Comparison of the Amplified *Mycobacterium tuberculosis* (MTB) Direct Test, Amplicor MTB PCR, and IS6110-PCR for Detection of MTB in Respiratory Specimens


Several nucleic acid amplification techniques (NAAT) have been developed for rapid and direct detection of *Mycobacterium tuberculosis* (MTB) from clinical specimens. This study compared the performance of the Gen-Probe Amplified MTB Direct Test (AMDT), Roche Amplicor MTB PCR test, and an IS6110-PCR assay with acid-fast smear and culture in the detection of MTB from 428 respiratory specimens from 259 patients. Patients’ charts were reviewed for clinical correlation. Of 98 specimens that were clinically positive for MTB, acid-fast smear was positive in 50% of cases, culture in 93%, IS6110-PCR in 83%, AMDT in 84%, and Amplicor MTB PCR in 80%. Of 337 specimens that were negative for MTB, 117 (35%) were positive for nontuberculous mycobacteria. Specificities were as follows: smear, 89%; culture, 100%; IS6110-PCR, 99%; AMDT, 98%; and Amplicor MTB PCR, 96%. The accuracies of the tests were 80%, 98%, 96%, 95%, and 92%, respectively. MTB culture-positive specimens that were smear-negative were detected by AMDT and IS6110-PCR in 77% of cases and by Amplicor MTB PCR in 70%. NAAT was less sensitive than was culture for detection of MTB, but all these techniques had acceptable accuracy and were completed within hours. NAAT may be useful for rapid screening of respiratory specimens to distinguish MTB from nontuberculous mycobacteria infection in order to isolate patients.

Because of the increase in the number of cases of tuberculosis and in drug resistance over the past few years, there has been a resurgence of interest in rapid diagnosis of *Mycobacterium tuberculosis* (MTB) infection. Rapid identification of cases has been emphasized as one of the strategies to control the spread of MTB infection [1]. New technology based on the use of specific DNA or RNA sequences offers the promise of rapid nucleic acid amplification techniques (NAAT) that may prove to be accurate, species-specific, and user-friendly. The traditional acid-fast smear for diagnosis has been problematic because of low sensitivity and nonspecificity, particularly in geographical areas where nontuberculous mycobacteria are commonly isolated. Culture methods are also imperfect because even with the most rapid methods (such as radiometric liquid systems paired with DNA hybridization techniques for identification) the average time for detection of MTB is 7–12 days [2]. Diagnosis of tuberculosis in cases of smear-negative specimens may take up to 8 weeks.

A number of rapid identification tests are being developed that rely on enzymatic amplification of specific nucleic acid sequences specific to the MTB complex. These tests include PCR, self-sustained sequences replication, ligase chain reaction, and Q beta replicase-amplified assay [3]. One of these tests, the Amplified MTB Direct Test (AMDT; Gen-Probe, San Diego), has recently been licensed for use in the United States. Two methods have been developed in a kit format for use in a clinical laboratory: AMDT and Amplicor MTB PCR (Roche Molecular Systems, Branchburg, NJ).

There is also a great deal of interest in the development of PCR techniques with use of a diversity of genes as selected targets. Targets include 16S ribosomal RNA gene sequences, single-copy genes encoding for structural proteins, and the repetitive insertion sequence IS6110 of the MTB genome, which is a multiple-copy element [4, 5]. Our in-house PCR assay uses primers and internal probe sequences described by Eisenach et al. [6]. This study is the first blinded comparison of the AMDT, Amplicor MTB PCR, and IS6110-PCR methods. Parameters analyzed included sensitivity, specificity, ease of use, appraisal
of clinical usefulness, and comparison with acid-fast smear and culture.

Materials and Methods

Patients and Specimens

Sputum and bronchoalveolar lavage specimens were obtained from Ochsner Foundation Hospital and the Medical Center of Louisiana (which includes University Hospital and the former Charity Hospital) in New Orleans. To facilitate comparison of NAAT, it was necessary to develop a study set with a higher percentage of MTB-positive specimens than that of the Ochsner Foundation Hospital population (estimated frequency of positive respiratory cultures for MTB is 1 of 200 at Ochsner Foundation Hospital and 1 of 20 at the Medical Center of Louisiana).

