Developing Molecular Amplification Methods for Rapid Diagnosis of Respiratory Tract Infections Caused by Bacterial Pathogens

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Current diagnostic methods for bacterial respiratory tract infections are slow and often of marginal value for patient management if the adequacy of the specimen is not confirmed before culture. Molecular amplification tests, which are highly sensitive, can provide results in hours rather than days but may not distinguish colonization from infection unless a quantification step is included. Defining the reference method to be used for evaluating a novel molecular assay, with input from the US Food and Drug Administration (FDA), is critical before initiating development of a potential product. Although expectorated sputum may be the clinician’s specimen of choice for testing because of ease of collection, the poor quality of such specimens may pose problems for clinical trials of novel amplification tests. There are still many gaps in our understanding of the interplay between colonization and infection and of the role that amplification tests may play in guiding anti-infective therapy. Thus, the performance parameters of a new diagnostic method should be closely matched to a precisely defined intended use statement.

Developing accurate methods for diagnosing bacterial respiratory tract infections has long been a challenge for the clinical microbiology laboratory [1, 2]. The semi-quantitative culture methods used in most clinical microbiology laboratories today, although adequate for recovering and identifying a wide variety of bacterial species from respiratory specimens, cannot differentiate between colonization and infection, especially when the majority of specimens submitted for testing either are contaminated with upper respiratory tract flora or are simply saliva, as shown by Gram stain [2, 3]. In fact, a Gram stain of expectorated sputum can be used both to ascertain the quality of a specimen and to guide empirical therapy [4], but the closing of satellite laboratories where residents, fellows, and attending physicians could perform and interpret their own Gram stains to aid in rapid decision making for patient management has significantly lengthened the time it takes for physicians to receive results [1, 5]. Recognizing infection in a ventilated patient is even more of a challenge, because colonization of the respiratory tree with a succession of gram-negative bacteria is common during prolonged hospital stays, and most of the species recovered from endotracheal aspirates would typically be considered true pathogens if recovered from the bloodstream [6]. Thus, many physicians feel compelled to initiate empirical antimicrobial therapy on the basis of culture results from endotracheal aspirates [7]. The key reason for performing bacterial culture is to optimize anti-infective therapy, which hinges not only on identifying the causative agent of infection in a timely manner but also on determining its antimicrobial susceptibility profile as well. In this age of antimicrobial resistance, the current turn-around times (ranging from 48 to 96 hours to complete testing) are often inadequate for optimal patient care [6, 8]. With the ever-expanding array of multidrug-resistant pathogens, including *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Acinetobacter* species, getting anti-infective therapy for bacterial pneumonia correct the first time is critical for
positive patient outcomes [4, 6, 7]. Thus, to many physicians, the rapid and accurate identification of bacterial respiratory pathogens, particularly for hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP), has become an unmet clinical need.

In 2005, the disease management guidelines published by the European Respiratory Society noted the potential role of molecular amplification methods in improving the diagnosis of lower respiratory tract infections [8]. Although many physicians find the potential for enhanced sensitivity and more rapid turn-around times attractive, there are both challenges and barriers that industry will face in developing and marketing molecular amplification tests for bacterial respiratory pathogens. This article will review those challenges and barriers.

