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Performance of Xpert MTB/RIF RUO Assay and IS*6110* Real-Time PCR for *Mycobacterium tuberculosis* Detection in Clinical Samples[▽]

Melissa B. Miller,[1]* Elena B. Popowitch,[1]† Michael G. Backlund,[1]† and Edward P. C. Ager[2]

Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine,[1] and Clinical Microbiology-Immunology Laboratory, University of North Carolina Health Care,[2] Chapel Hill, North Carolina

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The Cepheid Xpert MTB/RIF research-use-only (RUO) assay and a laboratory-developed test (LDT) targeting IS*6110* were evaluated and compared to mycobacterial culture as the gold standard. The performance characteristics of both molecular assays were determined by using 112 specimens from 90 patients, including 89 pulmonary specimens and 23 extrapulmonary specimens. Of the specimens tested, 37 (33%) were culture positive for the *Mycobacterium tuberculosis* complex; 29 were pulmonary, and 8 were extrapulmonary. Of these culture-positive specimens, 83% of the pulmonary specimens and 50% of the extrapulmonary specimens were smear positive. There was complete concordance between the smear-positive culture-positive specimens, independent of the anatomical site (100% sensitivity). The sensitivity of the MTB/RIF RUO assay for smear-negative specimens was 60% for pulmonary and 75% for extrapulmonary specimens, while the IS*6110* LDT sensitivities were 40% and 0%, respectively. There was also complete concordance among the culture-negative specimens tested. Both assays showed 95% specificity, with four culture-negative specimens testing as positive. A review of patient records indicated that there was a high likelihood of the presence of *M. tuberculosis* complex DNA in the false-positive specimens. Biosafety analysis was performed and showed an acceptable reduction in organism viability using the processing methods described above. Both molecular assays are suitable for the detection of *M. tuberculosis* isolates in smear-positive pulmonary and extrapulmonary specimens, while the sensitivity of the detection of *M. tuberculosis* isolates in smear-negative specimens was variable.

Tuberculosis remains an important worldwide health concern, with over 9 million new cases and approximately 2 million deaths annually. Currently in the United States, there is only one FDA-approved nucleic acid amplification test (NAAT) for the *Mycobacterium tuberculosis* complex, the Gen-Probe Amplified Mycobacterium Tuberculosis Direct (MTD) test (San Diego, CA). The Roche Amplicor *Mycobacterium tuberculosis* complex (MTB) test was discontinued in 2010. A recently introduced research-use-only (RUO) NAAT for the *M. tuberculosis* complex is the GeneXpert MTB/RIF assay (Cepheid, Sunnydale, CA), which simultaneously detects the presence of the *M. tuberculosis* complex and rifampin resistance directly from respiratory specimens. The Xpert MTB/RIF assay is a semiquantitative, nested, real-time PCR designed to amplify a 192-bp segment of the *M. tuberculosis* complex *rpoB* gene using five overlapping molecular beacon probes that span the entire 81-bp rifampin-resistance-determining region. The MTB/RIF assay also contains an internal control in which the detection of lyophilized *Bacillus atrophaeus* subsp. *globigii* spores serves as an internal processing and amplification control. Previous work has demonstrated that the Xpert MTB/RIF assay displays high percentages of sensitivity and specificity for the detection of *M. tuberculosis* complex isolates, particularly in smear-positive respiratory specimens. Previously reported sensitivities for smear-positive pulmonary specimens range from 95% to 100% (1, 4, 8, 11, 16), while sensitivities for smear-negative pulmonary specimens vary between 55% and 75% (1, 4, 8, 11, 13, 16). Extrapulmonary specimens have shown 37% to 96% sensitivity depending on the smear result and specimen type (1, 5, 9, 17). The specificities reported in all of the above-mentioned studies are excellent, ranging from 95% to 100%.