During the 5-month period from 1 October 1994 to 1 March 1995, the following specimens were entered into the study: all respiratory specimens submitted to the Ochsner Foundation Hospital laboratory for mycobacterial culture (n = 299); all smear-positive respiratory specimens from the Medical Center of Louisiana (n = 83); and all acid-fast bacilli smear-negative respiratory specimens from the Medical Center of Louisiana that were ultimately positive for MTB in culture (these specimens were stored at —70°C until they were tested by NAAT) (n = 46).

Specimen Processing

All respiratory specimens were digested and decontaminated by the standard N-acetyl L-cysteine NaOH-Na citrate method used for processing respiratory mycobacterial cultures [7]. After the specimen was processed for smear and culture, the remaining volume was revortexed and divided into three aliquots of 100 µL each. These aliquots from all Ochsner Foundation Hospital specimens and the smear-positive Medical Center of Louisiana specimens were assigned a random study number and stored at —70°C. Specimens were stratified by month of acquisition and randomly pulled and thawed as NAAT was performed and were incubated for 8 weeks. Mycobacteria were identified primarily by DNA hybridization (Accuprobe; Gen-Probe) and biochemical tests when necessary. Acid-fast smears were fixed and stained with auramine fluorochrome and examined under a fluorescence microscope (400×) [5]. Findings of these smears were reported as follows: no MTB seen = negative; 1–2 per 300 fields = indeterminant; 1–9 per 100 fields = 1+; 1–9 per 10 fields = 2+; 1–9 per field = 3+; and 9 per field = 4+. If a smear was indeterminant, it was fixed and examined again. Specimens from the Medical Center of Louisiana were inoculated into BACTEC 12-B bottles after the digestion/decontamination process was performed and were incubated for 8 weeks. Mycobacteria were identified by DNA hybridization (Accuprobe). Direct smears were stained by the Ziehl-Neelsen method and examined under an oil immersion lens; findings of these smears were reported as outlined above.

IS6110-PCR Technique

IS6110 is a 1,361-bp repetitive insertion sequence that is usually present 6–20 times in the MTB genome, although as few as one copy has been observed [6]. Our PCR method amplifies a 123-bp target sequence within IS6110. Amplification product (amplimers) were detected by visualizing a 123-bp fragment on the ethidium bromide-stained gel and by hybridization in a dot blot format to a digoxigenin-labeled internal oligonucleotide probe with detection by chemiluminescence [5].

Additional features of the assay include use of d-UTP-uracil-N-glycosylase (UNG) method to prevent carry-over contamination [9], use of β-actin primers as a PCR amplification control [10], and use of a monoclonal antibody–based “hot-start” protocol to reduce primer-dimer formation and generation of nonspecific side-reaction products [11]. The in-house IS6110-PCR has a detection limit of 10 fg (approximately two mycobacteria) of purified MTB DNA from the H37Rv isolate in agarose gel electrophoresis and of 1 fg after hybridization with an internal probe. This isolate is known to have at least 13 copies of IS6110 per MTB genome [12].

DNA extraction. DNA was extracted from specimens by treatment with heat and detergent. Cell pellets were washed once with TE buffer (10 mM Tris-HCl with a pH of 8.0, 1 mM EDTA) containing 1% Triton X-100. Samples were centrifuged at 12,000 g for 10 minutes, and the pellets were resuspended in a small volume of Triton X-100 (50–100 µL, depending on the size of the pellet) and then heated at 95°C for 30 minutes. Lysates were centrifuged to remove cellular debris, and 5 µL of the supernatant was added directly to the PCR mixture.

Primers and Probe Sequences

The primers used were T4 5′-CCTGCAGGCGTAGGC-GTCGG-3′ and T5 5′-CTCGTCCAGCGCCGCTTCGG-3′ [7].
The Probe LK229 5'-GTAGGCCAACCCTGGCCAGGT-CGACACATAGGTAAGCCT-3' was used.