CURRENT DIAGNOSTIC METHODS FOR RESPIRATORY TRACT INFECTIONS

The current semiquantitative agar plate-based culture method used for analyzing expectorated sputum from patients with suspected community-acquired pneumonia (CAP) or HAP is slow and results may be misleading, particularly if a Gram stain is not performed in parallel to ascertain specimen adequacy [6, 8]. Expectorated sputum samples that contain >10 squamous epithelial cells and <25 polymorphonuclear leukocytes per low power field are likely to contain saliva and not lower respiratory tract material from the site of infection [2]. Unfortunately, however, only 32% to 76% of sputum samples obtained from hospitalized patients recently admitted with severe CAP met the above acceptance criteria, according to a recent meta-analysis of published CAP studies [8]. Even if the sputum sample is deemed to be adequate using Gram stain criteria, the culture may still not yield an obvious pathogen [2, 8]. The highest predictive value for a culture of expectorated sputum occurs when Gram stain shows a predominant morphotype (e.g., gram-positive lancet-shaped coci in pairs) and the culture yields predominant growth of a single recognized respiratory pathogen of that morphotype (e.g., Streptococcus pneumoniae) [9]. Unfortunately, such concordance decreases rapidly when specimens are collected after initiation of antimicrobial therapy [9] or when their arrival at the microbiology laboratory is significantly delayed. On the other hand, for aspiration pneumonia, which is often caused by a mix of anaerobic bacterial species, a Gram stain of expectorated sputum is often the only diagnostic test of value, because sputa are not cultured anaerobically and aerobic cultures will yield only mixed normal respiratory flora.

The other issue that impacts patient care is the slow turn-around time of the test procedure. When antimicrobial susceptibility testing is included, the culture method requires a minimum of 48 hours to complete. This is often too late to be effective in guiding anti-infective therapy, especially when the bacterial pathogen is multidrug resistant. Urine antigen tests for pneumococci and Legionella pneumophila can provide rapid results, but the available commercial tests have variable sensitivity (range 66%–92% [4, 10]) and antigen tests for L. pneumophila are limited to serogroup 1 [10].

CHALLENGES FOR DIAGNOSTIC TEST MANUFACTURERS

One approach that may improve the diagnosis of respiratory tract infections and shorten the time necessary to place patients on appropriate therapy is the use of nucleic acid amplification methods. Real-time polymerase chain reaction (PCR) assays [11–13], pyrosequencing [14, 15], and nucleic acid arrays [16] are all options for improving the speed and accuracy of laboratory results. The key challenge for industry is to develop assays that are not only rapid but also readily accessible and perceived by either laboratories or health care systems as cost-effective. Development of an assay that is rapid but unavailable on evening or night shifts or on weekends because of its technical complexity limits the medical value of the test. From the industry perspective, cost-effectiveness should be determined not only by comparison to costs of performing slower conventional methods in the laboratory but also by consideration of the cost savings achieved from optimized antimicrobial therapy, decreased use of additional diagnostic tests, and shorter hospital stays.

To have a positive impact on patient management, molecular amplification tests will need to provide clear, definitive results that will give physicians the data necessary to start, or in some cases withhold, antimicrobial agents [6]. To be successful, industry must determine (1) which combination of molecular targets and clinical specimens will produce results that will effectively guide anti-infective therapy regimens for patients with pneumonia or other respiratory tract diseases; (2) which reference method will provide the most meaningful results for test development and clinical trials; and (3) which assays companies will be able to afford to develop, given both the costs of development and clinical trials and the ever-changing reimbursement schedules from both private insurers and the federal government. The potential return on investment is a key factor when industry chooses which assays to develop. Input from the US Food and Drug Administration (FDA) regarding each of these determinations can be helpful and should be sought. The development of novel diagnostic assays, especially when the reference methods are not obvious, is certainly one of the major challenges facing the industry.

CHOOSING MOLECULAR TARGETS

Molecular assays may target either a single pathogen, such as Mycobacterium tuberculosis [17], or multiple respiratory...
pathogens in a single assay. Several multiplex assays for viral respiratory pathogens have been described [12, 13, 16], a few of which have been commercialized and have received FDA clearance. There are merits to both single-pathogen and multiplex approaches.

**DETECTION OF A SINGLE BACTERIAL SPECIES**

Certain bacterial respiratory pathogens cause such distinct clinical syndromes that assays that target them individually still have clinical utility. These include organisms such as *M. tuberculosis*, *L. pneumophila*, and *Bordetella pertussis*. Molecular assays have been developed for all of these organisms, although for the sake of brevity, discussion will be limited to the issues surrounding the development of assays for *M. tuberculosis*, where there is little doubt that detection of the organisms is highly associated with disease.

