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Evaluation of the Abbott RealTi*me* MTB and RealTi*me* MTB INH/RIF Assays for Direct Detection of *Mycobacterium tuberculosis* Complex and Resistance Markers in Respiratory and Extrapulmonary Specimens

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The Abbott RealTi*me* MTB (RT MTB) assay is a new automated nucleic acid amplification test for the detection of *Mycobacterium tuberculosis* complex (MTBC) in clinical specimens. In combination with the RealTi*me* MTB INH/RIF (RT MTB INH/RIF) resistance assay, which can be applied to RT MTB-positive specimens as an add-on assay, the tests also indicate the genetic markers of resistance to isoniazid (INH) and rifampin (RIF). We aimed to evaluate the diagnostic sensitivity and specificity of RT MTB using different types of respiratory and extrapulmonary specimens and to compare performance characteristics directly with those of the FluoroType MTB assay. The resistance results obtained by RT MTB INH/RIF were compared to those from the GenoType MTBDR*plus* and from phenotypic drug susceptibility testing. A total of 715 clinical specimens were analyzed. Compared to culture, the overall sensitivity of RT MTB was 92.1%; the sensitivity rates for smear-positive and smear-negative samples were 100% and 76.2%, respectively. The sensitivities of smear-negative specimens were almost identical for respiratory (76.3%) and extrapulmonary (76%) specimens. Specificity rates were 100% and 95.8% for culture-negative specimens and those that grew nontuberculous mycobacteria, respectively. RT MTB INH/RIF was applied to 233 RT MTB-positive samples and identified resistance markers in 7.7% of samples. Agreement with phenotypic and genotypic drug susceptibility testing was 99.5%. In conclusion, RT MTB and RT MTB INH/RIF allow for the rapid and accurate diagnosis of tuberculosis (TB) in different types of specimens and reliably indicate resistance markers. The strengths of this system are the comparably high sensitivity with paucibacillary specimens, its ability to detect INH and RIF resistance, and its high-throughput capacities.

Rapid and accurate diagnosis of tuberculosis (TB) and fast detection of drug resistance are essential to ensure early initiation of appropriate antituberculotic treatment, adequately manage the disease, and control further transmission. Worldwide, one-third of all TB cases and almost three-quarters of the 480,000 cases of multidrug-resistant (MDR; defined as resistance toward rifampin [RIF] and isoniazid [INH]) TB are not reported, with the vast majority of them occurring in high-burden countries (1). Molecular tests are the most promising tools to close this diagnostic gap. Consequently, nucleic acid amplification tests (NAATs), such as PCR assays that allow for the fast and accurate detection of *Mycobacterium tuberculosis* complex (MTBC) DNA directly in clinical specimens, have become an indispensable tool in TB diagnostics over the last several decades. Most commercial tests show excellent specificity and sensitivity rates with smear-positive specimens while sensitivity rates range from 49% to 78% with smear-negative samples (2–7).

Particularly in regions with high prevalences of MDR-TB, the molecular detection of genetic markers of resistance directly in the clinical specimen is playing a pivotal role in early notification of cases of resistant TB. MDR-TB is increasingly spread in local outbreaks, epidemic scenarios, or even pandemic scenarios (8). Only the fast notification of resistance allows for the rapid interruption of transmission by adequate isolation and treatment and thereby slows the emergence of MDR-TB. The fully integrated, cartridge-based Xpert MTB/RIF (Cepheid, Sunnyvale, CA) can detect TB bacteria as well as resistance markers for RIF in clinical specimens and was endorsed by the World Health Organization (WHO) in 2010. Since then, it has been rolled out in all high-prevalence countries with particular emphasis in peripheral and remote areas (9–11). Despite its outstanding design, ease of use, and almost fully automated system, it has two major weaknesses. First, it only detects the genetic markers of RIF resistance and completely ignores INH resistance, which is found in 5% to 15% of RIF-susceptible cases worldwide and has a significant impact on treatment outcome (12, 13). Very few commercial assays, such as GenoType MTBDR*plus* (HAIN Lifescience, Nehren, Germany) or Anyplex plus MTB/NTM/MDR-TB (Seegene, Soul, South Korea), also allow for the detection of resistance markers for INH with sufficient reliability, although only the GenoType assays are widely used in high-prevalence countries (14, 15). Second, the module-based GeneXpert is not designed as a high-throughput system. However, high capacities are required at large laboratory centers of high-burden countries, such as South Africa, India, China, or other Asian countries (14, 16).

