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Comparison of Two Nucleic Acid Amplification Assays, the Xpert MTB/RIF Assay and the Amplified Mycobacterium Tuberculosis Direct Assay, for Detection of *Mycobacterium tuberculosis* in Respiratory and Nonrespiratory Specimens[▽]

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We compared the performance of the Xpert MTB/RIF assay, a new real-time tuberculosis (TB) PCR test, with that of the Amplified Mycobacterium Tuberculosis Direct (MTD) assay using 162 respiratory and nonrespiratory specimens. Based on culture as the gold standard, the overall sensitivity and specificity for all sample types for the Xpert MTB/RIF assay were 90.9 and 89%, respectively, while for the MTD assay, the overall sensitivity and specificity were 97.3 and 87.1%, respectively. A higher proportion of total equivocal results were obtained for the MTD assay, at 10.5% (17/162), while the Xpert MTB/RIF assay generated 5.5% (9/162) of invalid reads.

Tuberculosis (TB) is an age-old scourge brought on by the bacterium *Mycobacterium tuberculosis*. In 2009, there were 1.7 million people deaths from the disease (9), with morbidity escalating significantly when TB patients were coinfected with HIV (7). In developing countries, TB diagnosis is largely based on smear microscopy of sputum samples. This method has low sensitivity, causing the infection to be misdiagnosed. TB culture, though highly sensitive, takes a prolonged time to obtain results due to slow growth of the organism (6). Consequently, the slow turnaround time hinders TB control efforts. Nucleic acid amplification tests (NAATs) have greatly facilitated TB rapid testing by bringing the turnaround time to within a day rather than 2 or more weeks for a culture result. Various molecular approaches have been exploited for NAATs. The Gen-Probe Amplified Mycobacterium Tuberculosis Direct (MTD) test (Gen-Probe Incorporated, San Diego, CA) is a transcription-mediated amplification nucleic acid probe test. Recently, a new TB test, the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA), based on a fully integrated real-time PCR platform, has been described to perform rapid and sensitive detection of *M. tuberculosis* and rifampin (RIF) resistance (1, 3, 5). In this study, we sought to evaluate the performance of the Xpert MTB/RIF assay and compare it with that of our existing TB molecular test, the MTD test.

A total of 162 nonduplicated clinical specimens were used in this study, of which 131 were respiratory specimens (124 sputum, 5 bronchoalveolar lavage [BAL] fluid, and 2 tracheal aspirate specimens). The remaining 31 were nonrespiratory specimens, which included 5 gastric aspirates, 3 urine samples, 13 samples of normally sterile body fluids (7 cerebrospinal fluid [CSF] samples, including pleural, pericardial, and ascites fluids), and 10 miscellaneous samples, such as pus and biopsy specimens. All respiratory specimens and extrapulmonary specimens of a fluid nature like urine and gastric aspirates were decontaminated according to standard methods using *N*-acetyl-L-cysteine–sodium hydroxide (NALC-NaOH) (4). Tissue specimens were thoroughly minced using a pair of sterile scissors before being used for smear microscopy, culture, and the molecular assays. For normally sterile body fluids (CSF, including pericardial, pleural, and ascites fluids), prior decontamination with NALC-NaOH was not performed. Specimens were then concentrated by centrifugation (4), and the precipitate was resuspended in 67 mM phosphate-buffered saline (pH 6.8) to a final volume of 2.0 ml. Aliquots of the resuspended sediment were used as follows. Part of the sediment was used for Ziehl-Neelsen acid-fast staining, 0.5 ml was used for inoculation into a MGIT tube (Becton Dickinson, Cockeysville, MD), 0.5 ml was inoculated onto a Lowenstein-Jensen (LJ) slant, 0.5 ml was used in Gen-Probe's Amplified MTD (Mycobacterium Tuberculosis Direct) test (Gen-Probe, San Diego, CA), and another 0.5 ml was used in the Xpert MTB/RIF assays. BBL MGIT tube (Becton Dickinson, Franklin Lakes, NJ) cultures were incubated at 37°C with ambient air for 42 days. LJ slants were incubated at 37°C and 5% CO_2 for 56 days and examined weekly for growth.

