empiric regimens other than amoxicillin. *S aureus* is a relatively rare pulmonary pathogen, except in some patients with superinfections and influenza.

Bacteria that cannot be detected

Clinicians have virtually no opportunity to identify cases of pneumonia caused by *Chlamydia pneumoniae* or *Mycoplasma pneumoniae*, because tests are not well standardized, and good tests are not generally available except through research laboratories. Uncontaminated specimens are needed for detecting anaerobes, and such specimens are not available in most cases, except in cases with the infrequent complication of empyema.

The future of diagnostic testing is somewhat optimistic because of the events of the past 3 years, which have emphasized the need for better diagnostics in pulmonary infections. The anthrax epidemic of 2001 brought reality to the concern for bioterrorism and carried new demands for better detection methods, including diagnostic studies of patients with enigmatic pulmonary infections. The SARS epidemic of 2003 was associated with about 916 deaths, a small fraction of the annual mortality attributed to influenza and *S pneumoniae*, but it was devastating to global economies and

brought about incredible demands for infection control, including hospital closures, quarantines, the introduction of respiratory etiquette for disease control and extensive temperature monitoring. This made detection one of the highest priorities. The threat of avian influenza has refocused the need for detection of this specific agent because of the potential for a major global epidemic and the high-mortality rate (60%) among the 55 reported cases in Hong Kong, Vietnam, and Thailand [12]. These agents are on the must-know list and are rare, but their combined effects have refocused attention on the relatively poor status of detection methods for the etiologic agent of CAP. The emphasis is pathogen specific, but these experiences are likely to help launch microbiology into the 21st century with respect to use of modern molecular techniques to detect agents of pneumonia.

Gram stain and culture

The gram stain is one of the oldest and most controversial tests to detect the etiologic agent of pneumonia. The controversy concerns differences in its reported accuracy, which varies widely depending on the expertise of the observer and adequacy of quality control in specimen collection. Even within these boundaries, the results are highly variable, and critics can support almost any position with substantial evidence from the literature. In general, most studies support its use [13–19]. Some of the variations in results depend on the question asked of the test. Three studies examined the usefulness of gram stain for detecting *S pneumoniae* and reported a sensitivity ranging from 57% to 70% and a specificity ranging from 79% to 100% [19–21]. Sensitivity for detection of all bacterial pathogens is reported to range from 65% to 80% [20,22,23], providing the results are restricted to the analysis of purulent samples [20,24]. This determination is based on the gross appearance of the specimen or microscopic examination to determine the concentrations of leukocytes. Some investigators have emphasized the usefulness of these specimens for distinguishing pneumonia caused by *M pneumoniae* and distinguishing viral pathogens from bacterial pathogens based on the cellular constituents (mononuclear cells are predominant in the former, and polymorphonuclear (PMN) cells are predominant in the latter) [21]. In patients with cystic fibrosis, sputum specimens have proved to be highly valuable in distinguishing infections caused by *P aeruginosa*, *Burkholderia cepacia*, *S aureus*, and *H influenzae* [25].

Despite these enthusiastic and supportive studies, other investigators dislike the process. A meta-analysis of various reports shows that, compared with culture results, the sensitivity of gram stain ranges from 15% to 100%, and its specificity ranges from 11% to 100% [26]. Culture results may be an inadequate comparison, however, because of its inherent problems, including quality control in specimen collection, delays in plating, and erroneous results because of previous antibiotic use [27]. Better comparators are used in studies involving patients with positive blood cultures [28] and patients with

transtracheal aspirates [1]. Both studies showed gram staining to have highly favorable results.

