Lower respiratory infections are the major cause of death due to infectious disease in the United States and worldwide. Most forms of community-acquired pneumonia (CAP) are treatable, and there is consensus that the selection of antimicrobial agents is notably simplified if the pathogen is defined. The rich history of CAP studies in the prepenicillin era showed that an etiologic diagnosis was established in >90% of cases, but the 2009 data from Medicare indicate that a probable pathogen is now detected in <10% according to a review of the records of >17,000 patients hospitalized with CAP. This review addresses the issue of the state of the art of microbiological studies of CAP in terms of the realities of current-day practice. Unfortunately, the desire for better data to achieve pathogen-directed treatment clashes with a multitude of harsh realities, including cost, Centers for Medicare and Medicaid Services (CMS) requirements for antibiotics to be administered within 6 h of disease onset, guidelines that discourage any microbiological studies in most cases, belief in empiricism that is well supported by at least 1 prospective study, the decline of microbiological analysis standards in most laboratories, and the devastating impact of the Clinical Laboratory Improvement Amendments (CLIA) regulations that led to the demise of “the house staff laboratory” and the distancing of microbiological analysis from the site of care. Microbiological principles are reviewed, with emphasis on specimen source, pathogenic potential of isolates, concentrations, impact of antecedent antibiotics, and the “Washington criteria” for expectorated sputum. The recommendation is that the high-quality microbiological analysis that is still achieved in some places should be retained but that to advance the field on the basis of the contemporary realities, two goals should be adopted: First is the broad use of antigen tests for *Streptococcus pneumoniae* and *Legionella pneumophila* with interpretation by clinical staff under the CLIA waiver for low-complexity tests. The second and more ambitious recommendation is the adoption of molecular techniques, with particular emphasis on nucleic acid detection, which is rapid and sensitive and has already been developed for virtually all recognized pulmonary pathogens. This may be the ultimate solution for many laboratories, and it is likely to have selected use.
HISTORY

Studies of CAP date to the time of R. Koch and L. Pasteur in the nineteenth century, when some of the legendary studies in medicine were performed. However, the most important, well-documented, in-depth analysis of patients with CAP dates to the first half of the twentieth century, which has been beautifully summarized in Heffron’s book titled Pneumonia, [1], a review that R. Austrian called the greatest book ever written about a single microbe [2]. This is the period when S. pneumoniae was recognized as “Captain of the men of death” [2], the art of sputum cultures was refined, pathogenic studies in rodent models were developed, Bullowa reported his experience with >1400 transthoracic aspirations performed to establish the etiologic agent, and serotyping of the major pathogen became standard practice [1, 3, 4]. A comment from one of his classic reviews is testimony of the quality of work in the early studies by Bullowa: “The determination of the pneumococcus type may be made in 76 percent of cases by a direct Neufeld examination of sputum. Where too few organisms are present, it may be necessary to propagate them in white mice, where they are usually found in peritoneal exudates after three or four hours or in mouse blood after eight to ten hours. The organism obtained from the sputum is responsible for the pneumonia in 93 percent of cases, as shown by comparison with simultaneous or subsequent lung aspiration and blood culture” [3]. A review of 3319 cases of lobar pneumonia was reported for the period 1917–1933 [4] with identification of the probable putative agent in 99.6% of cases as reported in 3 series by Avery et al [5], Cecil et al [6], and Sutliff & Finland [7].

