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Cepheid GeneXpert MTB/RIF Assay for *Mycobacterium tuberculosis* Detection and Rifampin Resistance Identification in Patients with Substantial Clinical Indications of Tuberculosis and Smear-Negative Microscopy Results[▽]

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The GeneXpert MTB/RIF assay was evaluated with microscopically negative and positive pulmonary and extrapulmonary specimens from patients with substantial clinical indications for tuberculosis. For the pulmonary samples, the sensitivity, specificity, and positive and negative predictive values were 90.6%, 94.3%, 93.5%, and 91.7%, and for the extrapulmonary samples, they were 100%, 91.6%, 50%, and 100%, respectively. For microscopically negative specimens, the respective values were 86.3%, 93%, 79%, and 95.6%. The assay correctly detected rifampin resistance in all but one specimen, which harbored a mixed population. The GeneXpert assay was highly effective for tuberculosis diagnosis and identification of rifampin-resistant strains in smear-negative samples.

Tuberculosis (TB) remains a major public health problem, accounting for more than 9.4 million incident cases and 1.3 million deaths every year, worldwide (10). The emergence and spread of multidrug (MDR) and extensively (XDR) drug-resistant *Mycobacterium tuberculosis* complex (MTBC) strains poses significant challenges to disease control (11). In order to overcome conventional methods' low sensitivity and diagnostic delays, nucleic acid amplification (NAA) tests have been introduced. The NAA tests' sensitivities are high for specimens that are acid-fast bacillus (AFB) microscopy positive but lower for AFB-negative specimens (3). The identification of mutations associated with drug resistance depends on additional NAA tests, whose application on clinical samples is indicated only for AFB-positive specimens.

The recently introduced Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) detects the presence of MTBC DNA and its susceptibility to rifampin (RMP) in a single reaction (4, 8). Monoresistance to RMP is rare; however, >90% of RMP-resistant isolates also exhibit resistance to isoniazid (INH). Therefore, the detection of RMP resistance may serve as a surrogate marker for MDR *M. tuberculosis* (9). The assay is based on a heminested real-time PCR (RT-PCR) that targets the *rpoB* gene hot spot region. Any deviation from the wild-type sequence resulting in a delay in the appearance of the signal exceeding a predetermined ΔC_T value (>3.5), between the earliest and latest cycle threshold (C_T) values, is reported as RIF resistant. The test is carried out within 2 h in a disposable cartridge. The only manual step is the mixing of a bactericidal buffer with the sample prior to addition to the cartridge. This preamplification step reduces the viability of MTBC organisms, making the assay suitable for use near patients in settings with limited biocontainment facilities (1).

A prospective study was conducted between September 2009 and May 2010 at the National Reference Laboratory for Mycobacteria (NRLM), Athens, Greece, in order to assess the performance of the Xpert MTB/RIF assay in AFB-negative respiratory and nonrespiratory specimens in a routine hospital laboratory setting. Specimens were selected from patients with strong clinical indications for TB. A small number of AFB-positive specimens was also included to serve as positive controls. Specimens were processed by the standard *N*-acetyl-L-cysteine and sodium hydroxide method. A smear of the processed sediment was prepared and examined for the presence of AFB. Solid (Lowenstein-Jensen [LJ]) and liquid (Bactec MIGT 960) culture media were also inoculated. Bacterial colonies were investigated with AFB smear-staining microscopy, the GenoType MTBDR*plus* assay (Hain Lifescience, GmbH, Nehren, Germany) for the identification of mutations involved in rifampin and isoniazid resistance, and the GenoType mycobacterium CM/AS assay (Hain, Lifescience GmbH, Nehren, Germany) for the identification of nontuberculous mycobacteria. The MTBDR*plus* results were confirmed by drug susceptibility testing (DST) by the proportion method on LJ culture medium and/or Bactec MGIT960, using the standard critical concentrations of 40 μg/ml and 1 μg/ml RMP, respectively.

The specimens analyzed and the culture results are presented in Table 1 (121 samples, 80 pulmonary and 41 extrapulmonary, from 108 patients; 105 AFB negative, 1 suspicious, and 15 AFB positive). Culture data were available for 107 samples. Overall, 35/107 (32.7%) cultures were positive, of which 34/35 belonged to the MTBC and one was identified as *Mycobacterium avium*. Twelve, 1, and 21 MTBC strains were isolated from AFB-positive, AFB-suspicious, and AFB-negative samples, respectively (3 extrapulmonary and 31 pulmonary).