The reaction mixture for PCR (20 μL) consisted of 10 mM of Tris-HCl with a pH of 9.0, 50 mM of KCl, 0.1% Triton X-100, 2 mM of MgCl₂, 0.1 mM each of deoxynucleosidetriphosphate (dATP, dCTP, dGTP, and dUTP), 0.1 U of UNG, 0.75 U Taq polymerase, and 165 ng of TaqStart antibody (Clontech Lab, Palo Alto, CA). The following controls were used in each PCR run: MTB DNA (100 fg) as a positive control, normal human genomic DNA as a negative control, and a reagent control without sample. PCR was carried out in a thermal cycler (model 9600; Perkin-Elmer, Norwalk, CT) according to the following program: hold at 50°C for 2 minutes; 35 cycles at 94°C for 45 seconds, at 68°C for 55 seconds, and at 72°C for 1.5 minutes; hold at 72°C. The total assay time for the IS6110-PCR protocol is ~12 hours.

In addition to the use of UNG, other measures were taken to avoid carry-over contamination. Three separate areas were used for each step of the PCR. Dedicated materials, including plugged tips to avoid aerosolization, were used in each area.

Roche Amplicor MTB PCR

The Amplicor MTB PCR test is based on the amplification of a conserved region of the 16S ribosomal RNA gene of Mycobacterium [13]. The PCR assay uses genus-specific primers to amplify a 584-bp sequence, which is then detected by hybridization with a MTB species-specific DNA probe bound to a microwell plate in a colorimetric enzyme-linked immunoassay format. The assay incorporates the use of AmpErase (Roche Molecular Systems), which contains the enzyme UNG to destroy carry-over amplicons from previous reactions. Amplicor MTB does not include a control to monitor for PCR inhibitors or for efficiency of the amplification reactor.

DNA extraction. The 100-μL processed sample was washed with 10 mM of Tris-HCl with a pH of 8.0, 0.05% sodium azide, and 1% solubilizer. Samples were centrifuged at 12,000 g for 10 minutes; the pellet was resuspended in 100 μL of lysis reagent (containing 0.4% sodium hydroxide and 0.05% sodium azide), heated at 60°C for 45 minutes, and neutralized with 100 μL of a reagent containing Tris-HCl with 0.05% sodium azide. A 50-μL aliquot was used in the PCR.

Additional details of the Amplicor MTB PCR are detailed in the Amplicor MTB package insert and by D’Amato et al. [14]. The laboratory that performed this assay passed proficiency testing with the Amplicor MTB test as outlined by the test’s manufacturer before the study. The same personnel performed the IS6110-PCR assays. The Amplicor MTB assay took ~7.5 hours.

Gen-Probe Amplified MTB Direct Test (AMDT)

The AMDT is an isothermal transcription-based amplification of rRNA of Mycobacterium species via DNA intermediates [15]. Amplification product is detected by hybridization with an MTB complex-specific DNA probe labeled with an acridinium ester. Chemiluminescence is read in a luminometer. The AMDT does not use a control to monitor for inhibitors or for efficacy of amplification and does not use a method to eradicate amplicon contamination.

RNA extraction. Fifty μL of the sample was placed into a lysis tube containing glass beads and 200 μL of specimen dilution buffer. The sample was sonicated at 200 kHz in a water bath sonicator for 15 minutes at room temperature. The 50-μL lysate containing free ribosomal RNA as a template was used for the amplification reaction. The AMDT was carried out as previously described [16, 17]. The AMDT was performed by the microbiology laboratory, which passed a proficiency test on the use of AMDT as outlined by the manufacturer before the study. This assay takes ~5 hours.

Analysis of Data

The discharge diagnoses were reviewed for all patients at Ochsner Foundation Hospital from whom specimens were obtained. For patients whose diagnosis of mycobacterial disease was coded or who had discordant results of smear, culture, or NAAT, the clinical record was reviewed to determine if there was a clinically relevant explanation of the discordance (e.g., whether the patient had received antituberculous therapy), to determine if there were other pertinent diagnoses, and to determine the clinical likelihood that they had active MTB infection.

The records of all patients from the Medical Center of Louisiana who had a positive culture for MTB or discordant results of smear, culture, or NAAT were reviewed to determine if there were any confounding factors. From these record reviews we determined whether specimens were from patients with “clinically positive” cases of MTB. Criteria for “clinically positive” cases (clinically resolved) included a composite picture of culture positivity and/or a radiographic picture with a history and laboratory findings consistent with MTB infection, historical risk factors for MTB infection, a positive tuberculin skin test, and the absence of other diagnoses to explain the clinical abnormalities.