**RAPID DETECTION OF MYCOBACTERIUM TUBERCULOSIS**

The World Health Organization has reported that in 2008 there were an estimated 9.4 million new cases of tuberculosis worldwide [18]. Although the overall number of cases of tuberculosis is decreasing in the United States, the proportion of cases among foreign persons is steadily increasing [19, 20]. Of particular importance from a diagnostic point of view is the fact that in more than 26,000 cases of tuberculosis diagnosed in US-bound refugees and immigrants, the acid-fast stains of expectorated sputum samples from the patients yielded negative results. Such infections are likely to be missed by tuberculosis screening programs that rely primarily on acid-fast staining of respiratory secretions instead of chest x-rays and slower culture methods [19]. In addition, infections among foreign-born persons are more likely to be caused by multidrug-resistant *M. tuberculosis* strains than are cases among US domestic persons [20, 21]. Thus, particularly from the standpoint of immigrant and refugee screening programs, there is a need for rapid tests that have adequate sensitivity for detecting *M. tuberculosis* among smear-negative samples. Although molecular diagnostics have been used for direct detection of *M. tuberculosis* in clinical samples for more than a decade, these assays typically show unacceptably low sensitivity levels for smear-negative specimens [17]. However, a recently released PCR-based commercial assay (which is currently available only outside of the United States) showed sensitivity of >90% on smear-negative culture-positive samples in a multicountry, multicenter study [22]. Assays that incorporate direct detection of resistance markers in addition to direct detection of *M. tuberculosis* DNA targets are important for guiding anti-tuberculosis therapy, particularly in an era of multidrug-resistant and extensively drug-resistant strains [11, 23, 24]. The current drawback to developing and marketing tests for *M. tuberculosis* in the United States is that tests for this organism are classified by the FDA as Class III (ie, having high impact on patient management). Thus, molecular tests to detect the organism require what is known as a Pre-Market Approval (PMA) application rather than the less rigorous, but still technically demanding and costly, Pre-Market Notification (510(k)) submission. In a PMA submission, the company must independently show that its device is safe and effective in the context of its intended use. This includes demonstrating through carefully conducted clinical trials that the results of the tests are highly accurate compared with a robust reference method and that the company has designed and implemented sufficient controls of its manufacturing process (including product design, production, and quality control) to assure the FDA that the method or device can be manufactured reproducibly. An FDA Expert Panel is typically convened to review the data to further assure the safety and effectiveness of the device. By contrast, a 510(k) submission requires only that the company demonstrate through clinical trials that its device or method is “substantially equivalent” to a “predicate device or method”—that is, one that previously has been cleared by the FDA for use in the United States. Although early molecular probe assays for *M. tuberculosis* did not require a PMA, the reclassification of *M. tuberculosis* tests as Class III under current regulations poses a significant barrier to test development and marketing in the United States primarily because of the complexity, breadth, and cost of the clinical trials required. Nucleic acid amplification tests for *M. tuberculosis*, including at least 2 that simultaneously identify antimicrobial resistance markers [11, 23], are widely available outside of the United States. However, although there are published data describing the performance of the GenProbe Amplified *M. tuberculosis* Direct (MTD) test, the Roche Amplicor MTB test, the Cobas Amplicor test, the Abbott LCx test, and the BD-ProbeTec Strand Displacement Amplification (SDA) test for detection of *M. tuberculosis* in clinical samples, as noted by Ling et al [25] in their recent meta-review of nucleic acid amplification testing methods, the GenProbe MTD is now the only FDA-cleared test available in the United States. The paucity of molecular diagnostic options for *M. tuberculosis* detection in the United States may reflect both real and perceived regulatory and market-related barriers that are substantial enough to keep many in the diagnostics industry on the sidelines.