Paradoxically, with the advent of antimicrobial agents to treat CAP, there has been a progressive decline in the perceived need to know the pathogen for which the patient was being treated. This statement is supported by a random review of reported experiences with microbiological studies of CAP for the yield of S. pneumoniae, which was selected because it has always been regarded as the major etiologic agent in virtually all series. The anticipated microbiological results on the basis of a literature review published in 1995 are summarized in Table 1 [8], and the results of sequential studies for the yield of S. pneumoniae are summarized in Table 2 [9–20]. The most recent data are from a summary of 17,435 patients sufficiently ill to require hospitalization for CAP in 2009 and showed that an etiologic agent was identified in 7.6% [D. Bratzler 2009, private communication]. The latter results are the reported experience for Medicare, which collects these data for the CMS audits of CAP in non-federal hospitals in the United States, thus providing perhaps the best data on contemporary practice relevant to etiologic diagnostic testing and yield. The assumption from this historical review is that either S. pneumoniae is disappearing as an important pulmonary pathogen or diagnostic microbiological characterization is disappearing as a component of high-quality care.

**PRINCIPLES OF MICROBIOLOGICAL ANALYSIS FOR CAP**

Contemporary standards for high-quality microbiological analysis for CAP include 4 components: specimen source, pathogenic potential of various organisms, concentrations of organisms recovered, and the influence of prior antibiotics.

**Table 1. Microbiological Characteristics of Community-Acquired Pneumonia (CAP)**

<table>
<thead>
<tr>
<th>Origin</th>
<th>North Americaa</th>
<th>British Thoracic Societyb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>20–60</td>
<td>60–75</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>3–10</td>
<td>4–5</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3–5</td>
<td>1–5</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>3–10</td>
<td>Rare</td>
</tr>
<tr>
<td>Legionella</td>
<td>2–8</td>
<td>2–5</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>1–6</td>
<td>5–18</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>4–6</td>
<td>–</td>
</tr>
<tr>
<td>Aspiration</td>
<td>6–10</td>
<td>–</td>
</tr>
<tr>
<td>Viruses</td>
<td>2–15</td>
<td>8–16</td>
</tr>
</tbody>
</table>

**NOTE.** Data from Mundy et al [8].

a Based on 15 reports from North America.
b Based on an analysis of 453 adults in a prospective study of CAP in 25 British hospitals. Ellipses indicate that no studies were performed to detect the designated agent.

**Table 2. Recovery of S. pneumoniae in Sputum from Adults With Community-Acquired Pneumonia**

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>No. of patients</th>
<th>S. pneumoniae present, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fekety et al [10]</td>
<td>1971</td>
<td>100</td>
<td>62</td>
</tr>
<tr>
<td>BTS [12]</td>
<td>1987</td>
<td>433</td>
<td>42</td>
</tr>
<tr>
<td>Holmberg [13]</td>
<td>1947</td>
<td>147</td>
<td>39</td>
</tr>
<tr>
<td>Fang et al [16]</td>
<td>1990</td>
<td>359</td>
<td>15</td>
</tr>
<tr>
<td>Farr et al [17]</td>
<td>1991</td>
<td>245</td>
<td>18</td>
</tr>
<tr>
<td>Bohle et al [18]</td>
<td>1995</td>
<td>181</td>
<td>23</td>
</tr>
<tr>
<td>Park et al [19]</td>
<td>2001</td>
<td>410</td>
<td>11</td>
</tr>
<tr>
<td>Medicare [D. Bratzler 2010, private communication]</td>
<td>2009</td>
<td>17,435a</td>
<td>7.6a</td>
</tr>
</tbody>
</table>

**NOTE.** BTS, British Thoracic Society.

a Includes all cases with records indicating that a likely pathogen was detected.
Specimen Source
The most readily available specimen and diagnostic standard in most cases is expectorated sputum. This specimen must traverse the upper airways, which are colonized with large concentrations with multiple bacteria (10⁶–10¹⁰ colony-forming units [CFU]/mL saliva), including some that are potentially agents of pneumonia, such as *S. pneumoniae*, *Haemophilus influenzae*, and, in patients with prior antibiotics or underlying diseases, enteric gram-negative bacilli [21]. In general, microbiological specimens subject to oropharyngeal contamination may engender particular difficulty in detecting relatively fastidious bacteria, such as *S. pneumoniae*, in part because there is no easy method to distinguish common pathogens from normal flora with Gram stain or selective media. Some laboratories continue to perform Quellung staining to distinguish *S. pneumoniae* from the massive number of viridians streptococci and some laboratories still report high rates of success with traditional methods of Gram staining and culture [22], but this has become relatively unusual. It should be acknowledged that, by contrast with the pneumococci, *Staphylococcus aureus* and aerobic gram-negative bacteria are relatively easy to detect using selective media, so these organisms are relatively rare but likely to be disproportionately represented as a result of ease of detection as contaminants, especially in specimens collected after antibiotic treatment.