Cellular analysis as a contingency for culture

Sputum purulence is an important factor in the accuracy of sputum bacteriology. Early investigators emphasized the importance of specimen collection by the physician and fleck picking by the microbiologist. These points were formalized in the analysis of cellular constituents of the sputum sample by microscopic analysis in the oft-quoted report by Murray and Washington in 1975 [29]. The report emphasized cellular analysis as a criterion for discarding specimens containing more than 10 squamous epithelial cells/low power field (LPF; 100 times the magnification on the gram-stain smear). This conclusion was based on the observation that cultures of such specimens yielded an average of more than four bacterial species, and potential pathogens were found in less than 15% [29]. Two years later, however, one of their colleagues from the Mayo Clinic, Robert Van Scoy, expressed his frustration as a clinician who had discarded most of the specimens from his patients with pneumonia [30]. He pleaded for acceptance of any specimen that showed more than 25 PMN cells/LPF, regardless of the number of squamous epithelial cells (SECs) based on reanalysis of the original data. There has been widespread acceptance of this concept for screening specimens but also substantial variation in the criteria used (Table 1) [1,29–32]. One of the best studies to determine appropriate screening criteria was by Geckler et al [1], who compared results of various criteria from expectorated sputa with results from a transtracheal aspiration. This study showed the best agreement with the criterion of less than 25 SECs/LPF. There was agreement between sputa samples and TTA results in 79% of specimens with less than 25 SECs, compared with 27% in specimens showing more than 25 SECs (a total of 96 specimens were examined). In a subsequent report [33], gram stains of sputum and transtracheal aspirate (TTA) specimens from 100 patients with acute pneumonia were interpreted by laboratory staff and house staff. A predicted pathogen grew in the TTA culture in 36% to 62% of cases based on sputum gram stain and in 37% to 62% of cases based on gram stain of the TTA. These results demonstrate

Table 1
Quality of sputum sample

Reference	Method	Minimum criteria
18	SEC/LPF	<10 SEF
19	PMNs/LPF	>25 PMN
1	SEC/LPF	<25 SEC
20	SEC: PMNs ratio	>10 PMN/SEC
21	SEC + bacteria	<10 SEC + bacteria

Abbreviation: SEC, squamous epithelial cells.

good correlation between gram-stain results with expectorated sputum and TTA, but there was also enormous variation in the interpretations of the same gram stains from either source.

The number of specimens that are acceptable for culture by cytologic screening depends to a large extent on the criteria used, but most laboratories show that 40% to 60% of specimens are rejected for culture using commonly accepted criteria [15,20,27]. Most laboratories also report an improvement in the quality of specimens once these standards are imposed. Two caveats regarding the use of these criteria are that cytologic screening is not appropriate for studies of *Legionella* spp [34] or acid-fast bacilli, and specimens obtained using hypertonic saline (induced sputum) generally provide inadequate specimens because of high concentrations of SECs [35].

Sputum culture

Routine cultures of expectorated sputum have received mixed reviews that are analogous in some ways to review of gram stain. Some studies have shown that these specimens have good correlations with more reliable specimens, such as blood cultures or transtracheal aspirates [36,37], and some studies have shown that they are of little or no value [38–41]. Limitations imposed by the reality of clinical practice are that up to 40% of patients with pneumonia fail to produce adequate specimens for any diagnostic studies, and many patients have received antecedent treatment with antimicrobial agents that precludes meaningful culture, especially for recovery of fragile common pathogens such as *S pneumoniae* and *H influenzae*. Another major problem concerns the adequate implementation of quality assurance, especially at a time when resources for microbiology studies are limited and many or most hospitals outsource this service. Issues that are important components of quality control include the following:

- Cultures should be performed rapidly after collection if they are retained at room temperature, although this requirement is disputed by some investigators. The standard in microbiology laboratories is to plate within 2 hours or to retain the sputum at 4°C if the length of the delay is 2 to 24 hours [42]. This practice prevents overgrowth of contaminants or minority species.
- Cytologic screening should be done as a contingency for acceptability for culture, although the specific criteria are variable (see earlier discussion).
- Standard laboratory media for respiratory secretions are 5% sheep blood agar, MacConkey agar, and chocolate agar. The chocolate agar generally is incubated in 5% carbon dioxide to facilitate recovery of *H influenzae*. *Legionella* spp and *Bordetella pertussis* require specialized media.
- Potential pathogens that should be identified and reported include *S pneumoniae*, *S aureus*, *Streptococcus pyogenes*, *H influenzae*, Enterobacteriaceae,

Moraxella catarrhalis, and *Neisseria meningitidis*. The only reportable yeast is *Cryptococcus neoformans*.

