Molecular Detection of *Mycobacterium tuberculosis*: Impact on Patient Care

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**Background:** Nucleic acid amplification technologies such as PCR are revolutionizing the detection of infectious pathogens such as tuberculosis (TB). Amplification technology offers the potential for the diagnosis of TB in a few hours with a high degree of sensitivity and specificity. However, molecular assays neither replace nor reduce the need for conventional smear and culture, speciation, and antibiotic sensitivity assays.

**Methods:** We undertook prospective studies of sputum samples to assess the performance of two PCR-based assays for the detection of TB as well as the impact of more rapid availability of test results on patient care.

**Results:** The sensitivity of both the in-house and Amplicor PCR assays was 100% for smear-positive sputa. For smear-negative sputa (two sputum samples collected during the first 24 h of hospitalization), the sensitivity was 85% for our in-house PCR assay and 74% for the Roche PCR assay. Approximately 10% of the smear- and culture-negative sputa yielded positive PCR results; however, more than one-half of these were positive with both the in-house and Amplicor assays, suggesting the presence of TB DNA or organisms. Several of these came from patients whose other samples grew *Mycobacterium tuberculosis* during the same admission, and others came from patients who had previously treated TB. Overall, the specificities of the in-house and Amplicor PCR assays in smear-negative patients were 86% and 93%, respectively.

**Conclusions:** Molecular detection of slow-growing pathogens such as *M. tuberculosis* have the potential to improve clinical care through a dramatic reduction in the time required for detection and may provide substantial savings in the overall cost of care of a patient compared with conventional smear, culture, and speciation alone, despite the fact that conventional assays must still be performed for speciation of nontuberculous mycobacteria and for full assessment of antibiotic sensitivity.

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Molecular approaches to the diagnosis of disease have begun to have a major influence on current clinical management of various disorders. In particular, detection of human microbial pathogens has been impacted by nucleic acid-based techniques, primarily amplification assays. Numerous amplification-based assays have been developed to offer improvements in the detection of slow-growing or unculturable organisms, or when information not provided by conventional diagnostic approaches is needed. Quantitative measurement of serum or plasma concentrations of several viruses is becoming increasingly useful for monitoring of disease response to treatment. Because of the potential and widespread application of molecular testing in microbiology, several commercial methods have been developed, whereas methods for nonmicrobiologic applications remain few.

However, there are several critical differences between nucleic acid-based testing and conventional microbiology. Nucleic acid-based tests generally target only a single organism, whereas cultures generally are more broad in coverage. Although the molecular assay might rule out a single microbe, continued analysis in culture may be required to detect other relevant organisms. Additionally, antimicrobial resistance studies may be required, also necessitating further analysis in culture. In some cases, molecular epidemiologic studies may also be required to investigate disease spread. Thus, with few exceptions, molecular assays do not negate the need for performance of routine cultures.

One of the most useful applications of molecular testing has been the detection of *Mycobacterium tuberculosis*. Tuberculosis (TB) is one of the major threats to public health worldwide, infecting more than one-third of the world’s population (1). The United States has seen an increase in incidence of TB over the past 1–2 decades, which has been attributed primarily to the effects of HIV, poverty, and homelessness (2). Although the last few years have seen a decrease in TB incidence, the emergence
of drug-resistant strains has generated increased concern over control of this disease. However, *M. tuberculosis* is not the only human pathogen in this genus. Several nontuberculous mycobacteria can cause disease in immunosuppressed patients, primarily *M. avium*, *M. intracellulare*, and *M. kansasii* (3). Clinical presentation and history can help in making a presumptive diagnosis, but ultimately, positive identification and speciation of the organism are required. Treatment regimens differ for the various mycobacterial species, as does the need for isolation.

