Lower respiratory tract infections (LRTIs) produce between 5 and 10% of all deaths reported to the CDC via the 122 Cities Mortality Reporting System (5). The clinical laboratory plays a vital role in the diagnosis of these infections but faces numerous challenges due to the complexity of LRTIs, including specimen quality and diversity; contamination of specimens with oropharyngeal flora; a diverse pathogen population that includes bacteria, viruses, and fungi; and the complex pathophysiology of respiratory tract infections, especially in special populations. Five current questions in the clinical microbiology (CM) of LRTI were discussed:

1. What is the real value of the Gram stain of expectorated sputum?
2. What is the value of quantitative culture techniques on bronchoalveolar lavage (BAL) and mini-BAL specimens, endotracheal (ET) aspirates, and transbronchial biopsy specimens?
3. What is the role of the clinical microbiology lab in the diagnosis of acute exacerbations of chronic bronchitis (AECB)?
4. How do we optimize the microbiological evaluation of cystic fibrosis (CF) patients with exacerbations?
5. What is the optimum laboratory evaluation of patients with possible pulmonary tuberculosis?

There were discussants from both industry and clinical practice and from a wide variety of clinical practice settings. Surprisingly, there were comparatively few areas of controversy or serious disagreement.

THE SPUTUM GRAM STAIN

The sputum Gram stain, a standard procedure in clinical microbiology, is used for assessment of specimen quality, for preliminary, rapid diagnostic information, and for laboratory quality assurance.

Several systems are used to assess specimen quality using the sputum Gram stain. A number of quantitative criteria have been developed to screen for specimen quality, all of which are based on the premise that an abundance of squamous epithelial cells is indicative of superficial oropharyngeal contamination (18). Samples judged by Gram stain to consist predominantly of upper respiratory tract material are rejected for routine bacterial culture. The Gram stain in this case has two functions: cost-effectiveness and avoidance of reporting of misleading information to the clinician, which may result in misdiagnosis, leading to either misguided or unnecessary treatment. Reporting of misleading clinical information is also avoided by rejecting sputa for culture that is contaminated with upper respiratory flora because many of the potential pathogens which cause pneumonia can also colonize the upper respiratory tract.

The value of the sputum Gram stain for preliminary diagnosis of respiratory disease is well established. Of significance, criteria for interpretation and reporting of microorganisms in Gram-stained smears of lower respiratory tract (LRT) secretions are variable. In addition, recommendations have been made that sputum culture results be correlated with direct Gram stain results (9, 11, 19) in order to provide more clinically relevant information in light of the limitations of culture. Finally, the Gram stain is a useful tool in laboratory quality assurance. Comparison of Gram stain and culture results can reveal errors in procedure, specimen collection and/or transport issues, or specimen identification and tracking errors.

Discussion. The discussion centered on making the sputum Gram stain an even more valuable clinical tool by reporting more clinically useful information than currently reported in most laboratories; see Table 1 for examples. Because such guided reporting carries some risk of misdirecting providers, guideline development aimed at supporting laboratories in consistent, evidence-based reporting schemes would be valuable.

QUANTITATIVE CULTURES

The microbiological assessment of pulmonary samples obtained during bronchoscopic procedures is complicated not only by the diversity of patients who undergo such procedures and the variety of specimen types obtained but also by the presence of colonizing flora, particularly in intubated patients. Current quantitative culture procedures vary by specimen type. In general, colony counts of <10⁴/ml suggest contamination, counts of 10⁴ to 10⁵/ml represent a gray zone result, and counts of >10⁵/ml of a major isolate suggest a potential pathogen. Bronchial brushings are placed in 1 ml of saline, and then quantitative culture is performed by plating 10 µl of the material. Counts of >10⁴ CFU/ml correlate with disease in suspected pneumonia (20).