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TABLE 1. Specimens analyzed and culture results

AFB result and source of specimen(s)	No. with indicated culture result			
	Contaminated	Negative	MTBC	*M. avium*
AFB negative				
Pulmonary				
Sputum	7	22	13	1
Bronchoalveolar lavage fluid	1	4		
Bronchial secretions	2	5	2	
Brushing		1	2	
Washing	1	3	1	
Total	11	35	18	1
Extrapulmonary				
Cerebrospinal fluid		7		
Gastric fluid	1	4		
Lymph node		4		
Pericardial fluid		2		
Pleural fluid		8	1	
Pus		2		
Synovial fluid		1		
Tissue	1	5	1	
Urine		2	1	
Total	2	35	3	
AFB positive				
Pulmonary				
Sputum	1		11	
Washing		1	1	
Total	1	1	12	
Extrapulmonary				
Pleural fluid		1		
AFB suspicious				
Sputum			1	

TABLE 2. Comparison between GeneXpert MTB/RIF and culture results[a]

Sample type or AFB result	Culture result	No. with indicated result in GeneXpert MTB/RIF		
		Negative	Positive	Invalid
All samples (n = 121)	Negative	65	5	2
	M. tuberculosis	3	32	
	M. avium	1		
	Contaminated	12	1	
Pulmonary (n = 80)	Negative	32	2	2
	M. tuberculosis	3	29	
	M. avium	1		
	Contaminated	10	1	
Extrapulmonary (n = 41)	Negative	33	3	
	M. tuberculosis		3	
	Contaminated	2		
AFB negative (n = 105)	Negative	64	5	1
	M. tuberculosis	3	19	
	M. avium	1		
	Contaminated	12		
AFB positive (n = 15)	Negative	1		1
	M. tuberculosis		12	
	Contaminated		1	
AFB suspicious (n = 1)	*M. tuberculosis*		1	

[a] One patient with an Xpert MTB/RIF-positive/culture-negative result had a subsequent culture-positive sample that was taken into consideration in the final test analysis.

One hundred nineteen samples (98.4%) gave an interpretable Xpert MTB/RIF assay result (Table 2). Thirty-eight were positive and 81 negative. Two of the 107 samples with informative culture results were invalid by the Xpert MTB/RIF assay. From the remaining 6 Xpert MTB/RIF-positive/culture-negative samples, we investigated culture results from additional specimens. Only 1 of the 6 had a subsequent positive culture that was taken into consideration in the final analysis.

All but one of the Xpert MTB/RIF-positive samples were informative for rifampin resistance. The one indeterminate result was an AFB-negative/culture-negative synovial fluid sample. Four Xpert MTB/RIF-informative samples were culture negative, and one was contaminated. A mutated/resistant strain was identified in 3/37 MTBC Xpert MTB/RIF-positive samples and 5/35 cultured strains by the MTBDR*plus* assay. Two strains harbored the S531L substitution, one the D516V substitution, and one a deletion covering the WT2/WT3/WT4 probes. Furthermore, in one additional case, a complex heteroresistant pattern was observed, as the patient's sample harbored strains with either the H526L or the S531L mutation. This sample gave a ΔC_T value of 3.3 and was characterized as wild type by the Xpert MTB/RIF assay. One culture-positive sample was negative by the Xpert MTB/RIF. Informative for both assays were 32 samples (Table 3). The MTBDR*plus* and DST results had a 100% agreement.

A summary of the Xpert MTB/RIF assay's performance is depicted in Tables 3 and 4. Among 106 samples, 35 were positive for MTBC. Overall, the combined sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values were 91.4%, 93%, 86.5%, and 95.6%, respectively. For pulmonary samples, the sensitivity, specificity, and PPV and NPV values were 90.6%, 94.3%, 93.5%, and 91.7%, whereas in the extrapulmonary samples, they were 100%, 91.6%, 50%, and 100%, respectively (Table 4). Two pulmonary and 3 extrapulmonary (1 urine, 1 gastric fluid, and 1 synovial fluid) AFB-negative samples were Xpert MTB/RIF positive/culture negative. An additional AFB-negative sample exhibiting the same pattern came from a patient proven to have pulmonary TB.

The main purpose of this study was to assess the effectiveness of the Xpert MTB/RIF assay in testing AFB-negative specimens originating from patients with clinical signs highly indicative of active TB. Our findings on the performance of the Xpert MTB/RIF assay correlate well with those reported by others regarding the effectiveness of the assay in accurately detecting the presence of MTBC bacilli in AFB-negative specimens (2, 5, 7). In these studies, performed exclusively or mainly on respiratory samples, the reported sensitivities

TABLE 3. Comparison between the GeneXpert MTB/RIF and MTBDR*plus*/DST assays in determining rifampin susceptibility

MTBDR*plus*/DST rifampin susceptibility result	No. with indicated rifampin susceptibility result in GeneXpert MTB/RIF	
	Sensitive	Resistant
Wild type/sensitive	28	
Mutated/resistant	1	3

TABLE 4. Sensitivity, specificity, and positive and negative predictive values of the GeneXpert MTB/RIF assay with the culture method as reference

Specimen type	No. (%) [95% confidence interval][a]			
	Sensitivity	Specificity	PPV	NPV
All samples	32/35 (91.4%) [76.9%–98.1%]	66/71 (93%) [84.3%–97.6%]	32/37 (86.5%) [71.2%–95.4%]	66/69 (95.6%) [87.8%–99%]
AFB positive	12/12 (100%) [73.3%–100%]	1/1 (100%) [16.5%–100%]	12/12 (100%) [73.35%–100%]	1/1 (100%) [16.5%–100%]
AFB negative	19/22 (86.3%) [65%–96.9%]	65/70 (93%) [84.1%–97.6%]	19/24 (79%) [57.8%–92.8%]	65/68 (95.6%) [87.6%–99%]
Pulmonary	29/32 (90.6%) [74.9%–97.9%]	33/35 (94.3%) [80.8%–99.1%]	29/31 (93.5%) [78.5%–99%]	33/36 (91.7%) [77.5%–98.1%]
Extrapulmonary	3/3 (100%) [30.5%–100%]	33/36 (91.6%) [77.5%–98.1%]	3/6 (50%) [12.4%–87.6%]	33/33 (100%) [89.3%–100%]