**Conventional Laboratory Diagnosis of TB**

Traditionally, diagnosis of mycobacterial infection is a two-stage process requiring weeks for completion. For diagnosis of pulmonary infection, patients are initially triaged into isolation if needed based on the results of acid-fast smears of sputum collected at the time of admission and on the next three consecutive mornings (4). Alternatives to the acid-fast smear include several other stains, such as Ziehl-Neelsen, or fluorochrome staining with auramine or auramine/rhodamine, which may be faster to screen. Acid-fast smears are a necessary first step but are neither specific nor sensitive; mycobacteria can be differentiated from other acid-fast organisms such as nocardia, but definitive discrimination of *M. tuberculosis* from other mycobacterial species can be difficult or impossible. Additionally, ~10 000 organisms/mL of sputum are required to yield a positive result (5).

Sputum samples, as well as samples from other sites in patients suspected of having nonpulmonary TB, are also placed into culture. The sample is decontaminated with *N*-acetyl-l-cysteine/sodium hydroxide to prevent overgrowth of the culture by bacteria. Today, the traditional Lowenstein-Jensen solid culture medium has largely been replaced by several liquid culture systems. These commercially available systems improve the speed of diagnosis by continuously agitating the cultures to promote growth, along with continuous monitoring to detect growth of the organism as early as possible. Once growth has been detected, determination of the species is still required. In the past, time-consuming biochemical methods were performed, although most laboratories today have replaced this approach with a commercially available DNA probe method in which the organisms are lysed and hybridized in solution to a species-specific fluorescently labeled nucleic acid probe. Available probes target the most common mycobacterial species and do not differentiate between members of the *M. tuberculosis* complex of organisms (*M. tuberculosis*, *M. microti*, and *M. africanum*, of which only TB is regarded as a human pathogen) is perhaps the most commonly used target for the molecular detection of TB in in-house assays (28–30). Current molecular assays amplify targets specific to these organisms, although possibly a more useful approach would be amplification of a common mycobacterial target, followed by speciation and detection via probe hybridization.

In general, performance of molecular assays is acceptable, although variation in assay performance is observed, attributable not only to the design of the amplification step but also to sample preparation and the detection method. A survey of the literature revealed sensitivities of 70–100%, which vary to some extent based on the presence of acid-fast bacillus positivity in the sputum sediment. In acid-fast bacillus-negative samples, the detection rate of PCR is lower (sensitivity usually 50–65% compared with 85–90% for smear-positive sputa) and is related to the number of organisms present in the sample (8, 9). In samples other than sputum, assay sensitivity has not been systematically studied. Nucleic acid amplification assays have become widely used, however, for the

**Nucleic Acid Amplification Assays for TB**

To provide more rapid diagnosis of TB, many laboratories have turned to nucleic acid amplification. Currently, two Food and Drug Administration (FDA)-approved commercial methods are available for this purpose. Roche offers a PCR-based test targeting a TB-specific portion of the 16S ribosomal RNA gene, along with a colorimetric detection system (7–12). GenProbe markets a method that utilizes transcription-mediated amplification of 16S ribosomal gene transcripts, with product detection performed via chemiluminescence (7, 8, 12). Both of these methods have received approval from the FDA for testing of smear-positive sputum samples, and the GenProbe assay was approved in the summer of 2000 for use on smear-negative sputum samples as well. Other manufacturers are developing molecular assays for TB that use other amplification methods (13–16).

Several laboratories continue to use methods developed in-house for the detection of TB. Great care must be exercised in validation of these assays and in maintaining proper quality control. The primary advantage of these assays, aside from familiarity with their use, includes use on sample types other than sputum. Although commercial methods can also be used to examine nonsputum samples, these are off-label uses with respect to FDA approval. Some laboratories have continued to use in-house assays validated in accordance with Clinical Laboratory Improvement Amendments of 1988 (CLIA), College of American Pathologists, and NCCLS guidelines (17–20). A wide variety of nucleic acid targets have been used in in-house assays, as summarized in Table 1. Targets include single-copy genes (21–27) as well as multicopy targets (28–33) such as repeat sequences, which offer the potential advantage of increased detection sensitivity based on the increased number of copies of the target at the outset of amplification. IS6110, which is present in 10–25 copies in organisms of the *M. tuberculosis* complex group (including *M. tuberculosis*, *M. bovis*, *M. microrni*, and *M. africannum*, of which only TB is regarded as a human pathogen) is perhaps the most commonly used target for the molecular detection of TB in in-house assays (28–30). Current molecular assays amplify targets specific to these organisms, although possibly a more useful approach would be amplification of a common mycobacterial target, followed by speciation and detection via probe hybridization.
detection of tuberculous meningitis, with generally superior performance compared with conventional culture (33, 34).