- The concentration of bacteria is an important but often neglected aspect of interpretation. This facet of analysis is formalized with quantitative cultures of bronchoscopy specimens and can be applied conceptually to expectorated sputum culture. Most true pathogens are recovered in moderate or heavy growth, meaning that recovery occurs in the third or fourth streak using a standard plating procedure.
- There should be correlation with the gram-stain results. Some laboratory protocols are gram-stain directed, meaning that the extent of microbiology studies is determined by the gram-stain results [42–44]. Sampling errors with fleck picking can introduce errors [45].

Quality control within the laboratory often requires advocacy from selected groups, such as infectious disease specialists, pulmonary physicians, infection-control specialists, and pharmacy committees. These groups need to communicate on a regular basis to establish methods to achieve optimal results and advocate for adequate resources. The current yield of a likely pathogen in reported studies of hospitalized patients with pneumonia is only about 20% [46]. It is likely that a concerted effort can improve that yield, but it will require a campaign to promote the need.

Specimens collected by invasive techniques

Patients with CAP rarely require invasive diagnostic techniques, but sometimes these techniques are done for exceptional cases or the diagnostic test is done for another indication in which microbiology studies may be a secondary goal. Experience with these techniques enriches the total knowledge of etiologic agents and microbiology principles. The following section is a brief review of some of these techniques.

Transtracheal aspiration

Transtracheal aspiration originally was described by Pecora and Yegian in 1959 as a method to obtain specimens from the lower airways that are free of contamination from the upper airways [47]. The procedure is performed using a 14-gauge needle with an intermediate-sized Intracath, which is inserted through the cricoid membrane. The catheter is passed to its full extent, the needle is withdrawn, and aspiration is performed with a 20- to 30-mL syringe. Saline may be instilled to facilitate specimen collection, but this step is infrequently necessary and should be avoided when possible, because dilution of the specimen makes semiquantitative culture results less meaningful. Only a few drops of secretions are necessary for microbiology studies. This procedure was used extensively by several investigators during the 1970s [48–55], but it fell into disfavor in the 1980s as a result of patient nonacceptance, concern for complications, and arguments that it was unnecessary for optimal

care given the adequacy of empiric antibiotic treatment for most patients. The major complications were bleeding at the site of needle insertion; coughing paroxysms that could compromise air exchange when the catheter was placed in the lower airways; and the profound irritation of the foreign body in the trachea, which was the most unacceptable facet of the procedure for patients [48,55]. Serious complications were rare if the operator was skilled and the patient had no contraindications. Numerous reports of results with this technique were uniformly favorable in terms of the diagnostic results, provided that the specimen was obtained before antibiotic treatment. Use of the technique in healthy medical students established the microbiologic principle that justified this approach, showing that the tracheobronchial tree is normally sterile below the larynx in healthy persons [49]. Studies of patients were uniformly favorable in terms of diagnostic yield. The largest published report included 383 patients who had satisfied criteria for probable bacterial pneumonia based on fever, pulmonary infiltrate on chest radiograph, and clinical response to antibiotic treatment [50]. Of these 383 patients, 335 had a likely pulmonary pathogen recovered in the transtracheal aspiration (TTA); of the 48 patients with false-negative cultures, 44 had received previous antibiotic treatment. The major cause of false-positive cultures is presumably the presence of chronic lung disease with colonization of the lower airways [54]. The technique was useful in studies of pulmonary infections involving anaerobic bacteria, because meaningful cultures for anaerobes require uncontaminated specimens [55]. Even patients with chronic lung disease do not have anaerobes in the lower airways (with the occasional exception of patients with bronchiectasis) [55].