[a] For the estimation of test performance, 1 *M. avium* isolate was considered culture negative.

ranged from 72% to 90%. The higher sensitivity for AFB-negative specimens was attained upon testing of additional samples that increased the initial value from 72.5% (one test) to 85.1% (two tests) and, finally, to 90.2% (three tests) (2). We performed one or two additional tests for 11 AFB-negative cases. Discrepancies between the first (Xpert-negative) and the second (Xpert-positive) sample were detected in two cases. All cultures from consecutive specimens of one of these patients were negative. In contrast, cultures from the second patient's consecutive sputa were both positive for an MDR *M. tuberculosis* strain. The Xpert MTB/RIF assay's semiquantitative result for MTBC DNA in one of these specimens was very low, and it correctly identified the deviation from the wild-type sequence in the region recognized by probe E (the S531L mutation was identified by MTBDR*plus*). This case of MDR *M. tuberculosis* points out the impact that testing of consecutive AFB-negative samples, when strong clinical evidence exists, may have on patient management.

Five of the 35 MTBC culture-positive samples showed rifampin resistance. The Xpert MTB/RIF assay detected MTBC DNA in 4 samples and found *rpoB* mutations in 3 of them. The rifampin-resistant strains identified in 2 of the samples were from AFB-negative specimens, which are not approved for MTBDR*plus* testing. The strain incorrectly identified as rifampin sensitive derived from a patient with a mixed heteroresistant population. However, and despite the presence of a mixed population, a delay in probe hybridization was also observed upon sample processing, but it was not sufficient for the correct interpretation. On the other hand, the Xpert MTB/RIF assay determined correctly the presence of a wild-type allele in all rifampin-sensitive strains (specificity, 100%). Discrepancies between Xpert MTB/RIF and DST results regarding rifampin resistance (false resistance or false sensitivity) have also been reported previously (2, 5, 6). Since the assay does not detect specific mutations but deviations from the wild-type sequence, it is expected that in certain cases, i.e., in the presence of mixed infections or silent mutations, discrepancies would occur. Nevertheless, in the most extensive study published so far, the Xpert MTB/RIF assay correctly detected rifampin resistance in 99.1% of patients (2).

In conclusion, the Xpert MTB/RIF assay demonstrated a high capability to detect MTBC DNA in AFB microscopy-negative samples of pulmonary and extrapulmonary origin. With the exception of a specimen harboring a mixed MTBC population, the assay correctly retrieved information from the *rpoB* hot spot region regarding RMP resistance. Accumulating published data, including those presented herein, indicate that this rapid and easy-to-perform fully automated NAA test could prove to be an extremely helpful diagnostic tool in the fight against tuberculosis.

Cepheid supplied the GeneXpert MTB/RIF assays through its local Greek agent (Medicon Hellas S.A.).

REFERENCES

1. **Banada, P. P., et al.** 2010. Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. J. Clin. Microbiol. **48:**3551–3557.
2. **Boehme, C. C., et al.** 2010. Rapid molecular detection of tuberculosis and rifampin resistance. N. Engl. J. Med. **363:**1005–1015.
3. **Centers for Disease Control and Prevention.** 2009. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb. Mortal. Wkly. Rep. **58:**7–10.
4. **Helb, D., et al.** 2010. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J. Clin. Microbiol. **48:**229–237.
5. **Hillemann, D., S. Rusch-Gerdes, C. Boehme, and E. Richter.** 2011. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J. Clin. Microbiol. **49:**1202–1205.
6. **Marlowe, E. M., et al.** 2011. Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. J. Clin. Microbiol. **49:**1621–1623.
7. **Moure, R., L. Munoz, M. Torres, R. Santin, and F. Alcaide.** 2011. Rapid detection of Mycobacterium tuberculosis complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J. Clin. Microbiol. **49:**1137–1139.
8. **Van Rie, A., L. Page-Shipp, L. Scott, I. Sanne, and W. Stevens.** 2010. Xpert® MTB/RIF for point-of care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? Expert Rev. Mol. Diagn. **10:**937–946.
9. **Watterson, S. A., S. M. Wilson, M. D. Yates, and F. A. Drobniewski.** 1998. Comparison of three molecular assays for rapid detection of rifampin resistance in Mycobacterium tuberculosis. J. Clin. Microbiol. **36:**1969–1973.
10. **WHO.** 2010. Global tuberculosis control: WHO report 2010. World Health Organization, Geneva, Switzerland.
11. **WHO.** 2010. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. WHO/HTM/TB/2010.3. World Health Organization, Geneva, Switzerland.