The interrogation of IS*6110* has long been a valuable tool for studying the epidemiology of the *M. tuberculosis* complex due to its variability in copy number and genomic location (15). In addition, it is a favored target for laboratory-developed tests (LDTs) aimed at detecting *M. tuberculosis* in patient samples, due largely to its multiple genomic copies and, therefore, increased sensitivity. Mdivani et al. previously described an IS*6110* LDT applied to sputa obtained from patients receiving anti-*M. tuberculosis* therapy (12). We adapted this LDT for use in the current comparative evaluation. When analyzing a different IS*6110* LDT, Armand et al. previously reported a 100% sensitivity for smear-positive pulmonary and extrapulmonary specimens and 68% and 71% sensitivities for smear-negative pulmonary and extrapulmonary specimens, respectively (1).

By use of archived processed pulmonary and extrapulmonary specimens, the Cepheid Xpert MTB/RIF RUO assay and an IS*6110* LDT were evaluated and compared to mycobacterial culture as the gold standard. The performance characteristics measured were analytical sensitivity and specificity, clinical sensitivity and specificity, and biosafety.

* Corresponding author. Mailing address: UNC School of Medicine, Department of Pathology and Laboratory Medicine, Campus Box 7525, Chapel Hill, NC 27599-7525. Phone: (919) 966-3723. Fax: (919) 966-0486. E-mail: mbmiller@unch.unc.edu.

† E.B.P. and M.G.B. contributed equally to the manuscript.

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TABLE 1. Clinical specimens used for comparative evaluation by *M. tuberculosis* complex culture result

Specimen	Sample types (no. of specimens)	
	M. tuberculosis complex negative ($n = 75$)	*M. tuberculosis* complex positive ($n = 37$)
Pulmonary	Bronchial brush (1), bronchial wash (5), bronchoalveolar lavage (11), expectorated sputum (24), induced sputum (17), lung biopsy specimen (4), nasopharyngeal aspirate (1), pleural fluid (2), sputum (unspecified) (3), tracheal aspirate (2)	Bronchial wash specimen (1), bronchoalveolar lavage fluid (3), expectorated sputum (9), induced sputum (15), pleural biopsy specimen (1), pleural fluid (1), sputum (unspecified) (1)
Extrapulmonary	Brain biopsy specimen (2), lymph node biopsy specimen (2), sinus biopsy specimen (1)	Endometrial biopsy specimen (1), lymph node biopsy specimen (1), neck abscess aspirate (1), neck mass biopsy specimen (1), retroperitoneal fluid (1), stool (1)

MATERIALS AND METHODS

Study specimens. Specimens ($n = 112$) used in this study were collected from 90 patients between August 2007 and April 2011 and stored at −70°C. Specimens from 19 unique anatomical sites were tested (Table 1). Thirty-seven specimens (33%) were positive for the *M. tuberculosis* complex by culture. Forty-seven (42%) of the specimens had *M. tuberculosis* NAAT testing performed by a reference laboratory per physician order (Gen-Probe MTD assay to May 2009 and Mayo Medical Laboratories LDT thereafter).

Mycobacterial culture and smear. All nonsterile specimens and normally sterile specimens (biopsy specimens and aspirates) that were visibly bloody or purulent were subjected to digestion and decontamination using the BBL MycoPrep system (NALC [*N*-acetyl-L-cysteine]-NaOH) according to the manufacturer's instructions (Becton Dickinson, Sparks, MD). After decontamination, specimens were concentrated by centrifugation at 3,200 × *g* for 15 min and resuspended in 2 ml of sterile 0.067 M phosphate buffer (10). All specimens used in this study were fully processed by digestion, decontamination, and concentration. Processed specimen sediment was used to inoculate a Becton Dickinson Bactec MGIT tube and a Lowenstein-Jensen slant (Remel, Lenexa, KS), which were incubated for 6 and 8 weeks, respectively. Smears of processed sediment were stained with BBL TB Auramine-Rhodamine T (Becton Dickinson) and reported as previously described (10).

Nucleic acid extraction. Total nucleic acids were extracted from processed samples (phosphate buffer matrix) by using the Qiagen (Valencia, CA) BioRobot EZ1 instrument. Briefly, specimens were treated with Qiagen buffer G2 at a ratio of 4:1 (400 μl G2 and 100 μl specimen), boiled at 100°C for 30 min, and centrifuged at 13,000 × *g* for 3 min. A 200-μl aliquot was removed from the supernatant for extraction by using the Qiagen BioRobot EZ1 instrument. The DNA tissue kit, DNA bacterial card, and bacterial protocol were utilized. Total nucleic acids were eluted in 50 μl of elution buffer, and 15 μl of each extract was used for nucleic acid amplification.