Bronchoscopy

Bronchoscopy has proved to be valuable in detecting the etiologic agents of pulmonary infections, particularly in patients with *Pneumocystis carinii* infections and in patients with *Mycobacterium tuberculosis* infection and no expectorated sputum samples. For detecting conventional bacteria, this technique is no better than expectorated sputum because of the inevitable contamination of the inner channel during passage to the airways. Alternative methods that now are used extensively and have better results employ quantitative cultures of BAL specimens or specimens collected with the double lumen brush catheter. This technique first was described by Wimberly et al [56] in 1979, and it has become a popular, though controversial, method to study patients with nosocomial pneumonia in ICUs, particularly in patients with ventilator-associated pneumonia. It occasionally is used for patients with CAP who have enigmatic pneumonia, but the requirement for technical expertise, the need to collect specimens before institution of antibiotic therapy, and the effectiveness of empiric antibiotics that is given rapidly generally preclude practical use for patients with acute pulmonary infections [57]. Among patients with CAP, bronchoscopy most commonly is used for

patients with more chronic and enigmatic pulmonary infections in which empiric antibiotics are difficult to select and rapid institution of treatment is not mandated by the clinical presentation. The use of quantitative cultures is based on the principle that pulmonary infections by bacteria almost always are associated with bacteria in concentrations exceeding 10^6 /mL. The concentration that is considered significant with the brush catheter is usually 10^3 /mL and with BAL is usually 10^4 /mL; these concentrations reflect the dilutional effect of the procedure and the processing [55].

Transthoracic needle aspiration

Transthoracic needle aspiration permits collection of uncontaminated specimens directly from the pulmonary parenchymal for cytologic and microbiologic analysis. The procedure initially was introduced in 1882 [58]. It can be done with CT guidance to permit sampling of small lesions. It usually is performed with an 18- to 22-gauge, thin-walled spinal needle. There is an extensive reported experience with this technique for detection of conventional bacteria from 20 to 50 years ago, but the technique rarely is performed, except in patients with usual lesions at the present time. The most extensive experience from the earlier period was reported by Bullowa [2], who examined lung aspirates in 1467 patients with suspected pneumococcal pneumonia. Positive results were obtained in 510 patients (diagnostic yield, 35%). Among 211 patients with bacteremic pneumonia, the yield of *S pneumoniae* was 165 (78%). The presumed explanation for these false-negative results was improper placement of the needle or nonviable organisms (136 patients). A review of 23 published reports on this technique from 1922 through 1994 showed that the yield of microbial pathogens ranged from 37% to 94%, and the rate of false-positive results was virtually nil [55].

Detection of specific microbes

Streptococcus pneumoniae

S pneumoniae always has ranked first among identifiable pathogens in patients with CAP, but there has been a decrease in the yield with sequential studies. In the pre-penicillin era, *S pneumoniae* accounted for more than 80% of all pneumonias and 96% of lobar pneumonias [1,2]. There has been a continuing reduction in the yield of the pneumococcus, so studies in the past decade generally show that these organisms account for only 10% to 20% of cases [5–9]. This decline often is regarded as an artifact of microbiology, reflecting the diminished quality and interest in laboratory diagnosis that result in the nondetection of a bacterial pathogen in most cases. A meta-analysis of 122 reports in the English-language literature on CAP for 1966 through 1995 showed that approximately 6000 of 33,000 patients (18%) had a defined bacterial pathogen. *S pneumoniae* accounted for

73% of all cases in this category and 66% of cases with a lethal outcome [46]. *S pneumoniae* accounted for approximately 65% of all patients with bacteremic CAP [46] and had a much higher frequency in cases in which more aggressive diagnostic methods, such as transtracheal aspiration, were used [47]. The British Thoracic Society Pneumonia Research Committee reviewed 148 cases with CAP and no identifiable pathogen and concluded that most of these cases probably were caused by *S pneumoniae* [59].