For the Xpert MTB/RIF assay, sample reagent was added to pretreated specimens in a 2:1 ratio. The mixture was incubated at room temperature for 15 min with vigorous shaking. A total of 1 ml of sample was introduced into a Xpert MTB/RIF cartridge, which was then loaded into the instrument, where the subsequent steps of sample lysis, nucleic acid extraction, and amplification occurred automatically. No repeat testing was carried out when an "Invalid" or "Error" result was obtained due to insufficient sample volume.

Respiratory samples were processed according to the Gen-Probe MTD protocol (Gen-Probe, San Diego, CA). Briefly, 50 μl of specimen dilution buffer was added to 450 μl of test material and sonicated in lysing tubes. For the amplification reactions, 25 μl of sonicated lysate was removed from lysing tubes and added to 50 μl of *Mycobacterium tuberculosis* amplification reagent. Amplified products were subsequently de-

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TABLE 1. Performance of the Xpert MTB/RIF assay compared with *M. tuberculosis* culture results

Specimen type (no.)	Culture result (no.)[e]												% sensitivity (95% CI)[d]	% specificity (95% CI)[d]	Amended result (%)[c]	
	Positive						Negative								Sensitivity (95% CI)[d]	Specificity (95% CI)[d]
	Smear positive			Smear negative			Smear positive			Smear negative						
	Xpert +	Xpert −	Invalid	Xpert +	Xpert −	Invalid	Xpert +	Xpert −	Invalid	Xpert +	Xpert −	Invalid				
Respiratory (131)	43	0	2	13	6	3	3	6[a]	0	2	49[b]	4	90.3 (80.4–95.5)	91.6 (81–96.3)	100 (94.3–100)	98.4 (91.4–99.7)
Nonrespiratory (31)	4	0	0	10	1	0	2	0	0	1	13	0	93.3 (70.2–98.8)	81.2 (57–93.4)	94.4 (74.2–99.0)	100 (72.2–100)
CSF (7)	1	0	0	1	1	0	0	0	0	0	4	0	66.6 (20.8–93.8)	100 (51–100)		
Gastric aspirate (5)	1	0	0	3	0	0	0	0	0	0	1	0	100 (51–100)	100 (20.6–100)		
Urine (3)	0	0	0	0	0	0	0	0	0	0	0	3		100 (43.9–100)		
Fluid (6)	0	0	0	1	0	0	0	0	0	0	5	0	100 (20.7–100)	100 (56.6–100)		
Miscellaneous (10)	2	0	0	5	0	0	2	0	0	1	0	0	100 (72.2–100)		100 (64.6–100)	

[a] All six cultures grew *Mycobacteria* other than *M. tuberculosis*.
[b] Seven of these cultures grew *Mycobacteria* other than *M. tuberculosis*.
[c] Amended results based on clinical diagnosis as the gold standard. Clinical diagnosis was based on patient symptoms, chest X-rays, and use of anti-TB treatment.
[d] CI, confidence interval.
[e] +, positive; −, negative.

TABLE 2. Performance of the Gen-Probe's Amplified MTD test compared *with M. tuberculosis* culture results