Discussion. Although quantitative culture standardizes the laboratory assessment of these valuable, complex samples, the discussants emphasized significant problems: (i) the methods are labor-intensive, (ii) collection methods, including the vol-
ume of saline used in the procedure, are not standardized; and (iii) the time of specimen transport to the laboratory, especially from referral sites and outpatient procedure centers, frequently impacts the subsequent organism load in the sample. Significantly, the studies on which our current quantitative culture practices are based are approximately 2 decades old and fail to reflect today’s patient populations, particularly the large number of solid organ and bone marrow transplant recipients undergoing bronchoscopy. The group recommended that new studies assessing the performance and interpretation of quantitative cultures of bronchoscopic specimens, with standardization of both the collection and microbiological analysis, in specific patient populations are necessary to document the value of these labor-intensive procedures.

WHAT IS THE ROLE OF THE CLINICAL MICROBIOLOGY LAB IN THE DIAGNOSIS OF AECB?

The chronic bronchitis syndrome consists of impaired exercise tolerance, dyspnea on exertion, and chronic productive cough without a specific identified etiology. Patients are usually older with a significant smoking history and may have other comorbidities. They are at risk for severe pneumonias, ischemic cardiac disease, and other illnesses, some of whose symptomatologies overlap significantly with acute exacerbation of chronic bronchitis (AECB).

AECB is a poorly understood condition. Clinically, it consists of an acute increase in symptoms beyond normal day-to-day variation, typically in one or more of the main symptoms of (i) increased frequency and/or severity of cough, (ii) increases in volume and/or change in character of sputum production, and (iii) increased dyspnea.

While 70 to 80% of cases of AECB are infection related, it is unclear whether microbiological laboratory diagnosis contributes significantly to management of the syndrome. The laboratory is rarely informed that a specimen is from a patient with AECB. Potential bacterial pathogens, such as Haemophilus influenzae, Streptococcus pneumoniae, and Moraxella catarrhalis, all of which can contribute to exacerbations, are also isolated from patients between exacerbations. Although these organisms most likely contribute to the underlying pathology, their isolation from sputum in the setting of an exacerbation is of little or no significance. Similarly, organisms such as Staphylococcus aureus, though infrequently isolated, may have little to do with the exacerbation when found (17). AECB is strongly associated with acquisition of new strains of some of these organisms, but strain typing is currently expensive, slow, and limited in availability. Antibiotics improve outcomes in AECB, particularly in the sickest patients, but therapy is empirical rather than pathogen driven. Respiratory viruses, including rhinovirus, influenza virus, parainfluenza virus, coronavirus, adenovirus, respiratory syncytial virus, and human metapneumovirus, are frequently associated with AECB; however, therapeutic options are limited, with the exception of modestly effective therapies for influenza. Thus, the value of viral testing is also dubious. On the face of it, it appears that the laboratory has little role in managing chronic obstructive pulmonary disease (COPD), and cost-effective practice would involve limiting microbiological workup. The 2007 Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines and 2001 guidelines from the American College of Physicians state that sputum cultures should not be performed during most exacerbations of COPD.

Discussion. Some laboratory tests show value in AECB. Testing for respiratory syncytial virus has prognostic significance, and for patients admitted to the hospital, results of viral diagnostics have infection control significance. It might be expected that detection of a viral etiology would allow discontinuation of antibiotics, but critically, the safety of this approach is yet unproven by clinical trials.

A barrier to limiting laboratory use is that the diagnosis of AECB is rarely straightforward. Other illnesses, including pneumonia, enter the differential diagnosis. Of patients with COPD who died within 24 h of admission with a COPD exacerbation, 28% died of pneumonia (21). So, while microbiological testing is of limited value in managing AECB, it may still be incumbent on the laboratory to test sputa from these patients as a part of a comprehensive evaluation that includes other diagnoses, at least in the sickest patients.

It was not felt that sputum culture is overutilized in AECB, but the microbiologists in the session acknowledged that such utilization might not come to their attention.

HOW DO WE OPTIMIZE THE MICROBIOLOGICAL EVALUATION OF CF PATIENTS WITH EXACERBATIONS?