IS*6110* real-time LDT. The IS*6110* primers and probe sequences were previously described (12). For the LDT, 15 μl of extracted nucleic acids was added to 35 μl of a reaction mixture containing 900 nM each primer, 200 nM fluorophore hydrolysis probe, and 25 μl of TaqMan Universal PCR Master Mix (Life Technologies, Carlsbad, CA). Human albumin was amplified separately as an internal control using the same concentrations and volumes described above (7). Cycling conditions were 1 cycle at 50°C for 2 min and 1 cycle at 95°C for 10 min followed by two-step PCR (40 cycles of 15 s at 95°C and then 1 min at 60°C). Amplification and analysis were performed with the Applied Biosystems 7500 real-time PCR system. Patient sample concentrations were determined by using a standard curve derived from quantified DNA obtained from Advanced Biotechnologies (Columbia, MD).

Xpert MTB/RIF RUO assay. The GeneXpert instrument (Cepheid, Sunnyvale, CA) and Xpert MTB/RIF assay were described in detail previously (3, 4, 8). According to the manufacturer's RUO package insert, the sample reagent was added to a decontaminated and concentrated specimen at a 3:1 ratio, the sample container was agitated twice during a 15-min incubation at room temperature, and 2 ml of the inactivated material was transferred into the Xpert cartridge.

Limit of detection and precision. The lower limit of detection (LLD) was determined by two methods. First, serial dilutions of *M. tuberculosis* H37Rv quantitated bacterial DNA (Advanced Biotechnologies) with a DNA copy number of 1.3×10^4 DNA copies/μl (determined by the manufacturer's in-house real-time PCR) was used unextracted. Second, *M. tuberculosis* (ATCC 27294) was grown in 7H11 broth (Remel, Lenexa, KS) for 7 days and then diluted in sterile saline, plated onto 7H11 agar (Remel), and extracted. Dilutions were stored at 4°C. CFU per ml were calculated based on the serial plating of dilutions prior to nucleic acid extraction. Precision calculations were performed according to CLSI guidelines (6), using either triplicate testing over three consecutive days or five replicates over five consecutive days, as indicated. All replicates had to be positive to define the LLD.

Analytical specificity. Bacterial strains were obtained from clinical cultures and ATCC stocks. Sterile saline solutions of bacterial strains were prepared at a 2.0 McFarland standard, and pools of three to five organisms were tested. For adenovirus, cytomegalovirus, enterovirus, herpes simplex viruses 1 and 2, metapneumovirus, rhinovirus, and varicella-zoster virus, stocks were obtained by harvesting cell cultures at a ~50% cytopathic effect. For Epstein-Barr virus (Acrometrix, Benicia, CA), human herpesvirus 6 (Advanced Biotechnologies), and parvovirus (ZeptoMetrix, Buffalo, NY), the estimated quantities tested were 2.5×10^4 copies, 1.2×10^9 viral particles, and 1.0×10^6 IU, respectively. The remaining respiratory viruses tested were ZeptoMetrix qualitative controls. Viruses were tested in pools of 5 to 8.

Biosafety assessment. Two experiments were conducted to assess postprocessing viability. To test the extraction method used for the IS*6110* LDT, 10 smear-positive (3+) *M. tuberculosis* complex culture-positive samples were plated onto 7H11 agar after the centrifugation step. In addition, the *M. tuberculosis* complex control strain (ATCC 27294) was also processed through centrifugation and plated separately onto five plates over 5 days. To assess the extraction method used for the GeneXpert assay, 3.4×10^9 CFU/ml of the *M. tuberculosis* complex control strain in sterile saline solution, 1.1×10^6 CFU/ml of a patient-sourced *M. tuberculosis* complex isolate in sterile saline solution, and a sedimented patient sample previously smear positive (2+ quantification) and culture positive for *M. tuberculosis* were obtained. The patient sample concentration based on quantification by real-time IS*6110* PCR was 3.7×10^4 CFU/ml. On three consecutive days, each sample was processed according to the Xpert MTB/RIF RUO package insert, and 100 μl of each lysate was then plated onto 7H11 medium in triplicate.

