Assessment of Laboratory Performance of Nucleic Acid Amplification Tests for Detection of Mycobacterium tuberculosis

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Procedures for rapid laboratory testing for diagnosing tuberculosis (TB) are a major component of public health policy for controlling TB in the United States (17). The nucleic acid amplification (NAA) test for direct detection of Mycobacterium tuberculosis in patient-derived specimens provides a rapid result to determine whether a patient has TB. Information from the M. tuberculosis NAA result may contribute to patient diagnosis and to decisions involving respiratory isolation within a hospital. Two M. tuberculosis NAA tests, Gen-Probe MTD and the Roche Amplicor MTB, were approved by the U.S. Food and Drug Administration in 1995 for use with acid-fast bacilli smear-positive respiratory specimens (3). In 2000, the modified Gen-Probe MTD test was approved for use with smear-negative respiratory specimens (4). The U.S. Department of Health and Human Services has advocated increased use of M. tuberculosis NAA tests for rapid diagnosis and control of TB in the Healthy People 2010 Objectives (18).

Commercial development of the Gen-Probe MTD and the Roche Amplicor MTB provides access to M. tuberculosis NAA tests for routine diagnostic testing in laboratories which may not have had experience with molecular testing. The NAA tests, however, require specific quality assurance (QA) practices to prevent contamination. While the performance characteristics of M. tuberculosis NAA tests were documented previously (1, 2, 6–11, 15, 16), there is little knowledge of laboratory practices and performance associated with these tests in routine diagnostic settings (13, 14).

To assess laboratory practices and performance with M. tuberculosis NAA tests, the Centers for Disease Control and Prevention (CDC) implemented a M. tuberculosis NAA testing performance evaluation program in 1997. This voluntary program uses samples containing M. tuberculosis and nontuberculous mycobacteria (NTM), providing an anonymous challenge for laboratories (http://www.phppo.cdc.gov/mpep/mtbaa.asp). An aggregate report of results is provided to participants with analyses of common problems. We describe here the laboratory participants, laboratory practices for QA reported, aggregate testing performance, and analyses of the association between performance and QA practices for the combined results from two 1997 shipments.

Test samples were developed from patient-derived isolates of M. tuberculosis and NTM (Mycobacterium kansasi and Mycobacterium gordonae). Stock suspensions were prepared from 8-day growth on Lowenstein-Jensen medium and adjusted to an absorbency of 0.2 at 420 nm. Dilutions were made in either sterile water or phosphate buffer (0.15 M, pH 6.8). Organism counts were measured using a Bryte H5 flow cytometer (Bio-Rad Laboratories). Viability was confirmed by plate counts on Middlebrook 7H10 agar. Sensitivity for M. tuberculosis, using the 50-μl analytic test volume recommended by Gen-Probe MTD, was determined using samples with concentrations ranging from 1.5 × 10⁶ organisms to less than 1 organism per ml. Sample stability at various concentrations was tested by holding unfrozen samples before assay at room temperature for 2 to 3 days, at −70°C for 12 weeks, and for 4, 6, or 8 days at both 4°C and at room temperature after thawing. In each shipment, a total of five samples including aliquots of M. tuberculosis and M. gordonae or M. kansasi were sent to participating laboratories. Participants were instructed to analyze the samples within 72 h.

Anonymous results were analyzed by the CDC and the Wisconsin State Laboratory of Hygiene. Univariate analysis of data from two shipments (April 1997 and September 1997) was conducted for laboratory demographics, methods, and appropriate use of the biological safety cabinet (BSC). The cumulative qualitative test results for the 10 samples were collated.

For assessment of the relationship between QA practices and specificity for M. tuberculosis NAA testing, results for the M. gordonae samples were compared with a composite score of the laboratory’s responses to questions about QA practices. Only the results from laboratories using the Gen-Probe MTD were included due to low numbers for other methods. Participants were asked to report on uses of their BSC for M. tuberculosis NAA testing. They were also asked whether they used unidirectional workflow. Responses to each question were scored in order from the least-preferred to most-preferred QA
practices. Composite scores were then compared with sample test results using Fischer’s exact test. Adverse responses to QA questions were as follows: use of the same BSC for Mycobacterium tuberculosis NAA testing and for Mycobacterium tuberculosis specimen processing for other uses; not using unidirectional workflow (or not knowing if used). Bivariate analysis at the 95% confidence level was used to determine whether adverse answers to QA questions were associated with false-positive results.

Of the 86 participants in the Mycobacterium tuberculosis NAA performance evaluation program in 1997, 55% (47 of 86) were hospital laboratories, 27% (23 of 86) were health department laboratories, 15% (13 of 86) were independent laboratories, and 2% (2 of 86) were other types of laboratories. Most laboratories (74% [64 of 86]) performed the Gen-Probe MTD test. Fourteen percent (12 of 86) of the laboratories performed the Roche Amplicor MTB test, 12% (10 of 86) used in-house methods, and 2% (2 of 86) used other methods.