In common with COPD patients, patients with cystic fibrosis (CF) have underlying lung disease which typically results in continual symptoms and important alterations in lung physiology and microbiota (10). Similarly, both environmental and microbial alterations can trigger increased symptoms in patients with CF.
A relatively predictable series of pathogens colonize the respiratory tract of CF patients. Nontypeable *Haemophilus influenzae* and *Staphylococcus aureus* are typically found early in life, with *S. aureus* being one of the most common bacterial pathogens recovered from the respiratory tract of persons with CF (14). On the other hand, the prevalence of infection caused by *Pseudomonas aeruginosa* varies significantly by age, ranging from about 25% for children age 5 years and younger to 80% for adults 25 to 34 years of age (6). Other bacterial opportunists include *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Burkholderia cepacia* complex, *Ralstonia* species, *Cupriavidus* species, *Pandorea* species, and nontuberculous mycobacteria (NTM), particularly rapid growers. In addition to bacteria, fungi such as *Aspergillus* species, *Scedosporium* species, and *Exophiala dermatidis* have been implicated as causes of chronic colonization or infection of the airways (14). A set of evidence-based guidelines for the use of selective media and procedures is available (16). Proper testing of CF specimens is materials and labor-intensive, with testing performed for both symptomatic disease and organism-specific surveillance.

The role of the microbiology laboratory in management of CF patients is complicated by the chronic nature of the illness and by the intricacy of the host-microbe interactions:

- The complex bacterial flora requires selective media and complex protocols for isolation of possible pathogens.
- It is rarely possible to eradicate colonizing pathogens such as *Pseudomonas* and *S. aureus*, although early eradication protocols for *P. aeruginosa* are successful in some patients. The goal of therapy is often clinical improvement rather than eradication of infection.
- Inhaled therapies that achieve high local antibiotic concentrations are employed both for chronic suppression and for treatment of exacerbations.
- Antibiotic pharmacokinetics are altered in patients with CF.
- Unusual or altered strains of common bacteria (e.g., mucoid *P. aeruginosa* [8]) and uncommon pathogens (*Burkholderia cepacia* complex [13], rapidly growing mycobacteria) provide challenges for bacterial identification and for routine antibiotic susceptibility testing.

**Discussion.** There was comparatively little discussion on this topic. Current guidelines, while complex and labor-intensive, are well crafted, evidence based, and effective for the current state of our capabilities in clinical microbiology and management of CF, especially if surveillance cultures can be limited to 4/year, as the guidelines recommend. CLSI is currently developing guidelines for antibiotic susceptibility testing of organisms isolated from CF patients, which should clarify some of the issues, or at least provide standard procedures for further assessment.

**WHAT IS THE OPTIMUM LABORATORY EVALUATION OF PATIENTS WITH POSSIBLE PULMONARY TUBERCULOSIS?**

The tubercle bacillus was described by Koch in 1882, but the diagnosis of pulmonary tuberculosis (TB) is still a complex, slow, uncertain, and expensive process. Optimal laboratory diagnosis requires multiple specimens taken at intervals for acid-fast staining, possibly molecular assays for direct detection of *Mycobacterium tuberculosis*, culture in rapid broth and on solid medium, subsequent identification employing either phenotypic or molecular methods, and where appropriate, antimicrobial susceptibility testing.

The canonical “three sputa rule” for ruling out pulmonary tuberculosis has come under fire from at least two directions. Shorter hospital stays, high hospital occupancy rates, and cost controls drive providers to move patients out of isolation rooms with airborne precautions as rapidly as possible. Increasingly, accessible molecular diagnostics promise better sensitivity than acid-fast smear and microscopy in rapid detection of *M. tuberculosis*, potentially decreasing the number of specimens required (2), but at a significant cost and with a still unproven impact on overall diagnostic yield and public health (15). The latest CDC guidelines (3) recommend that all patients suspected of having pulmonary tuberculosis have at least one sample tested by a nucleic acid amplification test (NAAT).