Recently, Abbott launched the new automated RealTi*me* MTB

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(RT MTB) PCR assay for the qualitative detection of MTBC in respiratory specimens. This high-throughput system consists of the Abbott *m*2000*sp* platform, which fully automatically extracts DNA from up to 94 clinical specimens per batch, and the *m*2000*rt* cycler, which performs real-time PCR that targets both the gene of protein antigen b (*pab*) and the multicopy insertion element IS*6110*. The performance characteristics of RT MTB with regard to the limits of detection of MTBC bacteria, analytical specificity, and diagnostic sensitivity and specificity have been recently investigated by a team of Abbott researchers (17). The system allows for further analysis of MTBC-positive samples in an add-on step (reflex mode) with the Abbott RealTi*me* MTB INH/RIF (RT MTB INH/RIF) resistance assay, which identifies genetic markers in the *katG* gene and in the *inhA* promoter region for INH resistance as well as in the *rpoB* gene for RIF resistance.

In our study, we have evaluated the performance characteristics of RT MTB in a high-volume mycobacteriology laboratory in Germany using different types of respiratory and extrapulmonary clinical specimens sent for and frequently encountered in routine diagnostics. The results of the RT MTB were directly compared to those of the FluoroType MTB (FT MTB) (HAIN Lifescience), which is another widely used commercial semiautomated NAAT. As a first evaluation of RT MTB INH/RIF, resistance markers for INH and RIF were reviewed against results from the line probe assay GenoType MTBDR*plus* (HAIN Lifescience) and from phenotypic drug susceptibility testing (DST).

MATERIALS AND METHODS

Study design and specimens. Annually, more than 40,000 clinical specimens from patients suspected to have TB are sent to our mycobacteriology diagnostic center in Gauting, Germany, for diagnostic analyses of suspected TB or mycobacterioses and follow-up investigation of patients under treatment. From April to November 2015, the residual materials of clinical specimens after decontamination were preserved in a −30°C freezer for later analysis by RT MTB provided that the FT MTB assay was included in the diagnostic analyses and that 500 μl or more residual material was available. To avoid bias, specimens were excluded from the study if the patient already received anti-TB therapy for more than 2 weeks before specimen collection. A maximum of three study specimens per patient were included. From the preserved specimen collection, all samples that grew MTBC ($n = 253$) or nontuberculous mycobacteria (NTM) ($n = 50$) and 412 random samples with negative cultures were taken for the study. The final set of study samples included 608 respiratory specimens (273 sputum samples, 274 bronchial aspirate samples, 45 bronchoalveolar lavage [BAL] samples, and 16 tracheal aspirate samples) and 107 extrapulmonary specimens (31 tissues/biopsy specimens, 23 puncture samples, 20 pleural fluid samples, 5 urine samples, 3 gastric aspirate samples, 1 cerebrospinal fluid [CSF] sample, 14 other fluid/puncture samples, and 10 swabs from operative sites and wounds). RT MTB was applied to all study samples. RT MTB-positive samples were additionally analyzed using the RT MTB INH/RIF. The study was approved by the ethics committees of the Ludwig-Maximilians University of Munich (no. 437-12).