A wide distribution in weekly patient specimen volume was observed among participants (Fig. 1), with many laboratories testing <1 specimen per week. Overall sensitivity for all methods for detecting Mycobacterium tuberculosis was 97.9% (417 of 426). For Mycobacterium gordonae samples, overall specificity was 93.5% (314 of 336); however, overall specificity for the Mycobacterium kansasii sample was 71.6% (58 of 81).

Approximately 27% of participants reported that the BSC used for Mycobacterium tuberculosis NAA testing was also used for TB specimen processing (Fig. 2), and 26% reported using the BSC for other purposes. Additionally, 34% and 24% of participants in the April 1997 and September 1997 shipments, respectively, reported either not using unidirectional workflow or not knowing whether unidirectional workflow was used in performing Mycobacterium tuberculosis NAA testing (Fig. 3). Among laboratories using Gen-Probe MTD that had false-positive Mycobacterium tuberculosis NAA results with Mycobacterium gordonae samples, there was at least one response to a QA question that indicated a practice that is not recommended (Table 1). In comparing false-positive qualitative results with a composite score of adverse responses to QA questions regarding BSC use and unidirectional workflow, false-positive results were significantly associated with an adverse quality assurance score (P = 0.04). When inconclusive results were included as incorrect responses, the association was weakened (P = 0.07). A total of 10 false-positive results were reported by nine different laboratories for both shipments.

The CDC Mycobacterium tuberculosis NAA performance evaluation program participants represent a large cohort of laboratories using commercial Mycobacterium tuberculosis NAA tests in the United States. Cross-reactivity with Mycobacterium kansasii has been previously documented with Gen-Probe MTD (10). The Gen-Probe MTD has since been modified by the manufacturer, which may have decreased cross-reactivity with NTM. Results from a subsequent shipment containing a strain of Mycobacterium celatum, which has also documented cross-reactivity, indicated improved test performance with the modified Gen-Probe MTD (19). However, false-positive and inconclusive results with Mycobacterium gordonae samples may indicate cross-contamination. Results from the first two shipments indicate concerns about specific Mycobacterium tuberculosis NAA testing QA practices. Although the NCCLS recommends separation of equipment and work areas for NAA testing (12), some participants reported processing...
specimens for *M. tuberculosis* smear or culture in the same BSC used for *M. tuberculosis* NAA testing and either not using unidirectional workflow or not knowing whether unidirectional workflow is used. False-positive results with Gen-Probe MTD were significantly associated (*P* = 0.04) with a decreased QA score based on participant responses about use of the BSC and unidirectional workflow. There are indications from previous studies that false-positive results due to cross-contamination may be a problem with some methods (2, 5, 6). These analyses highlight potential problems with the use of commercial NAA tests in laboratories that may not have the equipment, facilities, or special QA expertise which NAA testing requires. Use of molecular diagnostics requires special attention to the configuration of workflow and multiple uses of laboratory space and equipment, which may require modifications in laboratory design and additional resources. Of special concern is the perception that the commercialization and Food and Drug Administration clearance of test kits provide laboratories protection from the same cross-contamination problems that are inherent with in-house-developed test methods.

These results represent the first two shipments of specimens. In subsequent shipments of this program, fewer false-positive results were observed, although there are remaining concerns about some of the QA practices (data not shown). This improved performance may possibly be due to increased attention to contamination based on the reports from this program and increased experience with test methods. The potential for cross-contamination with commercial NAA tests, however, still exists. These results demonstrate that laboratories should be vigilant and follow standard QA practices. The false-positive test results encountered in this program also support the need to interpret positive *M. tuberculosis* NAA test results with caution, especially if the specimen is acid-fast bacilli smear negative (4). Based on results, we recommend the following: that laboratories follow NCCLS recommendations for NAA testing; that manufacturers include QA recommendations (e.g., unidirectional workflow and separate equipment and areas) in package inserts; and that proficiency testing organizations increase the number of samples with NTM in their *M. tuberculosis* NAA testing programs to assist laboratories in detecting problems with contamination.

Use of trade names and commercial sources is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

**REFERENCES**


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**TABLE 1. QA survey of laboratories reporting false-positive results (Gen-Probe MTD)**

<table>
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<tr>
<th>Biosafety cabinet use</th>
<th>No. of responses on use of unidirectional work flow</th>
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<th>No</th>
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<td>5</td>
<td>7</td>
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<td>10</td>
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**FIG. 3. Use of unidirectional workflow by laboratories participating in the *M. tuberculosis* NAA PE in April and September 1997.**, numbers at the end of each bar represent the frequency of results.