The key that joined the randomly ordered study numbers to the patient data was known only to the statistician until the study was completed. To resolve discrepancies in clinical diagnoses, univariate and multivariate comparisons of smear, culture, and NAAT were done by Cochran χ² and logistic regression analyses.

Results

Analysis of sensitivity, specificity, and accuracy of tests by specimen. Of the 428 specimens that were studied, 98 were determined to be clinically positive (i.e., patients from whom these specimens were obtained had active cases of pulmonary tuberculosis) (table 1). Of 98 specimens, 91 (93%) that were
Table 1. Results of culture, smear, and NAAT performed for 428 respiratory specimens: correlation with the clinical diagnosis of *Mycobacterium tuberculosis* infection.

<table>
<thead>
<tr>
<th>Result for specimen (n = 428)</th>
<th>Culture</th>
<th>Smear</th>
<th>Amplicor</th>
<th>AMDT</th>
<th>IS6110-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for MTB</td>
<td>91</td>
<td>49</td>
<td>78</td>
<td>82</td>
<td>81</td>
</tr>
<tr>
<td>Negative for MTB</td>
<td>7</td>
<td>49</td>
<td>20</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>93</td>
<td>50</td>
<td>80</td>
<td>84</td>
<td>83</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>89</td>
<td>96</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>100</td>
<td>58</td>
<td>86</td>
<td>92</td>
<td>98</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>98</td>
<td>86</td>
<td>94</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>98</td>
<td>80</td>
<td>92</td>
<td>95</td>
<td>96</td>
</tr>
</tbody>
</table>

NOTE. AMDT = Gen-Probe amplified MTB direct test; Amplicor = Roche Amplicor MTB PCR; MTB = *Mycobacterium tuberculosis*.

clinically positive were positive for MTB in culture. Of the seven clinically positive but culture-negative specimens, three were accounted for by antituberculous treatment (smears and all NAAT were positive for these three culture-negative specimens).

The sensitivity of acid-fast smear for this population was low (50%). Of 85 smear-positive cases, 36 (42%) were clinically negative for MTB, indicating a high prevalence of nontuberculous mycobacteria recovery in our patient population.

The sensitivities of the Amplicor MTB PCR, the AMDT, the IS6110-PCR, and culture were 80%, 84%, 83%, and 93%, respectively. Likewise, these tests had (good) specificities of 96%, 98%, 99%, and 100%, respectively. These parameters were calculated using the "clinically resolved" results as the standard.

IS6110-PCR and AMDT were equivalent by logistic regression, which was used to statistically compare the performance of all tests for accuracy (accuracy = (true positive + true negatives)/total specimens) (table 2). Amplicor MTB PCR was 92% accurate whereas AMDT was 95% accurate (not statistically different). Amplicor MTB PCR was less accurate than IS6110-PCR (92% vs. 96%, P < .05). Culture was more accurate than AMDT and IS6110-PCR (98% vs. 95% and 96%, respectively; P < .05) and also more accurate than Amplicor MTB PCR (98% vs. 92%, P < .01).

A separate analysis of the 388 specimens collected from patients who had received <7 days of antimycobacterial therapy is presented in table 3. Forty specimens from 11 patients were excluded from the original data set presented in table 1.

Another way of analyzing the impact of therapy on the results of NAAT is by studying the group of 40 specimens excluded because of therapy (duration, 7–82 days; mean, 27 days). Of these 40 specimens, 20 (50%) were smear-positive; 36 (90%) were positive by culture, IS6110-PCR, and Amplicor MTB PCR, and 37 (93%) were positive by AMDT. Sensitivity, specificity, and positive and negative predictive values were calculated with use of culture rather than with use of "clinical resolution" as the standard.