An established diagnosis of pneumococcal pneumonia requires recovery of this organism from an uncontaminated specimen source, such as blood, pleural fluid, transtracheal aspirate, or transthoracic needle aspirate. As noted, this organism accounts for about 65% of bacteremic CAP cases, and these cases account for 5% to 12% of hospitalized patients with CAP [46]. Because most patients do not have empyema, have bacteremia, or undergo invasive diagnostic testing, this diagnosis is pursued only with gram stain and culture of expectorated sputum. As noted, gram stain is subject to substantial subjective variation depending on the quality of the specimen and expertise of the observer. Culture is problematic and produces false-positive and false-negative results; false-positive results occur as a result of the 5% to 10% of adult patients who are colonized with *S pneumoniae* in the pharynx, and false-negative results occur because of the imperfection of sputum bacteriology and multiple studies showing a diagnostic yield of 50% or less in patients with bacteremic pneumococcal pneumonia [41]. Specialized techniques to improve this yield of false-negative results include stains of expectorated sputum with specialized methods to detect pneumococcal polysaccharide, including the Quellung test, latex agglutination, and counterimmunoelectrophoresis in urine or respiratory secretions. A potentially important development is the commercial availability of a urinary antigen test using a rapid immunochromatographic assay. One prospective, controlled trial showed positive results in 88 of 107 adults with pneumococcal bacteremia and 3 of 106 (3%) adults with septicemia caused by other microbes [60]. This study, which showed a 80% sensitivity and 97% specificity, is at least as good as gram stain or culture, and it has the advantages of rapid results, diagnostic usefulness after antibiotic treatment, and specimen availability in patients who have no expectorated sputum. The disadvantages are that the sensitivity is less in children who have higher carriage rates for *S pneumoniae*, the sensitivity may be less in adults with nonbacteremic pneumonia, and there is no pathogen for sensitivity testing.

Chlamydia pneumoniae

The estimated frequency with which *C pneumoniae* is responsible for hospitalized cases of CAP ranges from 10% to 20% [5–9]. When this organism is found, there are often questions regarding relevance because of inadequacies in testing and the frequency of copathogens that seem to dictate the course [61]. Detection methods include culture for *C pneumoniae*, PCR,

and serology. Culture requires the use of cell cultures and is not generally available, except in research laboratories. PCR techniques may be used, but the reagents are not cleared by the Food and Drug Administration (FDA) and are poorly standardized [62]. The most frequently used assay is the microimmunofluorescence (MIF) assay for detection of IgM or a fourfold increase in IgG titer. The Centers for Disease Control and Prevention reviewed these diagnostic methods and concluded that only 4 of 16 PCR assays are adequate and that all are “home grown” (meaning that they are not commercially available). With regard to the MIF assay, the diagnostic criteria were an IgM titer exceeding 1:16 or a fourfold increase in the IgG titer [62]. Most reports of the cause of CAP have not obliged these criteria, so reliable incidence data are not available. Studies have shown poor interobserver consistency in the interpretation of the MIF assay [63]. Interpretation by two experts of specimens from 392 patients showed poor correlation (κ -value for dichotomous titers, 0.53 [$<1:16$ versus ≥ 16]). The conclusions are that the gold-standard method is culture, which is not routinely available; the laboratory standard is the MIF assay, which is poorly standardized; and the most promising test is PCR.

Mycoplasma pneumoniae

Laboratory tests to detect *M pneumoniae* infection include culture, serology, and PCR. Serology with IgM and IgG become elevated in many or most cases, but the response often is delayed, so the usefulness of these tests for early detection is limited [64]. Its usefulness also is limited by the reproducibility of the tests. Some authorities consider PCR to be promising [42,65], but there is substantial variation in specimen collection, sample preparation, amplification procedures, and reagents. No reagents for PCR detection have been approved by the FDA. In one report of 27 patients with atypical pathogens, including 19 patients with *M pneumoniae* infection, the atypical pathogens occurred more frequently in patients with typical pneumonia than in those with atypical pneumonia (as determined by clinical features) [64]. These observations emphasize the difficulty in establishing the diagnosis of *M pneumoniae* infections on the basis of clinical features and laboratory studies.