Processing of specimens and mycobacteriology analyses. All clinical specimens were decontaminated using the *N*-acetyl-L-cysteine (NALC)-NaOH method (18) with a final NaOH concentration of 1%. After concentration by centrifugation (20 min at 3,000 × *g*), the sediment was resuspended in 1.5 ml of 0.5 M phosphate buffer (pH 6.8) and inoculated for culture in mycobacteria growth indicator tubes (500 μl; MGIT; Becton-Dickinson) with Lowenstein-Jensen medium (25 μl) and Stonebrink medium (25 μl). Smears were stained with Auramine O, read under a fluorescence microscope at ×400 magnification, and interpreted following WHO standards (19), i.e., negative, no acid-fast bacilli (AFB) in 300 fields; scanty, 1 to 19 AFB per 40 fields; 1+, 20 to 199 AFB per 40 fields; 2+, 5 to 50 AFB per field; and 3+, >50 AFB per field. Routine TB NAAT was performed using FT MTB with 500 μl of decontaminated sample as described previously (6). Cultures were incubated for 8 weeks before they were declared negative. From positive cultures, species were identified using the line probe assays GenoType CM and GenoType MTBC (HAIN Lifescience). RIF and INH resistance markers were determined in smear-positive samples or in MTBC-positive cultures using the GenoType MTBDR*plus*. The phenotypic susceptibility of MTBC isolates toward first-line drugs was tested in MGIT using the Becton Dickinson SIRE kit following the manufacturer's recommendations.

RT MTB and RT MTB INH/RIF assays. Frozen decontaminated specimens (500 μl) were thawed, and an inactivating reagent was added to reach final concentrations of 1.2% NaOH, 45% 2-propanol, and 0.135% Tween 20. They were then vortexed thoroughly and incubated for 1 to 24 h at room temperature. After a final vortexing step, samples were loaded on the Abbott *m*2000*sp* instrument, which fully automatically extracted the DNA as described elsewhere (17). An internal control (IC) added to the lysis buffer was extracted together with each sample serving as extraction and amplification control. One negative and one positive control for each RT MTB and RT MTB INH/RIF were added and processed together with each batch of clinical samples. Then, the *m*2000*sp* automatically prepared and dispensed 25 μl of master mix together with 25 μl of extracted DNA eluate into each well of a 96-well optical reaction plate; the remainder eluate was stored at −30°C. The reaction plate was manually sealed and transferred to the *m*2000*rt* for real-time PCR and automated analysis. Results of the RT MTB run were reported as "MTB not detected" or "MTB detected." Threshold cycle numbers (CNs) were recorded for the MTBC target and IC of each test. For the RT MTB INH/RIF reflex testing of RT MTB-positive samples, the *m*2000*sp* automatically prepared the PCR mixes, including a negative and a positive control. Real-time PCR and automated analysis were performed on the *m*2000*rt* device. Resistance to RIF is detected by using eight *rpoB* wild-type probes, and resistance to INH is detected by using wild-type and mutant (315T1) *katG* probes as well as wild-type and mutant (−15T) *inhA* promoter probes. All mutations reported by the *m*2000*rt* were directly compared to the results of the GenoType MTBDR*plus* line probe assay, which has been previously evaluated in depth confirming its very high analytical specificity (20). The identities of the underlying *rpoB* mutations, which were not clearly identified by the GenoType MTBDR*plus*, were verified by Sanger sequencing of the RIF resistance-determining region of the *rpoB* gene using standard protocols and primers rpoB-F 5′-GGG AGC GGA TGA CCA CCC A-3′ and rpoB-R 5′-GCG GTA CGG CGT TTC GAT GAA C-3′.

Data analysis. The RT MTB and FT MTB results were compared to the outcomes of the cultures. In cases of discrepant results, the medical history, clinical data, and histological data of the patients were included in the final evaluation upon receiving consent from the patient. RT MTB INH/RIF results were compared to the results from the line probe assay GenoType MTBDR*plus*. Ninety-five percent confidence intervals (CIs) were determined using the VassarStats calculator. Differences of parametric distributions were tested with the unpaired Student's *t* tests on the OpenEpi version 2.3 platform. A P value of <0.05 was considered statistically significant.

RESULTS

RT MTB was applied to 715 precharacterized clinical specimens. Of these, 253 specimens (253/715; 35.4%) grew MTBC (246 grew *M. tuberculosis*, 6 grew *Mycobacterium africanum*, and 1 grew *Mycobacterium bovis* BCG), 50 specimens (50/715; 7.0%) grew NTM (9 grew *Mycobacterium abscessus*, 11 grew *Mycobacterium avium*, 3 grew *Mycobacterium gordonae*, 10 grew *Mycobacterium intracellulare*, 7 grew *Mycobacterium kansasii*, 1 grew *Mycobacterium malmoense*, 4 grew *Mycobacterium szulgai*, and 5 grew *Mycobacterium xenopi*), and 412 specimens (412/715; 57.6%) were culture negative (Table 1). Of the MTBC culture-positive samples, 84 (84/253;