**Analysis of false-positive NAAT.** Thirteen clinically negative specimens were positive by Amplicor MTB PCR. Of the

<table>
<thead>
<tr>
<th>Test</th>
<th>Smear</th>
<th>Culture</th>
<th>Amplicor</th>
<th>AMDT</th>
<th>IS6110-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear</td>
<td>↑↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Culture</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Amplicor</td>
<td>↑↑</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>AMDT</td>
<td>↑↑</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>IS6110-PCR</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

NOTE. Baseline is shown in the columns. Key: ↓↓ = less accurate, P < .01; ↑↑ = more accurate, P < .01; ** = not statistically different; ↓ = less accurate, P < .05; ↑ = more accurate, P < .05. AMDT = Gen-Probe amplified MTB direct test; Amplicor = Roche Amplicor MTB PCR.
Table 3. Results of smear and NAAT (using culture as the standard) for 388 respiratory specimens from patients who were not treated or who received antimycobacterial therapy for <7 days.

<table>
<thead>
<tr>
<th>Direct smear</th>
<th>Culture report</th>
<th>NAAT</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Smear</td>
<td>Amplicor</td>
<td>AMDT</td>
<td>IS6110-PCR</td>
</tr>
<tr>
<td>Positive (n = 65)</td>
<td>No. of specimens positive for MTB (n = 28)</td>
<td>25 (89)</td>
<td>26 (93)</td>
<td>25 (89)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of specimens positive for nontuberculous mycobacteria (n = 32)</td>
<td>3 (9)</td>
<td>4 (12)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of negative specimens (n = 5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Negative (n = 323)</td>
<td>No. of specimens positive for MTB (n = 28)</td>
<td>16 (57)</td>
<td>18 (64)</td>
<td>19 (68)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of specimens positive for nontuberculous mycobacteria (n = 84)</td>
<td>4 (5)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of negative specimen (n = 211)</td>
<td>8 (4)</td>
<td>3 (1)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Total no. of specimens (n = 388)</td>
<td>No. of specimens positive for MTB (n = 56)</td>
<td>41 (73)</td>
<td>44 (79)</td>
<td>44 (79)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of specimens positive for nontuberculous mycobacteria (n = 116)</td>
<td>7 (6)</td>
<td>5 (4)</td>
<td>3 (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of negative specimens (n = 216)</td>
<td>8 (4)</td>
<td>3 (1)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

| Sensitivity* (%) | 50 | 73 | 79 | 79 |
| Specificity* (%) | 89 | 96 | 98 | 99 |
| Positive predictive value* (%) | 43 | 73 | 85 | 94 |
| Negative predictive value* (%) | 91 | 96 | 96 | 97 |

NOTE. AMDT = Gen-Probe amplified MTB direct test; Amplicor = Roche Amplicor PCR; MTB = Mycobacterium tuberculosis; NAAT = nucleic acid amplification techniques.

* Parameters were calculated based on culture as the "gold standard."

In the laboratory may be an explanation for the false-positive results for the few specimens judged to be clinically negative when one or more of the NAAT were strongly positive.

Discriminatory value of NAAT for smear-positive nontuberculous mycobacterial specimens. Of the 428 specimens, 116 (27%) yielded nontuberculous mycobacteria (65 yielded M. avium complex, 27 yielded M. kansasii, 12 yielded Mycobacterium chelonae, 5 yielded Mycobacterium gordonae, 2 yielded Mycobacterium terrae, 1 yielded Mycobacterium fortuitum, and 5 yielded acid-fast bacilli that were not viable for identification). Of 117 specimens, 32 (27%) that yielded nontuberculous mycobacteria in culture were smear-positive. Of these 31 smear-positive specimens (all of which were considered clinically negative for MTB), Amplicor MTB PCR was positive for two, AMDT was positive for three, and IS6110-PCR was positive for one. All of these NAAT are effective for discriminating MTB from nontuberculous mycobacteria in smear-positive specimens. The positive predictive values of all NAAT were impressive (86%–98%) in our geographic area, in which a high number of nontuberculous mycobacteria are isolated.

Performance of NAAT used to test smear-negative MTB culture-positive specimens. Of the 91 specimens that yielded

13, 12 had relatively low optical density values of 0.351–0.496 (positive value: ≥0.350). Most of the true-positive Amplicor MTB PCR results (MTB culture-positive) had optical density values of ≥3.0. Culture of these 12 specimens with borderline optical density readings yielded six nontuberculous mycobacteria (four Mycobacterium avium complex, one Mycobacterium kansasii, and one unidentifiable Mycobacterium species). Of the 13 false-positive Amplicor MTB PCR tests, two were positive by IS6110-PCR and none was positive by AMDT PCR.