Legionnaires' disease

Legionella spp are implicated in 2% to 6% of CAP cases; may cause epidemics, including nosocomial epidemics; and result in a substantial mortality rate even when treated with the appropriate antibiotics. Laboratory methods for detection include culture, serology, direct immunofluorescent antibody staining, the urinary antigen assay, and PCR. The relative merits of these tests are outlined in Table 2 [66]. A confounding issue in noncultural assays is that there are more than 40 *Legionella* spp and a total of

Table 2
Diagnosis of Legionnaires' disease

Test	Time	Sample ^a	Sensitivity (%)	Specificity (%)	Comment
Culture	3–7 d	LRT	<10–80	100	Detects all strains
DFA stain	<4 h	LRT	25–70	>95	Technically demanding
Urine antigen	<1 h	Urine	70–90	>99	Detects only <i>L pneumophila</i> serogroup 1
PCR	<4 h	LRT	80–100	>90	Detects <i>L pneumoniae</i> (all serogroups)

Abbreviations: DFA, direct immunofluorescent antibody; LRT, lower respiratory tract specimen; NPS, nasopharyngeal swab.

^a Expecterated sputum, bronchoscopic aspirate, nasotracheal suction specimen.

Adapted from Murdoch DR. Diagnosis of *Legionella* infection. Clin Infect Dis 2003;36:64.

64 serogroups [67]. *L pneumophila* causes about 90% of cases, and serogroups 1, 4, and 6 account for most within species. Guidelines from the Infectious Diseases Society of America [5,68] recommend two tests: (1) culture, which detects all of the species but is technically demanding (ie, large numbers of false-negative results by many or most laboratories); and (2) the urinary antigen assay, which is easy to perform and provides rapid results. The main limitation of the urinary antigen assay is that it detects only *L pneumophila* (serogroup 1), but this organism accounts for about 80% of sporadic cases of Legionnaires' disease. The diagnostic usefulness of the assay is summarized in the report by Helbig et al [69]. Among 472 patients with culture-proven *Legionella* spp infections, the urinary antigen assay had a sensitivity exceeding 80% in patients with community-acquired or hotel-associated Legionnaires' disease, but only 45% in patients with nosocomial disease (Table 3). The inference is that *L pneumophila* may occur less frequently in hospital-acquired cases, which has important implications for test secretions. The FDA has approved a PCR method that detects *L pneumophila*, including all serotypes, using respiratory tract secretions.

Anaerobic bacteria

Anaerobic bacteria that dominate in the oropharyngeal flora are relatively common causes of aspiration pneumonia and lung abscess. The

Table 3
Sensitivity of the *Legionella* spp urinary antigen assay

Category	No. of positive cultures	No. of urinary antigen
Community acquired	214	172 (80%)
Travel associated	169	159 (94%)
Nosocomial	89	40 (45%)

Adapted from Helbig JH, Uldum SA, Bernander S, et al. Clinical utility of urinary antigen detection for diagnosis of community-acquired, travel-associated, and nosocomial Legionnaires' disease. J Clin Microbiol 2003;41:838–40.

major mechanisms to establish this diagnosis requires specimens that are devoid of upper airway flora, such as transtracheal aspirates, pleural fluid, transthoracic needle aspirates, or uncontaminated specimens from metastatic sites [70,71]. Most studies of anaerobic pulmonary infections were done with TTA from 1970 to 1980; this procedure is no longer common, and anaerobic lung infections are almost never proved unless an empyema is present. There is a limited experience with bronchoscopy specimens using BAL [56,57] or the protected brush catheter [4]; the total experience is sparse and is limited to laboratories with the specialized skills necessary to successfully culture anaerobes. Anaerobic infections of the lung now rarely are confirmed by bacteriologic studies in the absence of empyema. Clinical suspicion of these infections is often helpful, however. Putrid sputum or empyema fluid is considered diagnostic of anaerobic infection, aspiration pneumonia with typical predisposing conditions usually is caused by anaerobes, and necrosis of tissue with lung abscess or a bronchoplural fistula strongly suggests this cause [71].