The specificity of molecular assays for the detection of TB is dependent on several factors. Primer design is, of course, important, and assays are implemented using primers and probes that show no cross-reactivity with other species. Unacceptable specificity is more likely to be the result of false-positive results. As with all molecular assays, laboratories must guard against false-positive results by use of appropriate laboratory practices, such as spatial separation of work areas, unidirectional workflow, and chemical inactivation of amplicons. However, there are other potential causes of false-positive results, such as contamination of the sample with TB organisms or DNA. Cross-contamination during DNA preparation is a concern, as is contamination with viable or nonviable organisms during the decontamination and processing of sputum samples. Contamination has been observed in mycobacteriology laboratories (35) and could certainly lead to DNA false positives.

The risk of a false-positive PCR result in a TB assay includes two other situations unique to molecular assays. One situation involves potential amplification of DNA from nonviable organisms present in samples during or after antitubercular treatment. We have observed positive results in patients up to 2.5 years after completion of a directly observed therapy program (unpublished results), and others have reported similar findings (13). Thus, communication with the clinicians involved in a patient’s care and clinical history is essential in avoiding overcalling a positive PCR result.

A second situation in which nonviable organisms can lead to a positive PCR result is in samples obtained via instrumentation, such as bronchoscopy. After collection of sterile prewashes of sterile bronchoscopes (to reflect material remaining in the barrel of the scope from previous patients), we have observed amplifiable human DNA in 20% and amplifiable TB DNA in 3.6% of samples, a number that exceeds our rate of false positives and the rate of bronchoscopy samples that are culture positive for TB at our institution (36). Similar results have been reported for Helicobacter pylori DNA in sterile gastrosopes (37). It is our policy to request a saline prewash of bronchoscopy samples to analyze in parallel with the actual patient sample.

### Performance of a PCR Assay

Despite these potential problems, molecular studies for the detection of TB can provide enormous potential benefits to patients. Previous studies have all been retrospective in nature, focusing primarily on determination of assay performance. We undertook a prospective study to examine the impact of molecular testing for TB in an effort to better determine the benefits and impact on patient care. Working with colleagues at Cook County Hospital in Chicago, we assayed multiple sputum samples from 85 consecutive consenting patients who were admitted to the pulmonary service with a presumptive diagnosis of TB (38). We performed PCR on each sputum sample, using both our in-house assay targeting IS6110 and the Roche Amplicor assay. PCR results were compared to conventional smear, culture, and speciation (using the nonamplified GenProbe Accuprobe method) results generated in the Cook County Hospital Mycobacteriology Laboratory.

In general, the performance of both our in-house and the Roche assays was good. In smear-positive patients, both assays showed 100% sensitivity and specificity, reassuring values because this is the primary application for which the commercial assays have received FDA approval.

However, the results in smear-negative sputa were also acceptable. Our in-house assay yielded a sensitivity of 89%. The Roche assay, which uses a somewhat smaller volume of sputum, had a lower sensitivity (85%) than our IS6110 method. However, the Roche method has a con-
current specificity that was better (93% compared with 86% for the IS6110 assay).

The implementation of PCR testing of the first two sputum samples collected during the first 24 h of hospitalization would allow a more rapid diagnosis of TB in these patients. Our in-house and the Roche Amplicor assays yielded PCR sensitivities (compared with culture) of 85% and 74%, respectively, along with specificities of 88% and 93%. In this study, if patients with paucibacillary (<20 colonies on solid medium) were excluded, the PCR sensitivity increased to 100% and 95% for the in-house and Roche Amplicor assays, respectively (38), illustrating that the performance of PCR is related to the burden of organisms present in the sample.