**DIRECT DETECTION OF MULTIPLE ORGANISMS IN RESPIRATORY TRACT SAMPLES BY SYNDROME**

Potential multiplex assays for respiratory tract disease caused by bacterial pathogens include those for CAP, HAP, VAP, acute
bronchitis, sinusitis, exacerbation of chronic obstructive pulmonary disease (COPD), and aspiration pneumonia. In designing assays, it is critical to understand whether an assay for a limited number of bacterial pathogens will meet physicians' needs and provide adequate data for initiating or altering antimicrobial therapy. The targets chosen for molecular assays are usually the key pathogens likely to be present in respiratory samples. However, in this age of multidrug resistance, expanding the target selection to include key antimicrobial resistance genes that would alter existing therapy or guide empirical therapy should also be considered. Potential groups of targets for respiratory tract samples obtained from patients with CAP, HAP, or VAP are presented in Table 1. The addition of resistance gene targets, particularly those that mediate carbapenem resistance (ie, resistance to doripenem, ertapenem, imipenem, and meropenem), may be very useful, especially because carbapenems now play a prominent role in empirical therapy of both HAP and VAP [6, 8]. For example, the emerging Klebsiella pneumoniae carbapenemase (KPC) β-lactamases (encoded by blαKPC genes, which mediate resistance to penicillins; cephalosporins, including extended-spectrum cephalosporins; and carbapenems) [26–28] can compromise therapy for infections caused by K. pneumoniae, Enterobacter aerogenes, and other species of Enterobacteriaceae [29, 30], whereas the metallo-β-lactamases, IMP and VIM (encoded by the blαIMP and blαVIM genes, respectively) that mediate resistance to all β-lactam drugs including carbapenems (with the exception of the monobactams), can compromise therapy for Pseudomonas aeruginosa infections [30]. Specific OXA β-lactamases (eg, OXA-40, which also mediates resistance to extended-spectrum cephalosporins and carbapenems) can also compromise therapy for Acinetobacter infections [31]. For gram-positive pathogens, the presence of mecA in samples positive for S. aureus suggests that vancomycin (or perhaps linezolid), either alone or in combination with other anti-infective agents, is necessary. If physicians have confidence in the results of molecular assays, then negative results for a specific pathogen should prompt physicians to withhold treatment for that infectious agent (eg, to withhold therapy for suspected methicillin-resistant Staphylococcus aureus [MRSA] if the molecular assay for MRSA is negative). Of course, laboratories may still culture respiratory specimens that are positive by molecular methods to recover the pathogen to perform standard antimicrobial susceptibility tests on organisms obtained in pure culture. Standard susceptibility testing provides a broader susceptibility profile that extends beyond the few antimicrobial resistance genes likely to be included in a molecular assay.

### RESPIRATORY SAMPLES FOR ANALYSIS

A variety of respiratory samples are amenable to molecular testing, including expectorated sputum, bronchoalveolar lavages (BALs), protected bronchial brushes, and endotracheal aspirates. Of these, expectorated sputum samples are by far the most common respiratory samples submitted to the clinical microbiology laboratory but are also the poorest in overall quality. As the Infectious Diseases Society of America/American Thoracic Society CAP guideline notes, one of the problems with diagnostic tests for respiratory tract infections “is driven by the poor quality of most sputum microbiological samples and the low yield of positive culture results” [4]. Getting specimens from the site of infection that are not contaminated with upper respiratory tract flora is a constant problem [3, 6, 32]. Endotracheal aspirates from ventilated patients are often of better quality, particularly when obtained in pure culture.