Agents of bioterrorism

The three category A agents that cause pneumonia are *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis* [72–76]. Most of the experience with inhalation anthrax comes from the 2001 epidemic attributed to bioterrorism, in which 11 patients developed this disease. Before this event, no cases of inhalation anthrax had occurred in the United States since 1976 [73,74]. The diagnosis of inhalation anthrax is made on the basis of clinical and laboratory observations, including several clinical clues that distinguish this disease from common forms of CAP [74]. Some laboratory tests are particularly suggestive: a wide mediastinum on chest radiograph, hyperdense mediastinal nodes on chest CT scan, and bloody pleural effusions. The best method to confirm the diagnosis is with blood cultures, which were positive in all eight patients who had specimens collected before antibiotic therapy; these cultures were positive within 18 hours [72]. With tularemia, the putative agent may be cultured from blood, sputum, or pharyngeal exudates, but only with difficulty and with the use of specialized media containing cysteine or other sulfa-hydroxyl compounds, such as thioglycolate broth or charcoal-yeast agar [75]. This organism can be a hazard to laboratory personnel and should be cultured only in a biologic safety level 3 (BSL-3) laboratory. Diagnostic methods include PCR, enzyme immunoassay (EIA), immunoblot, and pulsed-field gel electrophoresis. These tests are generally available only in research or public health laboratories. *Y testis* is cultured from sputum or blood [76]. On gram stain, it typically appears as a safety-pin-shaped, bipolar-staining, gram-negative coccobacillus. When this diagnosis is suspected, the specimen should be divided for incubation at 28°C (for rapid growth) and at 37°C (for identification of the capsular antigen).

Table 4
Pathogen detection

Organism	Pulmonary specimen (usual)	Microscopy (stain)	Culture ^a	Serology	Other
Bacteria					
Aerobes	Sputum, bronch, blood	Gram	Conventional	—	<i>S pneumoniae</i> urinary Ag
Anaerobes	TTA, pleural fluid	Gram	Anaerobic media	—	—
<i>Legionella</i> spp	Sputum, bronch blood	FA (<i>L pneumophila</i>)	Selective media	IFA	Urinary Ag: <i>L pneumoniae</i> SG#1 Respiratory secretions: PCR: <i>L pneumoniae</i> (all serotypes)
<i>C pneumoniae</i>	NPS, sputum, bronch	—	(Cell culture)	CF: <i>C psittaci</i> MIF: <i>C pneumoniae</i>	PCR (experimental)
<i>M pneumoniae</i>	NPS, sputum	—	(Culture)	CF, EIA	PCR (experimental)
Mycobacteria	Sputum, bronch	AFB	Selective media	—	PCR
Viruses					
Influenza	NPS or aspirate	FA	(Cell culture)	CF, EIA, FA, LA	Rapid Ag detection
RSV	NPS	FA (pediatric only)	(Cell culture)	—	—
Parafu	NPS	—	(Cell culture)	—	—
SARS	NPS	—	(Cell culture)	—	PCR (experimental)

Abbreviations: AFB, acid-fast stain; Ag, antigen; bronch, bronchoscopy aspirate or BAL; CF, complement fixation; EIA, enzyme immunoassay; FA, fluorescent antibody; IFA, immunofluorescence assay; LA, latex agglutination; MIF, microimmunofluorescence assay; NPS, nasopharyngeal swab; SG, serogroup.

^a Entries in parentheses are not usually available.

Summary

Diagnostic tests for the detection of the etiologic agent of pneumonia are neither recommended nor done for most outpatients with CAP (Table 4). Most of these patients have no clear diagnosis but seem to do well with empiric antibiotic treatment, which often costs less than the diagnostic tests. For hospitalized patients, a pre-treatment blood culture and an expectorated sputum gram stain and culture should be done. Testing for *Legionella* spp is appropriate in hospitalized patients, especially those who are seriously ill. New tests that merit use in selected patients are the urinary antigen assay for *S pneumoniae* and the PCR test for *L pneumophila*. Anticipated developments in the near future are PCR tests for detection of *C pneumoniae* and *M pneumoniae*.

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