False-positive PCR results in comparison with culture were observed for both assays. Although initially disappointing, many of these results were seen to be positive in both assays. Because these assays target different fragments of DNA, this suggests that actual organisms or TB DNA could be present in the sample. In fact, when full clinical data were examined on these patients, two were found to have been previously treated for TB infection, but before the 1-year study exclusion period we were using. Because these false-positive results occurred in smear-negative patients who would have been discharged from isolation (and from the hospital were they otherwise healthy), this would have led to unneeded continuation of isolation and medication had the PCR results been used to dictate treatment. Thus, false-positive molecular results represent a major problem in the widespread implementation of these assays. On the other hand, our assay still had a very high negative predictive value, so that patients could have been confidently released from isolation as a result of a negative PCR.

Impact of PCR Detection on Patient Care
Possible outcomes on patient care and clinical management as a result of PCR testing are summarized in Table 2. In essence, in smear-positive patients, a positive PCR would indicate that the mycobacterial species was in fact TB, and patients would be appropriately retained in isolation and treated until their sputum samples became smear negative. A negative result would indicate the presence of a nontuberculous mycobacterial infection, and the patient could be released from isolation and treated more appropriately. The potential for positive impact on patient care with a reduction in the cost of care is apparent.

In summary, nucleic acid amplification assays are warranted on smear-positive sputum samples and potentially on smear-negative samples if the clinical presentation is highly suggestive of TB. Recent guidelines from the Centers for Disease Control support this approach (12). In smear-negative patients, the PCR may also be of value, although it should be used with more caution. Most positive PCR results will be indicative of a real TB infection. If clinical suspicion is high, treatment could be begun, but in the face of uncertainty, waiting until the culture results become available before beginning treatment is perhaps the best course. Because the patient is smear negative, there is presumably less risk of infecting others than in the smear-positive situation. Additionally, PCR may be useful in the diagnosis of extrapulmonary TB infections, particularly for meningitis (33, 34).

Future Directions
Thus, the rapid availability of results obtained by molecular assays can be a considerable advantage to patient management in certain situations. More rapid and appropriate treatment can lead to fewer ultimate complications and hospitalization in patients who have the disease, and avoidance of unnecessary treatment and isolation of those who do not have TB. Similar pressures for more rapid diagnostic capabilities have driven the development of other molecular assays, particularly those for slow-growing viruses, and particularly for the diagnosis of infections of the central nervous system.

There is, however, substantial room for further advancement. At the present time, most molecular assays are fairly work-intensive, requiring analysis of amplicons by one or more gels and probes. Colorimetric, chemiluminescent, and fluorescent detection methods have been put to use, primarily in the commercial systems, but more rapid methods as well as the automated equipment to perform these techniques are needed. New real-time PCR instrumentation is expected to have a very positive impact on the laboratory performance of PCR, but additional changes in the practice of molecular pathology will still be

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*a Neg, negative; Pos, positive; MOTT, mycobacteria other than tuberculosis.
needed. Rather than detection of single microbes, in the future molecular assays will need to be aimed at multiple organisms. Multiplex amplification reactions may be useful for the simultaneous detection of multiple pathogens in a single sample. Alternatively, consensus primers may be used for amplification of related microbes, such as mycobacteria, with species-specific detection performed using probes or DNA arrays.

It is likely that future detection systems may be sufficiently sensitive to detect small numbers of pathogens without any amplification. Whatever the format and instrumentation for the assays, the impact of molecular testing on patient care will be substantially greater in the future.

Collaborators in our PCR studies include Dr. Robert Cohen, Shirin Muzaffar, and David Schwartz at Cook County Hospital in Chicago. Scott Luke provided expert technical assistance. I would also like to acknowledge the financial support of the American Society of Clinical Chemistry and the Agency for Health Care Policy and Research. Additionally, Dr. Lemuel Bowie provided invaluable professional inspiration and advice on outcome studies in the clinical laboratory.

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