### Table 1. Potential Targets for Multiplex or Individual Molecular Amplification Assays by Syndrome [2, 4, 6, 8, 41]

<table>
<thead>
<tr>
<th>CAP/exacerbations of COPD</th>
<th>HAP/VAP</th>
<th>Individual organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Staphylococcus aureus</td>
<td>Mycobacterium tuberculosis</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>mecA gene^</td>
<td>Bordetella pertussis</td>
</tr>
<tr>
<td>blαTEM gene^b</td>
<td>Pseudomonas aeruginosa</td>
<td>...</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>blαVIM, blαIMP genes^c</td>
<td>...</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Acinetobacter spp</td>
<td>...</td>
</tr>
<tr>
<td>mecA gene^d</td>
<td>blαOXA genes^d</td>
<td>...</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>Enterobacteriaceae</td>
<td>...</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>blαKPC gene^e</td>
<td>...</td>
</tr>
<tr>
<td>Chlamydia psittaci</td>
<td>Stenotrophomonas maltophilia</td>
<td>...</td>
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<tr>
<td>Legionella pneumophila</td>
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**NOTE.** CAP, community-acquired pneumonia; COPD, chronic obstructive pulmonary disease; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia.

^ Mediates resistance to all β-lactam agents with the exception of the novel anti-methicillin-resistant S. aureus cephalosporins.

^b Mediates resistance to penicillins and first-generation cephalosporins.

c Mediates resistance to cephalosporins and carbapenems; metallo-β-lactamases, such as VIM and IMP, typically do not mediate resistance to monobactams.

d Some OXA β-lactamases can mediate resistance to carbapenems.
quality than that of expectorated sputum from patients with HAP but may still be contaminated with upper respiratory tract flora [6, 32]. BALs and protected brush samples are more likely to yield samples from the site of infection but require significantly more effort to obtain and thus offer a much smaller market for a new molecular test. In fact, there is a significant gap in our knowledge as to how well molecular tests for bacterial pathogens would perform on expectorated sputum samples, compared with performance on BALs or protected brush samples from the same patient collected within a similar period. This knowledge gap is also a barrier to test development, because a molecular test that cannot be performed on expectorated sputum (given all the problems with specimen quality) may not have broad enough appeal among physicians to make it a financially viable product (from the industry perspective).

**CHOOSING A REFERENCE METHOD FOR TEST DEVELOPMENT AND CLINICAL TRIALS**

Choosing a reference method for both test development and clinical trials again highlights the issue of the ability of multiple bacterial species to colonize and to infect the respiratory tract [4, 32]. Antimicrobial stewardship programs frequently stress the need to treat infections but to withhold antimicrobial agents from patients who are asymptptomatically colonized [6, 33]. The enhanced sensitivity of molecular methods is essentially negated if physicians perceive the test as “overly sensitive,” detecting colonization in the absence of infection. Balancing sensitivity and specificity (and by analogy positive and negative predictive values) for molecular tests is another example of a critical gap in our understanding of the best manner in which to apply molecular diagnostics. Is quantification of the targets the answer for molecular tests? A recent Cochrane report concludes that the use of quantitative cultures did not improve the outcomes of patients with VAP, compared with the outcomes of patients who were managed with qualitative results [34]. However, the decreased turn-around time of molecular test results may well prove advantageous over the two culture methods (ie, qualitative and quantitative) that produced results in similar, and significantly slower, time frames.