With respect to the problem of contamination, there have been numerous attempts to decrease it by means of sputum washing, careful fleck picking, and cytologic screening. Sputum washing consisted of placing the mucoid sample in the equivalent of a tea strainer and washing it to eliminate the salivary contaminants [23]. The freeze-crack system is a variant of this and involved flash freezing the specimen and scooping the purulent core for culture. Neither of these procedures attracted much attention, in large part because they did not have established merit, were technically tedious, and raised concern for possible exposure to selected pathogens, such as *Mycobacterium tuberculosis*.

The one system that survived this period of attempts at quality improvement is the molecular analysis of cellular constituents of sputum, as initially reported in the frequently quoted 1975 paper from Murray and Washington [24]. This report recommended discarding specimens that contained >10 squamous epithelial cells (SECs) per low-power field (LPF) with a 100× magnification of the gram-stained smear. However, one of their colleagues from the Mayo Clinic, R. Van Scoy, expressed great frustration as a clinician who had most of his specimens from patients with pneumonia discarded on the basis of the original criteria [25]. He pleaded for acceptance of any specimen that showed >25 polymorphonuclear cells per LPF regardless of the number of SECs on the basis of his reanalysis of the original data. Since then, there has been widespread acceptance of the concept for specimen screening but also substantial variation in the criteria used. Particularly important among the multitude of studies examining the various criteria was the report by Geckler et al [26], who compared the results from various methods of expectorated sputum screening with results from transtracheal aspiration. This supported the use of <25 SECs/LPF, which became the criterion that was subsequently endorsed by Washington [27].

Recommended procedures at present are for cultures to be performed on deep cough–produced sputum specimens obtained before antibiotic treatment and plated within 2 h of collection or stored at 4°C to prevent overgrowth of contaminants. The cytologic screening should be performed as a contingency for acceptability for culture to detect the usual pathogens. Note that this screening is not necessary for *Legionella* [28], *Mycobacteria*, or any specimens obtained using hypertonic saline (induced sputum) [29]. Potential pathogens to be identified and reported include *S. pneumoniae*, *S. aureus*, *Streptococcus pyogenes*, *H. influenzae*, *Enterobacteriaceae*, *Moraxella catarrhalis*, *Neisseria meningitidis*, and pneumonads.

Because of the problem of contamination with upper airway flora, multiple alternative specimen collection methods that eliminate or reduce contamination have been attempted. These include transtracheal aspiration, transthoracic aspiration, and the collection of specimens at bronchoscopy.