TABLE 1 Diagnostic accuracy of Abbott RT MTB and HAIN FT MTB using conventional culture as the reference method

Culture results	Abbott RT MTB				HAIN FT MTB			
	No. negative ($n = 480$)	No. positive ($n = 235$)	% specificity (95% CI)	% sensitivity (95% CI)	No. negative ($n = 491$)	No. positive ($n = 224$)	% specificity (95% CI)	% sensitivity (95% CI)
MTBC negative ($n = 462$)	460	2	99.6 (98.3–99.9)		462	0	100 (99–100)	
Culture negative ($n = 412$)	412	0	100 (98.9–100)		412	0	100 (98.9–100)	
NTM ($n = 50$)	48	2	95.8 (86.1–99.3)		50	0	100 (91.1–100)	
MTBC positive ($n = 253$)	20	233		92.1 (87.9–95)	29	224		88.5 (83.8–92.1)
Smear negative ($n = 84$)	20	64		76.2 (65.4–84.5)	29	55		65.5 (54.2–75.3)
Smear positive ($n = 169$)	0	169		100 (97.2–100)	0	169		100 (97.2–100)

33.2%) were smear negative and 169 (169/253; 66.8%) were smear positive.

The RT MTB assay became positive with 235 (32.9%) and negative with 480 (67.1%) specimens (Table 1). There were no invalid results. Using mycobacterial culture as the method of comparison, the positivity rates of RT MTB were 92.1% (95% confidence interval [CI], 87.9% to 95.0%), 100% (95% CI, 97.2% to 100%), and 76.2% (95% CI, 65.4% to 84.5%) for all, smear-positive, and smear-negative samples, respectively. The CN values were negatively correlated with the grade of AFB in smear microscopy (Fig. 1), allowing us to clearly discriminate between smear-negative (mean ± standard deviation, 36.1 ± 2.6) and the different smear grades of AFB-positive samples, i.e., scanty (30.6 ± 3.1), 1+ (26.9 ± 2.6), 2+ (22.9 ± 2.9), or 3+ (19.6 ± 1.6) (unpaired *t* test, $P < 0.0001$). Ten of the 20 smear-negative samples, which were false negatives in RT MTB, showed MTBC growth only in liquid culture, underlining the low number of MTBC bacilli in the clinical specimens.

Diagnostic specificity reached 99.6% (95% CI, 98.3% to 99.9%) when calculated for all MTBC-negative specimens and 100% (95% CI, 98.9 to 100%) in the subgroup of culture-negative samples (Table 1). With samples growing NTM, specificity was 95.8% (95% CI, 86.1% to 99.3%) due to two discrepant positive RT MTB results with relatively high CN values (≥35). Both specimens contained high loads of NTM (*M. abscessus* and *M. avium*, respectively), as indicated by high grades in smear microscopy, and were derived from patients suffering from severe known NTM disease without a documented history of TB.

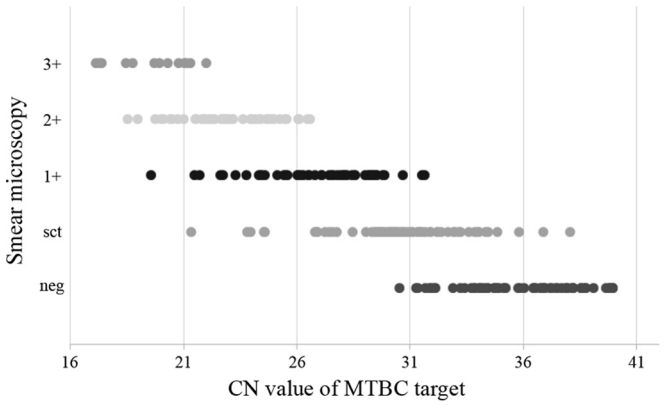

FIG 1 Threshold cycle number (CN) values of MTBC-positive samples are depicted according to the grade of acid-fast bacilli in smear microscopy, i.e., negative (neg), scanty (sct), 1+, 2+, or 3+.