Specimen type (no.)	Culture result (no.)[e]												% sensitivity (95% CI)[d]	% specificity (95% CI)[d]	Amended result (%)[c]	
	Positive						Negative								Sensitivity (95% CI)[d]	Specificity (95% CI)[d]
	Smear positive			Smear negative			Smear positive			Smear negative						
	MTD +	MTD −	Equivocal	MTD +	MTD −	Equivocal	MTD +	MTD −	Equivocal	MTD +	MTD −	Equivocal				
Respiratory (131)	44	0	1	17	2	3	2	6[a]	1	3	46[b]	6	96.8 (89.1–99.0)	91.2 (81.1–96.2)	98.4 (91.5–99.7)	93 (83.3–97.2)
Nonrespiratory (31)	4	0	0	8	0	2	2	3	0	2	6	4	100 (75.8–100)	69.2 (42.3–87.3)	100 (79.6–100)	90 (59.6–98.2)
CSF (7)	1	0	0	1	0	0	0	3	0	1	0	1	100 (34.2–100)	75 (30.1–95.4)		
Gastric aspirate (5)	1	0	0	3	0	0	0	0	0	0	1	0	100 (51.0–100)	100 (20.7–100)		
Urine (3)	0	0	0	0	0	0	0	0	0	0	3	0		100 (20.7–100)		
Fluid (6)	0	0	0	1	0	0	0	0	0	0	2	3	100 (20.7–100)	100 (34.2–100)		
Miscellaneous (10)	2	0	0	3	0	2	2	0	0	1	0	0	100 (56.5–100)	66.7 (20.8–93.9)	100 (61–100)	

[a] All six cultures grew *Mycobacteria* other than *M. tuberculosis*.
[b] Seven of these cultures grew *Mycobacteria* other than *M. tuberculosis*.
[c] Amended results based on clinical diagnosis as the gold standard. Clinical diagnosis was based on patient symptoms, chest X-rays, and use of anti-TB treatment.
[d] CI, confidence interval.
[e] +, positive; −, negative.

TABLE 3. Analysis of discrepant results among the MTD assay, Xpert MTB/RIF assay, and culture

Specimen type	Result[a]				Clinical diagnosis[b]
	Xpert MTB/RIF	MTD	Smear	Culture	
Sputum	Pos	Neg	Neg	Pos	Active TB
Sputum	Pos	Pos	Pos	Neg	Active TB
Sputum	Pos	Equivocal	Pos	Neg	Active TB
Sputum	Pos	Pos	Pos	Neg	Active TB
Sputum	Pos	Equivocal	Neg	Neg	Active TB
Sputum	Pos	Neg	Neg	Neg	No TB
Sputum	Neg	Pos	Neg	Pos	Active TB
Sputum	Neg	Pos	Neg	Pos	Active TB
Sputum	Neg	Pos	Neg	Pos	Active TB
Sputum	Neg	Equivocal	Neg	Pos	Active TB
Sputum	Neg	Neg	Neg	Pos	Active TB
Sputum	Neg	Pos	Neg	Pos	Active TB
Sputum	Invalid	Pos	Neg	Neg	No TB
BAL	Neg	Pos	Neg	Neg	Active TB
BAL	Neg	Pos	Neg	Neg	No TB
CSF	Neg	Equivocal	Neg	Pos	Active TB
CSF	Neg	Pos	Neg	Neg	No TB
Biopsy	Pos	Pos	Pos	Neg	BCG lymphadenitis
Biopsy	Pos	Pos	Pos	Neg	Active TB
Biopsy	Pos	Pos	Pos	Neg	Active TB
Biopsy	Pos	Pos	Neg	Neg	Active TB

[a] Abbreviations: Pos, positive; Neg, negative.
[b] Clinical diagnosis was based on patient symptoms, chest X-rays, and use of anti-TB treatment.

tected on a luminometer, with readings interpreted according to the manufacturer's recommendations. The MTD protocol for tissue specimens was modified compared with that of respiratory samples. For minced tissue samples, a nucleic acid extraction step was performed using MasterPure Complete DNA and RNA purification kit (Epicentre Biotechnologies, Madison, WI), according to the manufacturer's instructions. The MTD protocol then proceeded with the amplification step (see above) using kit-extracted DNA.

Equivocal and invalid results obtained from either assay were excluded from sensitivity and specificity calculations. The McNemar test was used to calculate the statistical difference between the Xpert MTB/RIF and MTD assays. P values of <0.05 (two sided) were considered statistically significant.