Statistical analysis. *P* values were determined for independent samples by using a two-tailed Student *t* test. Confidence intervals (CIs) were determined by using GraphPad software (GraphPad, La Jolla, CA).

This study was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill.

RESULTS

Analytical sensitivity and specificity. For the IS*6110* LDT, the LLD using the commercial quantified DNA was determined to be 0.2 copies per reaction or 3.3 copies per ml, with a mean cycle threshold (C_T) of 35.6 (95% CI, 34.9 to 36.3). The LLD for the ATCC strain of *M. tuberculosis* was determined to be 1,241 CFU/ml, with a mean C_T value of 35.4 (95% CI, 35.1 to 35.7). Precision testing of the 1,241 CFU/ml over 5 days demonstrated an intrarun precision of 0.56 C_T (coefficient of variation [CV], 1.6%), and the total precision was 0.75 C_T (CV, 2.1%). Lower limit analysis of the Xpert MTB/RIF assay was performed using only the *M. tuberculosis* ATCC strain, as the quantified DNA could not be analyzed by the GeneXpert system. The Xpert LLD was determined to be 786 CFU/ml, with

TABLE 2. Organisms tested for analytical specificity[a]

Organism tested (no. of isolates)
Bacteria
Acinetobacter baumannii (1)
Bordetella holmesii (1)
Bordetella parapertussis (1)
Bordetella pertussis (1)
Haemophilus influenzae (1)
Klebsiella pneumoniae (1)
Moraxella catarrhalis (2)
Mycobacterium avium (1)
Mycobacterium fortuitum (1)
Mycobacterium gordonae (1)
Mycobacterium intracellulare (1)
Mycobacterium kansasii (1)
Mycobacterium scrofulaceum (1)
Oropharyngeal flora (3)
Pseudomonas aeruginosa (1)
Staphylococcus aureus (4)
Staphylococcus spp., coagulase negative (2)
Streptococcus agalactiae (1)
Streptococcus pneumoniae (1)
Streptococcus pyogenes (2)
Streptococcus sp., viridans group (1)
Viruses
Adenovirus (1)
Cytomegalovirus (1)
Epstein-Barr virus (1)
Enterovirus (2)
Herpes simplex viruses 1 and 2 (1)
Human herpesvirus 6 (1)
Influenza A virus (1)
Influenza B virus (1)
Metapneumovirus (1)
Parainfluenza viruses 1, 2, and 3 (1)
Parvovirus (1)
Respiratory syncytial viruses A and B (1)
Rhinovirus (1)
Varicella-zoster virus (1)
Clinical specimens
Actinomyces sp. (1)
Aspergillus spp. (5)
Candida spp. (4)
Haemophilus sp. (1)
Klebsiella pneumoniae (1)
Mycobacterium abscessus group (2)
Mycobacterium avium complex (14)
Mycobacterium fortuitum group (1)
Mycobacterium gordonae (3)
Mycobacterium nonchromogenicum (1)
Nocardia spp. (4)
Penicillium spp. (3)
Pseudomonas aeruginosa (5)
Serratia sp. (1)
Staphylococcus aureus (5)
Streptococcus agalactiae (1)

[a] Bacteria and viruses were tested directly at high titers. Clinical specimens are organisms that were cultured from the *M. tuberculosis* complex-negative specimens.

a mean C_T of 36.4 (95% CI, 35.9 to 36.9). The within-run precision of the 786 CFU/ml performed over 3 days was 0.86 C_T (CV, 2.1%), and the total precision equaled 0.94 C_T (CV, 2.4%). Previous studies have found the LLD of the Xpert assay to be 131 CFU/ml using spiking experiments with residual sputa (8).