To illustrate this point, a brief review of the semiquantitative culture method for expectorated sputum is appropriate. Assessments of bacterial growth are performed on a series of agar plates without an enrichment step. The constellation of growth media usually includes blood agar, chocolate agar (for fastidious organisms), MacConkey agar (or a similar selective medium for gram-negative organisms), and colistin-nalidixic acid agar (or a similar selective medium for gram-positive pathogens). Results such as rare (often recorded as “one plus”) growth of pneumococci mixed with other respiratory flora from an expectorated sputum sample on blood or chocolate agar would likely be reported as “mixed respiratory flora” (often with no specific mention of pneumococci on the report), whereas heavy growth of pneumococci on the same agar plate may be reported as “four plus growth, *S. pneumoniae*.” Although both samples contain *S. pneumoniae*, in the former example, the organism is regarded as normal flora and is not reported, whereas in the latter it gains “pathogen” status and is reported. It is likely that both samples would yield a positive molecular amplification test result. Thus, quantification of the amount of target present with a defined threshold value that correlates with infection rather than colonization may be necessary to provide confidence in the molecular results [4, 32]. This adds complexity to the test development process (because the test result is not simply the presence or absence of the target organism) but is technically feasible. High concentrations of target organism sequences recognized through the amplification process are highly suggestive of infection, but can one trust the absence of amplification to be an indication of lack of infection if there was no initial assessment of the specimen to ensure that it was of adequate quality? Will it be necessary to include some host cell marker to indicate that the specimen originated from the site of infection and is not saliva? Although Gram stains of specimens are critical for interpretation of culture results, requiring a Gram stain of a specimen before performing a molecular assay would defeat the purpose of having a rapid molecular test, especially one that may ultimately be used outside of the clinical microbiology laboratory as a “point of care” test [10]. Once again, we have identified a gap in our knowledge that is critical to assay development. It is imperative, particularly for clinical trials of novel molecular assays, that the end points that represent positive results for each bacterial species be established, especially for organisms that can both colonize and infect the respiratory tract. Quantification would not be required for organisms such as *M. tuberculosis* or *B. pertussis*, because the presence of these organisms is synonymous with disease. The issue of whether quantitative bacterial cultures would be necessary for clinical trials must be answered. Appropriate cut-offs for quantitative culture are debated, but several sources suggest that the following numbers of organisms of a single bacterial species indicate infection and not colonization: for expectorated sputum, >1 x 10^7 colony forming units (CFU)/mL; for endotracheal aspirates, >1 x 10^6 CFU/mL; for bronchoalveolar lavage, >1 x 10^5 CFU/mL; and for protected brush specimen, >1 x 10^5 CFU/mL [6, 32, 35]. It likely will be necessary to evaluate the semiquantitative analysis of expectorated sputum and quantitative methods (such as with BAL specimens) in parallel to indicate whether a molecular amplification assay could be used successfully on expectorated sputum specimens. Because molecular assays are more sensitive than culture, one may ask whether enrichment broths should be required to maximize detection by culture during clinical trials. However,
there are no defined enrichment broths for the majority of bacterial respiratory pathogens; thus, this is not likely to be of value.

ASSAY DEVELOPMENT—WHAT IS THE GOLD STANDARD FOR BACTERIAL IDENTIFICATION?

One question that arises when using amplification methods during both test development and clinical trials is whether the amplification products truly represent the target organism sequences. To establish this, it is often necessary to determine the nucleic acid sequence of a sample of the amplification products for the targeted pathogens. DNA sequencing is often assumed to be completely accurate, showing 100% sensitivity and specificity (particularly by non–molecular biologists). However, because of a variety of technical factors, including the quality of the sequencing template (ie, the amount of contaminating DNA from other sources), the selection and quality of the primers used for amplification and sequencing, the robustness of the base-calling software, and the method for compiling the “consensus sequence” from multiple forward and reverse reactions, sequencing does not always represent the ultimate reference standard [36–38]. Nonetheless, companies must now consider what measures of sequence quality (eg, using the quality scores obtained by means of the “Phred” and “Phrap” computer programs [37, 39]) would constitute acceptable standards if sequencing were included in clinical trial design. For example, for individual sequencing runs, a length of sequence that has <100 cumulative “Phred 20” quality score bases (where the Phred software estimates the statistical likelihood of an inaccurate base determination) is often considered to be unacceptable, because it represents a probability of error level of 1% [40]. On the other hand, using another software program, a “Phrap” quality score of 40, which is the quality score for the consensus sequence assembled from multiple reads of a DNA sequence (equivalent to an error rate of ~1 base for every 10,000 bases sequenced), is often considered acceptable—that is, of publication quality (http://www.phrap.com/background.htm). Such key parameters of quality have yet to be promulgated by regulatory agencies, although sequencing data have been included in multiple 510(k) submissions. Such guidelines would be of value to diagnostic test developers as they contemplate beginning clinical trials of novel diagnostic products.