For the respiratory samples tested, 43 of the 45 (95.6%) smear- and culture-positive samples were positive by the Xpert MTB/RIF assay, while the MTD assay identified 44 (97.8%) of these (Tables 1 and 2). Of the smear-negative and culture-positive samples, Xpert MTB/RIF detected *M. tuberculosis* complex in 13 of the 22 samples (59%), while 17 (77.3%) were identified as positive as by MTD. Lower sensitivities observed for smear negative samples were a consistent and expected result for the molecular tests (1, 2, 5). Out of the 55 smear and culture-negative samples, 2 tested positive by Xpert MTB/RIF and 3 were positive by MTD (Tables 1 and 2). Invalid reads for respiratory samples using the Xpert MTB/RIF assay accounted for 6.9% (9/131), while the equivocal results for MTD were 8.4% (11/131). There was no significant difference in the number of equivocal and invalid samples obtained from both assays ($P = 0.80$). Molecular assays still increase case detection rates compared with smear microscopy, since of the 67 respiratory samples that grew *M. tuberculosis*, the Xpert MTB/RIF and MTD assays positively identified 56 and 61 specimens, respectively, whereas smear microscopy identified only 45 of these. A previous Xpert MTB/RIF study found that 3.7% of the samples were indeterminate (1), although it is not clear why we had a higher rate in our setting.

For the nonrespiratory specimens, all smear- and culture-positive specimens ($n = 4$) were positive by the Xpert MTB/RIF and MTD assays (Tables 1 and 2). Of the 11 smear-negative and culture-positive samples, 10 were detected by Xpert MTB/RIF, and 8 were picked up by MTD (Tables 1 and 2). Among the 14 smear- and culture-negative samples, 1 was positive by the Xpert MTB/RIF assay, and 2 were positive by the MTD assay (Tables 1 and 2). There were no invalid results using the Xpert MTB/RIF assay, while 19.4% (6/31) of the MTD results were equivocal, resulting in a significant difference in the number of equivocal and invalid samples obtained from each assay ($P = 0.031$). However, when all sample types were taken into consideration, there was no significant difference ($P = 0.14$) in the number of indeterminate samples for the Xpert and MTD assays.

In 6 smear-positive and culture-negative samples and 7 smear- and culture-negative samples, mycobacteria other than *M. tuberculosis* (MOTT) were isolated (3 samples with *Mycobacterium fortuitum*, 4 samples with *Mycobacterium avium*, 2 samples with *Mycobacterium abscessus*, and 4 samples not identified to the species level) (Tables 1 and 2). There were 8 culture-negative isolates, of which 6 were smear positive and all were positive by molecular assays (Table 3). Two of these specimens were obtained from patients who were already on antituberculosis treatment.

Discrepant results for both of the molecular assays were resolved by reviewing the clinical findings (Table 3), and the sensitivity and specificity of both assays were adjusted accordingly (Tables 1 and 2). Taking into account all specimen types and using culture as the gold standard, the overall sensitivity and specificity of the Xpert assay were 90.9 and 89.5%, respec-

tively. For the MTD assay, the sensitivity and specificity were 97.3 and 87.1%, respectively. The differences in the sensitivities and specificities of the two assays were not significant, yielding *P* values of 0.37 and 0.61, respectively, by the McNemar test. There was very good agreement between the Xpert MTB/RIF and MTD assays, as the kappa coefficient was calculated to be 0.86.

In this study, a single respiratory sample isolate was detected as RIF resistant by the Xpert assay. Sequencing through the *rpoB* gene (8) revealed a 526(His→Cys) codon mutation. The limited number of rifampin-resistant isolates present in this study hindered a proper assessment of the efficacy of the Xpert MTB/RIF assay in detecting rifampin resistance.

In our laboratory context, the MTD test has a performance similar to that of the Xpert assay. The MTD test is, however, a fully manual test. In contrast, the Xpert MTB/RIF assay is a self-contained, integrated test that offers minimal hands-on time, with low potential for PCR contamination. The concurrent detection for RIF-associated mutations is also an added advantage (1).

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