TABLE 3. Clinical sensitivity and specificity of the Xpert MTB/RIF RUO assay and the IS*6110* LDT using culture as the gold standard

Test	% value (no. of specimens positive by NAAT/no. of specimens positive by culture)	
	Xpert MTB/RIF RUO assay	IS*6110* LDT
All sites (n = 112)		
Sensitivity, all	92 (34/37)	81 (30/37)
Sensitivity, smear positive	100 (28/28)	100 (28/28)
Sensitivity, smear negative	67 (6/9)	22 (2/9)
Specificity	95 (71/75)	95 (71/75)
Pulmonary sites (n = 89)		
Sensitivity, all	93 (27/29)	90 (26/29)
Sensitivity, smear positive	100 (24/24)	100 (24/24)
Sensitivity, smear negative	60 (3/5)	40 (2/5)
Specificity	97 (58/60)	97 (58/60)
Extrapulmonary sites (n = 23)		
Sensitivity, all	88 (7/8)	50 (4/8)
Sensitivity, smear positive	100 (4/4)	100 (4/4)
Sensitivity, smear negative	75 (3/4)	0 (0/4)
Specificity	87 (13/15)	87 (13/15)

Table 2 lists the bacteria and viruses that were tested to assess the analytical specificities of both molecular assays. No cross-reactivity was observed. In addition, the specimens used in this study were also culture positive for a variety of organisms, which are listed in Table 2. None of these clinical specimens was positive for the *M. tuberculosis* complex by either molecular assay.

Clinical sensitivity. Of the 112 specimens used in this study, 37 (33%) were culture positive for the *M. tuberculosis* complex; 9 (24%) were smear negative, and 28 (76%) were smear positive at the following quantities: 1+ smear (n = 5), 2+ smear (n = 8), and 3+ smear (n = 15). Both the IS*6110* LDT and the Xpert MTB/RIF assay detected all smear-positive specimens, which included 24 respiratory specimens (Table 1) and 4 nonrespiratory specimens, including a neck abscess aspirate, stool specimen, lymph node biopsy specimen, and retroperitoneal aspirate. The reference laboratory NAAT was performed on 20 of the smear-positive specimens, including 3 of the nonrespiratory specimens (neck abscess aspirate, stool specimen, and lymph node aspirate), and all were positive.

There was, however, a difference in the performances of Xpert MTB/RIF assay and the IS*6110* LDT for the smear-negative culture-positive specimens (n = 9) (Table 3). The IS*6110* LDT detected only two of nine specimens (22%), while the Xpert MTB/RIF assay detected six (67%). The three specimens missed by both assays were expectorated sputum, induced sputum, and a pleural biopsy specimen. In addition, the IS*6110* LDT missed a bronchial wash specimen, a neck mass biopsy specimen, an endometrial biopsy specimen, and pleural fluid. None of the smear-negative culture-positive specimens were tested by the reference laboratory molecular test. The time to culture positivity was calculated for each of the following groups: positive by both tests, positive by the Xpert assay only, and negative by both tests. There was not a statistical difference in the time to culture positivity for any comparison. The time of freezer storage was also compared for each group.

Culture-positive specimens that were negative by both assays ($n = 3$) were on average 915 days old (95% CI, 762 to 1,068), whereas those detected by at least one of the assays ($n = 6$) were on average 443 days old (95% CI, 116 to 770). This difference was statistically significant ($P = 0.03$). Thus, the length of the specimen storage time may have contributed to the decrease in sensitivity seen with smear-negative specimens, but it is also well established that smear-negative specimens have a lower sensitivity due to the lower organism load present in the sample.

Clinical specificity. Seventy-one of 75 specimens (95%) previously determined to be culture negative for the *M. tuberculosis* complex were negative by both the IS*6110* LDT and the Xpert MTB/RIF assays. Of the *M. tuberculosis* culture-negative specimens, 30 (40%) were smear positive and either grew another organism ($n = 22$) (see "Analytical sensitivity and specificity" above) or showed no growth ($n = 8$). The four false-positive specimens included brain (negative smear), induced sputa ($n = 2$) (1+ and 3+ smears), and lymph node aspirate (1+ smear).