Seven clinically negative specimens were positive by AMDT. These specimens all had fairly low relative light unit values of 30,569–169,448 (positive value: ≥30,000). The mean AMDT value for clinically positive specimens was 1,752,000.

Of these seven specimens that were false-positive by AMDT, four yielded M. kansasii in culture and the other three did not yield any mycobacteria in culture. All seven of these false-positive AMDT tests were negative by IS6110-PCR and Amplicor MTB PCR.

Two clinically negative specimens were positive by IS6110-PCR. Both of these specimens were negative by AMDT and positive by Amplicor MTB PCR, and both cultures yielded M. avium complex. Cross-contamination with MTB cells or DNA in the laboratory may be an explanation for the false-positive results for the few specimens judged to be clinically negative when one or more of the NAAT were strongly positive.
MTB in culture, 47 (52%) were smear negative for acid-fast bacillus. With use of AMDT and IS6110-PCR, MTB was detected in 36 (77%) of 47 specimens; with use of Amplicor MTB PCR, MTB was detected in 33 (70%) of 47 specimens. If the results had been reported prospectively, MTB would have been detected earlier, which would have significantly altered the management of a number of these patients.

For example, one patient with a known diagnosis of HIV infection was admitted to the Medical Center of Louisiana for 8 days beginning on 29 November 1994 for evaluation of fever and lymphadenopathy. Findings on a chest radiograph were questionably abnormal. Sputum specimens were submitted for detection of acid-fast bacilli on hospital days 2, 3, and 8, and culture and smears of these specimens were all negative for acid-fast bacilli. The patient was discharged from the hospital without a diagnosis of the cause of his fever.

He was readmitted on 16 December 1994 after his condition deteriorated further. Sputum samples were again submitted for detection of acid-fast bacilli, and smears were negative again on 21 December 1994. Ultimately, all sputum cultures yielded MTB. NAAT studied retrospectively were positive for MTB for two of the three specimens from the first hospital admission. Had this information been available to the clinicians at the time of the first hospital admission, treatment would have been initiated earlier.

Inhibitors in clinical specimens. Ten percent of respiratory specimens demonstrated inhibited amplification with the β-actin control with use of the initial sample preparation. Reextraction with phenol-chloroform resulted in successful amplification for 90% of specimens. Those specimens that repeatedly failed to amplify the β-actin gene (in-house control) were reported as negative in this study.

Analysis of performance of smear, culture, and NAAT by patient. Twenty-seven patients (from whom the 98 clinically positive specimens were obtained) in this study were considered to have a diagnosis of active pulmonary MTB infection (table 4). An average of 3.63 respiratory specimens per patient (range, 1–14 specimens) were submitted for evaluation. Sixty-three percent (17 of 27) of patients had at least one positive smear, 100% had at least one positive culture, 78% (21 of 27) had at least one positive Amplicor MTB PCR test, and 85% (23 of 27) had at least one positive AMDT or IS6110-PCR test. NAAT was positive for at least one specimen submitted for 89% (24 of 27) of patients. As expected, the higher the number of specimens examined by the various tests for MTB, the higher the diagnostic yield. It appears that in this study all NAAT was positive for 26 of 27 patients for whom three or more specimens were submitted.

Seven specimens in this study were negative in culture for patients who were believed to have clinically active cases of pulmonary MTB infection. Of these seven specimens, three were from a patient who had been receiving antituberculous therapy for at least 68 days. He had had multiple prior positive sputum cultures for MTB, and all NAAT and smears were positive with use of the culture-negative specimens obtained while he was receiving therapy. The other four culture-negative specimens in “clinically positive” cases were believed to represent poor-quality sputum specimens (saliva vs. sputum) or other sampling error phenomena because there were other culture-positive specimens within several days before or after the culture-negative specimen.

If all specimens from patients who were receiving treatment for 7 days are excluded (table 4) and these data are reanalyzed by patient, 40 specimens from 11 patients would be taken out of the analysis; two patients (total of six specimens) would be dropped from analysis because all specimens received from these patients for the study were collected after more than 1 week of therapy. Although one might think that the sensitivity of NAAT would be better in untreated patients, the percentage of patients with one or more positive NAAT is higher for all three NAAT if all specimens are included regardless of treatment status. This finding is a consequence of a higher average number of specimens submitted per patient in the group that was not excluded on the basis of their treatment status (3.63 or 2.32 specimens per patient).