Transtracheal aspiration was originally described by Pecora and Yegian in 1959 [30]. The procedure involves a 14-gauge needle with an intermediate-sized intracath that is inserted through the cricothyroid membrane; the catheter is passed to its full extent, the needle is then withdrawn, and aspiration is performed with a 20–30 mL syringe. Transtracheal aspirations were used extensively by several investigators to bypass the contaminating upper airway flora during the 1970s [31–38]. Use of the procedure in healthy medical students established the fact that the lower airways are sterile in the absence of infection in healthy persons [31], and multiple studies provided testimony to utility for meaningful results, but transtracheal aspiration fell into disfavor in the 1980s because of patient nonacceptance, concern for complications, and a sentiment that the procedure was unnecessary. The largest published series included 383 patients with probable bacterial infections and showed that likely pulmonary pathogens were recovered for 335 patients; 44 of the 48 negative culture results were from specimens from patients who had received prior antibiotic treatment that could account for the lack of pathogen detection [33]. Among patients who had false-positive culture results, most had chronic airway disease, indicating bacterial colonization below the level of the larynx, thus decreasing the diagnostic utility of these specimens in patients with chronic lung disease [37].
Transthoracic needle aspiration was introduced in 1883 [39], and the largest subsequent experience was reported by Bullowa [3, 4], who summarized results with 1467 patients with suspected pneumococcal pneumonia. Culture of specimens from 510 patients yielded a suspected pathogen; among 211 patients with bacteremic pneumococcal pneumonia, the yield with transthoracic needle aspiration was 165 (78%). The presumed explanation for false-negative results was improper placement of the needle. A subsequent summary of 19 reports from 1922–1991 with a total of 1489 patients showed 850 (57%) with positive results for a likely pathogen [40]. This procedure is now rarely performed, in large part as a result of the same concerns as noted for transtracheal aspiration—patient safety, patient acceptance, and need.

Bronchoscopy was initially viewed as an excellent method to sample the lower airways directly, but early work with the procedure showed clear evidence of contamination by oral flora on the basis of the demonstration of oral flora in bronchoscopic aspirates, visualization of saliva in the inner channel during the procedure, and by methylene blue in the specimen following staining of the posterior pharynx prior to the procedure [41]. The alternative methods that subsequently gained favor were quantitative cultures of bronchoalveolar lavage (BAL) specimens (in 1978) [42] or specimens collected with the double-lumen brush catheter (in 1979) [43]. These procedures are now largely restricted to patients with nosocomial pneumonia and ventilator-associated pneumonia; they are rarely used for CAP, in large part because it is considered impractical and generally unnecessary to obtain a pretreatment specimen [44].

Techniques Designed to Detect Specific Pathogens

*S. pneumoniae* has always been the most common identifiable pathogen in CAP, but the data in Table 2 show the remarkable reduction in frequency of detection since the 96% diagnostic yield achieved in the preantibiotic era. More recent reports indicate a yield of only 10%–20% [16–20]. The studies from the CMS are certainly more representative of care standards in the United States than are the other recent reports, and the former indicate a yield of *S. pneumoniae* <5% [21]. The difference between the reported experience and the CMS data presumably reflects the fact that the reported experience often represents results of therapeutic trials or academic centers where there is a more concerted effort to identify pathogens, such as *S. pneumoniae*. A meta-analysis of 122 reports in the English-language literature on CAP for 1966–1995 showed that approximately 6000 (18%) of 33,000 had a bacterial pathogen [45]. In this summary, *S. pneumoniae* accounted for 73% of all cases and 66% of cases with a lethal outcome. Pneumococcus also accounted for about 65% of all patients with bacteremic pneumonia. As noted previously, Gram staining is subject to substantial subjective variation depending on the quality of the specimen collected and the skill of the observer. The culture is also problematic for this pathogen as a result of false-positive and false-negative results. The point emphasized is that our ability to detect pneumococci in CAP in 2010 is very poor.

An important development for detection of this specific pathogen is the urinary antigen assay, which has the advantages of ease of getting a diagnostic specimen and substantial improvement over sputum in terms of diagnostic yield and ability to establish this diagnosis after antibiotic treatment. One prospective, controlled trial showed positive results in 88 (82%) of 107 adults with bacteremic pneumococcal pneumonia and false-positive results in just 3 (3%) of 106 adults with septicemia due to other microbes [46]. This study showed a sensitivity of 82% and a specificity of 97%. Other studies have yielded similar results [47–48]. Disadvantages are that sensitivity and specificity are less in children and in adults with nonbacteremia pneumonia [48].