Discrepant analysis. The four seemingly false-positive specimens were obtained from three patients. A clinical chart review of these three patients indicated the following. Patient 1 had a brain biopsy specimen that was smear negative and culture negative but positive by both the IS*6110* LDT and Xpert MTB/RIF assay. The reference laboratory NAAT was also positive. The patient had a history of noncavitary pulmonary tuberculosis 6 months prior to presenting with seizures and an enlarging brain lesion. The patient was receiving rifampin and isoniazid at the time of presentation, although drug levels were determined to be low. The clinical diagnosis was a brain tuberculoma, which was treated and improved. Patient 2 had two induced sputa that were smear positive but culture negative. Both specimens were also positive by the reference laboratory NAAT. This patient had a history of pulmonary tuberculosis for which therapy was completed 14 months earlier. The patient presented with a 1-week history of fever, night sweats, and dark urine. Both the patient's sister and daughter were currently being treated for tuberculosis. The final diagnosis of the patient was community-acquired pneumonia. However, the patient received four-drug *M. tuberculosis* therapy through the health department until sputa were smear negative. Patient 3 was recently diagnosed with uterine carcinoma. She presented with hilar lymphadenopathy not responding to her current chemotherapy. The lymph node aspirate was smear positive, culture negative, and NAAT positive by both the IS*6110* LDT and Xpert MTB/RIF assay but negative by a reference laboratory NAAT. Of note, the referred NAAT was performed on the native specimen, while the evaluation NAATs were performed on a decontaminated and concentrated specimen. The pathology of the lymph node showed both necrotizing and nonnecrotizing granulomatous inflammation. The patient's father was diagnosed with active tuberculosis 3 months prior, and the patient had recently converted her purified protein derivative (PPD) reaction. The patient had no lung abnormalities, and thus, three induced sputa were negative for the *M. tuberculosis* complex by smear and culture. The patient received four-drug therapy for tuberculosis. Based on the above-described clinical findings, these culture-negative, NAAT-positive specimens are likely truly positive for *M. tuberculosis* complex DNA, although the organisms may not have been viable.

Biosafety. To test the biosafety of the processing protocol for the IS*6110* LDT, processed smear-positive patient specimens ($n = 10$) and the *M. tuberculosis* control strain were cultured prior to the placement of the lysates onto the extraction equipment to assess organism viability. No growth was observed for any of the replicates after 3 months of incubation. To ascertain safety for the extraction method used for the Xpert MTB/RIF assay, we assessed the viability of high titers of an *M. tuberculosis* control strain, a patient *M. tuberculosis* isolate, and a sedimented positive patient sample. Cultures of the lysate prior to pipetting into the GeneXpert cartridge showed no growth except for two of three replicates for one sedimented patient specimen. Cultures indicated that 30 CFU/ml survived the lysis process. These data indicate a complete inactivation of the high-concentration *M. tuberculosis* complex solutions and a 1,000-fold reduction in the viability of *M. tuberculosis* in the patient sample. This is in line with previously reported standards (14). Likewise, Banada et al. previously demonstrated a >6-log-unit reduction in viability when Xpert lysates were tested (2).

DISCUSSION

A summary of the sensitivity and specificity data for the IS*6110* LDT and the Xpert MTB/RIF assay can be found in Table 3. Both assays detected smear-positive respiratory and smear-positive nonrespiratory specimens (neck abscess aspirate, stool sample, lymph node biopsy specimen, and retroperitoneal aspirate) accurately. The overall sensitivity was higher for the Xpert MTB/RIF assay due to a better detection of smear-negative specimens, although this was not statistically significant ($P = 0.18$). The LLD was observed to be lower for the Xpert MTB/RIF assay than for the IS*6110* LDT (786 CFU/ml and 1,241 CFU/ml, respectively), which likely contributes to the improved sensitivity of the Xpert assay. However, the LLDs were determined by using a sterile saline matrix as opposed to negative processed sediments. While our reported LLD values may not truly reflect the actual values for clinical specimens (and this may vary by specimen type), the LLD analysis of the two molecular tests provides a means for comparing the relative analytical sensitivities of the assays. Using quantification by real-time IS*6110* PCR, the range of *M. tuberculosis* loads in patient samples was 187 to 2.0×10^{12} CFU/ml, with a median of 1.6×10^{6} CFU/ml and mean of 1.0×10^{11} CFU/ml. Specimens that were Xpert MTB/RIF positive and IS*6110* LDT negative had estimated quantities of *M. tuberculosis* of 996 to 4,000 CFU/ml. The specificities of both tests relative to culture were 95%, but a discrepant analysis was performed by chart review. Clinical history and patient management indicated that although these patients had culture-negative specimens, they likely had *M. tuberculosis* complex DNA present in the sample. It is well accepted that molecular-based approaches to the diagnosis of infectious disease will lead to positive DNA results in the absence of viable organisms. Three of the four patient samples were also smear positive. Negative and positive predictive values could not be calculated because this was a study of convenience samples, not