SENSITIVITY AND SPECIFICITY, POSITIVE, AND NEGATIVE PREDICTIVE VALUES

The key parameters that define the accuracy of a novel test, compared with the accuracy of the reference method, include sensitivity, specificity, and positive and negative predictive values. At present, there is no consensus as to which of these parameters is most critical for potential molecular assays for respiratory pathogens. Is a highly sensitive test that rarely misses the presence of a given pathogen in a respiratory sample desirable, or is a test with a high negative predictive value indicating the absence of a given pathogen of greater value? The former scenario indicates that an antimicrobial agent is likely necessary, whereas the latter indicates that antimicrobial agents can be withheld. Although both high sensitivity and high specificity rates are desirable (and are sometimes achievable in an assay), the predictive values depend on disease prevalence and cannot be manipulated by assay design. Thus, it is critical to define the intended use of the assay before initiation of any clinical trials. This is best accomplished in consultation with representatives of the appropriate FDA center. Other issues that arise concerning the design of clinical trials include the age ranges, ethnicity, and clinical severity of the populations to be studied. Will trials need to be conducted in both high prevalence and low prevalence populations, stratified not only by age but also by gender and even socioeconomic group? The broader and more diverse the study population is, the larger the cost of conducting the trial. Clearly, such clinical trials, especially if conducted in accordance with the guidelines of a PMA, can become prohibitively expensive rather quickly. Thus, the intended use of the assay is perhaps one of the most critical decisions industry needs to make before any development is initiated.

A POSSIBLE VAP ASSAY

To illustrate the above issues, a possible molecular amplification assay for VAP is described. The intended use for the test is as a diagnostic aid where the results of the test are considered in association with other laboratory and clinical information to establish a diagnosis of VAP. Whether the intended use includes the ability of the test to guide anti-infective therapy has to be considered carefully (eg, can this aspect of the test be proven in a clinical trial?). The target organisms are S. aureus, P. aerugino- nosa, Acinetobacter baumannii, a DNA sequence common to all Enterobacteriaceae, and target sequences that are highly specific for the following antimicrobial resistance determinants: mecA, blakPC, blakIMP, blavIM, and blaOXA. The target specimens are protected bronchial brush samples and endotracheal aspirates. The reference methods include quantitative cultures on blood chocolate, MacConkey, and CNA agar; a Gram stain of the specimen; and a clinical diagnosis of VAP (fever, leukocytosis, and purulent respiratory secretions) supported by radiologic findings [32]. If a high degree of correlation between culture results, Gram stain results, molecular test results, and clinical diagnosis are achieved in alpha trials, beta trials could be conducted to gather information on the potential cost effectiveness (and physician acceptance) of the assay. If the assay results are promising, a molecular test that ultimately could be performed
on sputum samples from patients with HAP (rather than VAP) may be feasible. Tests for CAP would require a different selection of targets.

**SUMMARY**

There are many challenges to developing molecular tests for bacterial respiratory pathogens, particularly organisms that can be both asymptomatic colonizers and overt pathogens of the respiratory tract. Defining the reference method and the intended use of the product before initiating clinical studies is critical. Input from the FDA also is highly desirable. Although expectorated sputum may be the clinician’s specimen of choice because of ease of collection, the poor quality of such specimens may require development of a unique host cellular target to ensure specimen adequacy. Molecular amplification assays will be costly to develop and clinical trials will be expensive, yet the need is great. There are still many gaps in our understanding of the interplay between colonization and infection. The key question industry must answer is: “Is the juice worth the squeeze?”

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