The urinary antigen test has also been developed and is now the favored method for detection of Legionnaires disease, which accounts for 2%–6% of CAP cases [47]. The urinary antigen assay for *Legionella* SG1 is the most common and, for most laboratories, the most practical test [47, 49–53]. One large study with the diagnosis based on positive sputum culture results in 212 patients showed that the sensitivity of the urinary antigen assay was approximately 80%, with most of the false-negative results due to *Legionella* species other than *L. pneumophila*, which accounts for about 80% of all cases [49]. For *chlamydia pneumoniae* and *Mycoplasma pneumoniae*, there is no test that has been cleared by the FDA [47]. Multiple laboratories have developed polymerase chain reaction (PCR) methods for detection of *C. pneumoniae*, but a review by the Centers for Disease Control and Prevention indicated that only 4 of these were acceptable in quality assessment [53]. A subsequent report indicated that 2 of the favored methods showed minimal correlation when they were compared [54]. According to one authority, there is no practical method to establish a diagnosis of *C. pneumoniae* infection at this time [55]. The same might apply to *M. pneumoniae* infection, at least in adults.

One recent development of particular note has been the PCR assay that has been cleared by the Food and Drug Administration (FDA) for detection of 12 respiratory tract viruses [56]. There has also been substantial progress for multiple tests, including rapid diagnostic tests for the detection of influenza viruses, which show good specificity but sensitivity of only 50%–70% [57]. The rapid tests that can be applied at the point of care for *S. pneumoniae*, *Legionella*, and influenza provide results within 20 min; all 3 appear to show good specificity and sensitivity of 50%–80%. Molecular methods for detecting respiratory viruses including influenza are clearly more sensitive [57–59], but the advantage of the rapid tests is that they can be performed at the point of care.
Concentrations
Multiple studies of bacterial infections indicate that pathogens almost invariably reach levels of $10^6$/mL or $10^6$/g at infected sites [60]. These concentrations apply to nearly all bacterial infections except for soft-tissue infections involving group A streptococci. This observation becomes important in the interpretation of clinical relevance, because likely contaminants in low concentrations can usually be ignored. A few agents assume pathogenic importance regardless of concentration; these include M. tuberculosis, Bacillus anthracis, Legionella, endemic fungi, Yersinia pestis, and Francisella tularensis. For others, quantitation is important for interpretation, particularly with organisms that commonly colonize the upper airways. It should be noted that these concentrations indicate easy detection by conventional Gram stain and recovery in moderate or heavy growth by conventional reporting [61]. With quantitative cultures of expectorated sputum and endotracheal aspirates, the expected threshold is at least $10^6$/mL [23, 61]; for brush catheter samples, the threshold is $10^4$/mL [42, 43], and for BAL specimens the threshold is $10^3$/mL [62, 63]. These latter concentrations are adjusted on the basis of dilution of the specimen with the procedure.

CURRENT REALITIES

CMS Requirements for CAP
The CMS has used CAP as a performance standard with potential application for public reporting and/or reimbursement [64]. The standard is to initiate antibiotic therapy according to Infectious Diseases Society of America (IDSA)/American Thoracic Society (ATS) guidelines within 6 h of registration in emergency departments (“door-to-needle time”). This standard is based on a retrospective analysis of 13,771 patients aged >65 years hospitalized with CAP that showed an increase in both hospital and 30-day mortality with further delays in treatment [65]. The relevance of this CMS policy to microbiology analyses is that it is defensible on the basis of the increase in mortality, but it notably impairs the ability of physicians to establish an etiologic diagnosis prior to the first dose of antibiotics because of other care needs in emergency departments, including the need for imaging to facilitate the diagnosis in possible CAP cases. Additional practical issues that have deterred diagnostic studies include CLIA 88, which was a well-intentioned congressional act that restricted the performance of Gram stain analyses used in the context of clinical care to licensed laboratory technicians. This led to the elimination of “house staff laboratories” in virtually all US hospitals in 1988. Furthermore, many hospitals have now outsourced microbiological analysis. The result is that laboratories that used to be several yards from the patient may now be several miles away. This makes diagnostic microbiological analyses feasible for reporting within hours quite unrealistic for many or most care facilities.