preserving the incidence of *M. tuberculosis* complex detection at our institution (~1%).

The rifampin screening component of the MTB/RIF assay was not fully evaluated during the study period due to the low incidence of rifampin-resistant *M. tuberculosis* in our patient population. Of the specimens tested, only four were positive for rifampin resistance by the Xpert assay. Three were rifampin resistant by phenotypic methods, while one was phenotypically rifampin susceptible. The Centers for Disease Control and Prevention Tuberculosis Molecular Detection of Drug Resistance service identified a silent mutation (Phe514Phe) in the rifampin-susceptible *M. tuberculosis* complex isolate. Based on the specimens tested in this study, the sensitivity of the rifampin resistance component of the assay was 100%. Further testing is warranted to fully assess the positive predictive value of rifampin resistance detection in areas of low prevalence.

Limitations of this study include the retrospective design, which was necessary due to the low prevalence of *M. tuberculosis* in our community. While cultures were performed in real time, the molecular analysis was done much later. As a result of the low prevalence, specimens were stored for an extensive time prior to testing. It appears that the length of storage had an impact on the sensitivity of the IS*6110* LDT. A prospective analysis of smear-negative specimens would provide a clearer view of the true sensitivity. Also, only processed (digested/decontaminated and concentrated) specimens were used in this evaluation. The waiting time for specimen processing increases the time to a result for both molecular tests. Ideally, direct unprocessed specimens should be tested for the greatest impact of results. Notably, the Xpert RUO package insert provides instructions for direct specimen processing. For assessing the performance characteristics of extrapulmonary specimens, we had a paucity of culture-positive specimens (n = 8), and only 50% of them were smear positive. Thus, the calculated performance characteristics with extrapulmonary specimens may not be representative of a larger sample size. Lastly, we used culture as the gold standard. While this is the most convenient reference method, it may not be the best standard, as indicated by a discrepant analysis performed by chart review. Since our reference laboratory used both the GenProbe MTD assay and an LDT during the course of this study, we could not include the analysis of an FDA-approved NAAT performed on study specimens. Furthermore, a reference NAAT was performed only upon physician request, leading to an incomplete data set for comparison.

The Xpert MTB/RIF RUO assay and the IS*6110* LDT performed identically for all smear-positive specimens (pulmonary and extrapulmonary). The MTB/RIF assay detected more of the smear-negative culture-positive specimens than did the IS*6110* LDT, likely due to differences in the analytical sensitivities of the assays. Although the sensitivity for smear-negative specimens with either assay is suboptimal, the Xpert MTB/RIF sensitivity is decent, at 67%, compared to 22% for the LDT (Table 3). In the context of high test specificity, a rapid positive result for a smear-negative respiratory specimen would positively impact patent care and public health efforts. However, multiple sequential tests may be needed to increase the sensitivity to provide actionable negative results for smear-negative specimens (4).

The Xpert assay has a 15-min hands-on time and a 113-min run time, while the IS*6110* LDT has a 30-min hands-on time and a 147-min run time (extraction and amplification). Unlike the IS*6110* LDT, which requires batch processing and molecular proficiency, the MTB/RIF assay is a random-access assay and does not require molecular expertise to perform. There is a great need for rapid, accurate *in vitro* diagnostic products for the early diagnosis of tuberculosis, even in low-prevalence areas such as the United States. Our evaluation of the Xpert MTB/RIF RUO assay demonstrates that such a test is realistic.

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