The development of gamma interferon release assays (IGRAs) has provided a new tool for assessing the TB infection status of patients (1, 3, 4). In contrast to the venerable tuberculin skin test (TST), which requires a carefully performed injection, a return visit, and a highly subjective reading procedure, clinicians need to perform only a simple venipuncture to collect specimens for IGRA. Conversely, from a laboratory perspective, IGRAs are awkward, requiring specialized collection materials and/or time-limited and often labor-intensive specimen processing prior to testing. IGRAs provide some performance advantages compared to the TST, as there is limited cross-reactivity with nontuberculous mycobacteria and none with *Mycobacterium bovis* BCG vaccine strains. Some IGRAs may be more sensitive than the TST, although the lack of a gold standard for latent TB infection makes this determination difficult (7). The relative sensitivity of IGRAs and the TST in immunocompromised populations and in young children is still unclear (12). Also, for individual patients, results of the TST and the two available IGRAs may disagree, further complicating the interpretation of these tests. Like the NAATs for pulmonary tuberculosis, IGRAs provide clinical convenience and some improvement in performance at significant cost to the laboratory.

**Discussion.** None of the discussants in the small group and few in the meeting at large have implemented routine testing by NAAT for all patients with a suspicion of TB, regardless of the acid-fast bacillus (AFB) smear result. Only one NAAT is FDA approved for smear-negative specimens, so access to appropriate testing is an issue, but the major concerns voiced were doubts about the effectiveness of the intervention and the cost to laboratories. Numerous studies have been published on the ability of nucleic acid amplification (NAA) assays to directly detect *M. tuberculosis* complex in clinical specimens. However, drawing definitive conclusions regarding the performance sensitivity, specificity, predictive values, and clinical utility of NAA assays from the myriad of studies is a daunting task. It is a given that the most significant advantage of NAA assays is their rapid turnaround time for the direct detection of *M. tuberculosis*, which may have important implications for patient management and tuberculosis control. However, assessing the performance of these tests on the basis of the current literature
is problematic. More clinical correlative studies are needed so that a better understanding of when and how to use NAATs in conjunction with available clinical information may become evident, such that these assays are utilized in a cost-effective manner with optimum patient management. The performance characteristics of most NAATs performed on smear-negative specimens have not been defined and during routine use might be expected to be poorer than in carefully controlled and limited-duration studies due to accumulating contamination and operator errors. The costs would likely be borne by laboratories, unless provider ordering can be efficiently driven by an electronic medical record or similar system; convincing hospitals to bear those costs is a daunting task. The cost to find one undiagnosed TB patient in most centers will be extremely high. The public health benefits are predominantly theoretical, on the basis of estimates of the fraction of smear-negative patients who would spread tuberculosis. The laboratory is rarely, if ever, informed of the diagnosis, so it is unclear how testing would be confined to patients tested for tuberculosis, as opposed to those in whom the likely diagnosis is NTM infection. This guideline will meet continued resistance from the laboratory community, unless more compelling data are provided, more streamlined and less expensive tests are FDA approved, ordering and utilization can be controlled, and reimbursement is provided. None of these conditions currently applies.

IGRAs clearly have a recognized role in detection of tuberculosis infection in TST-positive, BCG-immunized individuals. However, more clinical data as to their performance are required before conclusions regarding their usefulness in children and various immunocompromised populations can be made. From a laboratory perspective, the role of IGRAs will also require recognition of the increased costs in labor and reagents to the laboratory, as weighed against the ease of use for clinics and improved compliance with screening and exposure management due to elimination of follow-up visits.

SUMMARY OF NEEDS

A number of needs were identified during this session:

- Evidence-based guidelines for interpretive reporting of the sputum Gram stain will allow laboratories to provide accessible, clinically relevant information to guide the management of pneumonia patients.
- Updated studies of the utility and performance of quantitative cultures of lower respiratory tract specimens in current patient populations with standardized sampling and analytical methods are required to document the value (if any) of these procedures.
- Clinical trials are needed to determine whether patients with AECB and in whom a viral agent is detected still benefit from empirical antibiotic therapy; the importance of routine diagnosis of respiratory viral infection in this population is currently underdocumented.
- Standardized protocols for susceptibility testing in CF patients, currently in development, will allow standardization of practice and will support further research on the therapeutic microbiology of CF.

- Outcome and cost-effectiveness studies of the routine employment of IGRAs for employee health and risk-based screening for latent tuberculosis will provide evidence to establish their appropriate use.
- Outcome studies of the public health impact of routine testing by NAATs on AFB smear-negative patients for tuberculosis are necessary to document the value, if any, of this extremely expensive, unfunded intervention.


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