Billing
The cost of testing is an important issue but difficult to address because of great differences in costs compared with charges, bundling, and the lack of studies that address the issue of cost of diagnostic studies to decrease downstream benefit in terms of length of stay, adverse effects, and resistance.

Guideline Recommendations
The recommendations of the IDSA/ATS for CAP are for “routine microbiology only for pathogens that would significantly alter empiric decisions” [66]. The major concerns for routine microbiological analysis expressed in this document were cost, the problems of poor quality specimens and low yield, and the impression that empiric treatment was usually effective. Exceptions noted included illnesses due to some selected pathogens, such as important viral pathogens (such as influenza and severe acute respiratory syndrome), agents of bioterrorism, M. tuberculosis, endemic fungi, and S. aureus. Sputum cultures were also recommended for patients sufficiently ill to require hospitalization in the intensive care unit. Another exception is the recommendation of sputum and blood cultures for patients whose empiric treatment failed, but that recommendation is obviously complicated by and often misleading because of the somewhat predictable problem of “sputum superinfection.”

Results With Empiric Treatment
With regard to the impression that empiric treatment is usually effective, there is at least 1 study that examined this issue. This report compared outcome in 262 patients hospitalized with CAP who were randomly assigned to either empiric antibiotics according to ATS guidelines or to pathogen-directed treatment based on a BAL culture result [67]. The results showed no difference in terms of response to treatment, length of stay, or mortality; there were substantially more adverse drug reactions in the group treated empirically, but the fact that erythromycin was used in the empiric regimen could account for that difference (Table 3). Thus, this report supports the empiric approach to antibiotic selection for the vast majority of patients with CAP.

Table 3. Pathogen-Directed Antibiotic Treatment Compared With Empiric Antibiotic Treatment for Community-Acquired Pneumoniaa

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Pathogen-directed treatment (n = 134)</th>
<th>Empiric treatment (n = 128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality, %</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Length of stay, mean days</td>
<td>14.3</td>
<td>13.2</td>
</tr>
<tr>
<td>Clinical failure rate, %</td>
<td>19</td>
<td>21</td>
</tr>
</tbody>
</table>

* Data from van der Berden et al [67]
DIAGNOSTIC TESTING TO DEFINE THE ETIOLOGICAL CHARACTERISTICS OF PNEUMONIA

Rationale
There is an impression of urgency to improve diagnostic microbiological analysis, reflecting concern for galloping empiricism in antibiotic selection. CAP is a leading example of a condition in which microbiological standards established early in the 20th century are now nearly gone. Paradoxically, since antibiotic therapy became available, there has been a disquieting loss of interest in defining the pathogen, so the physician now rarely knows what is being treated. Defense of this change is based on the assumptions that empiricism usually works and that microbiological standards are unrealistic because of the need to initiate antibiotic treatment rapidly. The 1988 CLIA ruling on specimen management, the devastation of traditional teaching methods, poor practice standards, and outsourcing have added to the difficulty in doing it right. The cases favoring the need for better microbiological analysis in CAP are as follows:

1. Desire for pathogen-directed therapy as an essential standard for managing an important infectious disease. Justification is based in large part on avoiding antibiotic abuse, which is an inevitable consequence of the empiric choices based on the IDSA/ATS guidelines.
2. Recognition of selected pathogens that are important to detect for purposes of epidemiologic studies and/or treatment. These include S. aureus, agents of bioterrorism, M. tuberculosis, endemic fungi, and influenza virus. Of note is a recent report indicating that 16 (43%) of 37 cases of pneumonia caused by methicillin-resistant S. aureus were initially treated with inappropriate antibiotics on the basis of use of the guideline-mandated empiric options [68]. The mean age of these patients was 16 years, and the mortality rate was 51%.
3. Need to track S. pneumoniae in terms of susceptibility testing and serotype determination to inform vaccine-related decisions and antibiotic guidelines [69–71].