Discussion

All three of the NAAT that were evaluated approximated each other in terms of accuracy; however, the Amplicor MTB PCR was less accurate statistically than the IS6110-PCR. All
NAAT were less sensitive than culture for detection of MTB in respiratory specimens. Although inexpensive and widely available, microscopy was nonspecific in our study population and less sensitive than NAAT and thus not satisfactory for rapid diagnosis. All of the NAAT demonstrated excellent specificities (96%–99%). Most of the false-positive results obtained with the Amplicor MTB PCR and the AMDT are probably due to nonspecific reactions because the optical readings were close to the cutoff threshold values for each assay. The only two false-positive results with IS6110-PCR were in samples that yielded M. avium complex in culture.

The relative sensitivity of the Amplicor MTB PCR test in our study was 80%, whereas it has been previously reported to have a sensitivity of 58% [18], 67% [14], and 83% [19]. The AMDT has been previously reported to have sensitivities of 71% [20], 82% [17], 86% [19], and 91% [5, 16], compared with 84% in the present report. The IS6110-PCR resulted in a sensitivity of 83%, whereas previous studies reported values ranging from 79% [21] to 92% [5].

Both Amplicor MTB PCR and AMDT are quicker and simpler than IS6110-PCR for use in a clinical laboratory. Amplicor MTB PCR and IS6110-PCR incorporate an amplification product inactivator (UNG). The lack of an equivalent method is a disadvantage of the Gen-Probe AMDT assay [20, 21]. Only IS6110-PCR has the advantage of a control for detection of amplification inhibitors or failure of DNA liberation. Unsuccessful amplification has been reported in ~10%–20% of clinical specimens [6, 22, 23].

All of the NAAT have analytical sensitivities for nucleic acid detection, quantitatively equivalent to 1–10 mycobacteria [6, 20]. The lower observed sensitivity suggests problems in the efficiency of mycobacterial cell lysis, the presence of amplification inhibitors, or perhaps an unequal distribution of mycobacteria in paucibacillary respiratory specimens. Fewer organisms are present in smear-negative specimens. Clumping of microorganisms is a common problem with mycobacteria; therefore, uniform dispersion in clinical specimens is difficult to obtain. In addition, all of the NAAT use a processed sample with a relatively small volume (50–100 μL) to minimize the effect of inhibitory substances. Analysis of multiple respiratory specimens should maximize detection of MTB [10, 23].

NAAT appears to be useful for rapid screening in cases in which smears are positive for acid-fast bacilli to distinguish MTB from nontuberculous mycobacteria in hospitals with limited isolation capabilities. Although NAAT is less sensitive than culture, the data on the accuracy of these tests suggest that NAAT could be used for the rapid prediction of the presence or absence of MTB. Examination of more than one specimen will improve the sensitivity of testing, as is indicated by the fact that specimens from 26 of 27 patients in this study were positive by NAAT if three or more specimens were examined.

NAAT cannot be expected to replace cultures because of the need for the isolation of mycobacterial organisms for susceptibility testing. A practical use for NAAT would be for patients who are at high risk for MTB infection and whose sputum smears are negative because, as our study shows, the conditions of 70%–77% could be rapidly diagnosed by NAAT. A negative smear for a patient with untreated pulmonary MTB infection has been used to indicate that this patient has lower infectivity for person-to-person transmission than does a smear-positive patient [24]. Since NAAT is more sensitive than smear, it seems reasonable to hypothesize that negative NAAT may be good predictors of the relative lack of infectivity in patients with MTB pulmonary infection. The process of nucleic acid liberation from the mycobacterial cell needs further optimization to increase sensitivity. Once increased sensitivity is achieved, the clinical usefulness of NAAT for the diagnosis of MTB would improve.

Acknowledgments

The authors acknowledge Lana Ancar, Susan Breaux, Janice Chatelain, Peggy Cherry, Jennifer Detiveaux, Wanda Eppling, Estelle Gordon, and Holly Simmons for their invaluable assistance in coordinating this study. They also appreciate Dr. Frédéric Nolte’s thoughtful review of the manuscript.

References