Options for Moving Forward
Faced with the realities of current practice, it does not seem likely that the historic standards are to be resurrected except as isolated examples. Two developments are seen as having the potential to move the issue forward:

1. Point-of-care testing on the basis of the CLIA waiver for low-complexity tests that do not require laboratory-trained personnel, which would permit the diagnosis of most infections due to Legionella, most influenza cases [57], and many S. pneumoniae infections. Antibiotic selection could be improved on the basis of the adoption of penicillins as preferred options for pneumococcal pneumonia [71] and data suggesting that Legionella is the only “atypical agent” of pneumonia in adults that requires specific therapy [72]. It should be noted that this type of CLIA-waived diagnostic testing is standard practice with the rapid tests for human immunodeficiency virus used in thousands of emergency departments and clinics with millions of patients in the United States and in the world [73]. Preliminary data have been reported with favorable results using clinical staff to read the Binax NOW rapid test for pneumococcal pneumonia [74].

Expanded use of point-of-care tests might notably improve the diagnostic yield for the 2 bacterial pathogens that can be detected with currently available tests, although it must be acknowledged that these tests are inadequate for detecting Legionella other than L. pneumophila serogroup 1 and the yield is disappointingly low in adults with nonbacteremic pneumonia and possibly too nonspecific in pediatric patients. The same limitations may apply to rapid tests for influenza. The major problems are the limited menu and inadequate sensitivity, but, more importantly, the specificity is good.

2. The important long-range goal should be the use of molecular diagnostics that are now in evolution toward clinical use. Available data suggest that currently available methods can detect virtually all clinically important pathogens with extraordinary sensitivity using nucleic acid detection methods, such as PCR [48, 75–79]. These have the ideal properties for speed and sensitivity and seem ideal for detecting pulmonary pathogens that do not colonize the airways, such as most viruses, agents of bioterrorism (B. anthracis, F. tularensis, and Y. pestis), presumably the 3 “atypical agents,” M. tuberculosis, endemic fungi, and possibly others. It is likely that this will become a diagnostic standard for detection of C. pneumoniae and M. pneumoniae and will finally provide some definitive data on the role of these somewhat mystic agents for which there are no FDA-cleared tests and great variation in yield in different studies using serology. A major problem with most of the common bacterial pathogens is that they are important to recognize but often colonize the upper airways to give false-positive results. Examples include S. pneumoniae, H. influenzae, S. aureus, Enterobacteriaceae, and pseudomonads. In these cases, the distinction may be facilitated by quantitation based on clinical correlates and the principles reviewed above [60].

Limitations to these developments in molecular diagnostics in pulmonary infections include (1) cost, (2) lack of adequate specimens from the respiratory tract in many patients, (3) lack of standards to validate results, (4) limited data on quantitation thresholds to define significance, (5) interpretation when there

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are multiple potential pathogens, (6) lack of antibiotic sensitivity data on pathogens, and (7) lack of realistic application for facilities that outsource laboratory services or have limited resources, night coverage, and so forth [77, 79].

Despite these limitations, it seems clear that the technology to make this advance is currently available, a commercially available test to detect 18 respiratory viruses has been cleared by the FDA [80–82], and molecular methods are also available to detect and quantitate all known respiratory bacterial pathogens [83]. Furthermore, there are encouraging preliminary data. A recent report from Karolinska University (Stockholm, Sweden) on the use of multiple diagnostic tests including qualitative PCR in 124 patients with CAP showed a likely pathogen in 89% of patients for whom all diagnostic tests were performed [84]. Of note, there were 43 patients (35%) with 2 or more pathogens. Given the reality that empiric selection based on current guidelines usually works, it is likely that this development may be painfully slow and possibly prioritized to patients who are seriously ill, nonresponsive to empiric antibiotics, or involved in an unexplained epidemic. The detection of 2009 influenza A (H1N1) is an example of the latter [81].

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References

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