About 15% of samples (107/715; 14.9%) were derived from extrapulmonary specimens. RT MTB performed as well with extrapulmonary specimens as with respiratory specimens (Table 2). Although specificity (100% versus 99.5%; chi-square test, $P > 0.5$) and sensitivity rates for smear-positive (100% for both) and smear-negative (76.0% versus 76.3%; $P > 0.95$) specimens were almost equal, a trend to lower overall sensitivity was simulated by a lower proportion of smear-positive samples among extrapulmonary samples (84.2%) compared to those among respiratory samples (93.5%). Correspondingly, mean CN values (± standard deviation) for MTBC targets were within similar ranges for extrapulmonary and respiratory specimens with both smear-negative (35.8 ± 2.6 and 36.2 ± 2.5, respectively) and smear-positive (29.7 ± 3.7 and 26.4 ± 4.4, respectively) samples (data not shown). Furthermore, extrapulmonary samples did not inhibit the PCR as indicated by the similar CN values (mean ± standard deviation) of the ICs for extrapulmonary (36.2 ± 1.4) and respiratory (35.5 ± 1.1) samples.

All 715 study specimens were initially subjected to routine TB NAAT using FT MTB. Direct comparison of RT MTB and FT MTB results showed concordance in 696 of 715 (97.3%) samples. Four smear-negative MTBC cases tested negative by RT MTB but positive by FT MTB, while 15 tested positive by RT MTB but negative by FT MTB, including the two discrepant positive samples growing NTM. RT MTB showed statistically insignificant trends toward lower specificity (99.6% versus 100%; chi-square test, $P = 0.15$) but higher sensitivity (92.1% versus 88.5%; $P = 1.7$) than FT MTB, particularly with smear-negative samples (76.2% versus 65.5%; $P = 0.13$) (Table 1).

All 233 RT MTB-positive samples (excluding the two discrepant positive NTM specimens) were subjected to resistance testing by RT MTB INH/RIF (Table 3). The assay detected resistance markers for INH but not for RIF in eight samples (8/233; 3.4%), i.e., five *inhA* promoter mutations (5/233, 2.1%) and three *katG* mutations (3/233, 1.3%), and markers of MDR, i.e., concurring mutations in the *katG* (± *inhA*) and the *rpoB* genes in 10 samples (10/233; 4.3%). No resistance markers were detected in 171 RT MTB samples (171/233; 73.4%). Incomplete or missing resistance profiles were observed in 44 samples (44/233; 18.9%) due to signals for one or more targets below the limit of detection (LOD). The vast majority of those samples (42/44; 95.5%) were negative and two were scanty in smear microscopy, suggesting that the invalidity of RT MTB INH/RIF results was induced by the paucibacillary nature of those specimens. This hypothesis is supported by the finding of significantly higher mean CN values for MTBC targets in the RT MTB assay with samples classified as below the

TABLE 2 Performance of Abbott RT MTB with respiratory and extra-pulmonary specimens

Specimen type	Culture results	Abbott RT MTB				
		No.	No. negative	No. positive	% specificity (95% CI)	% sensitivity (95% CI)
Respiratory	MTBC negative	393	391	2	99.5 (98–99.9)	
	MTBC positive	215	14	201		93.5 (89.1–96.2)
	Smear negative	59	14	45		76.3 (63.1–86)
	Smear positive	156	0	156		100 (97.0–100)
Extrapulmonary	MTBC negative	69	69	0	100 (93.4–100)	
	MTBC positive	38	6	32		84.2 (68.1–93.4)
	Smear negative	25	6	19		76 (54.5–89.8)
	Smear positive	13	0	13		100 (71.7–100)
Tissues	MTBC negative	24	24	0	100 (82.8–100)	
	MTBC positive	7	0	7		100 (56.1–100)
	Smear negative	5	0	5		100 (46.3–100)
	Smear positive	2	0	2		100 (19.8–100)
Body fluids	MTBC negative	39	39	0	100 (88.8–100)	
	MTBC positive	27	5	22		81.5 (61.2–92.8)
	Smear negative	17	5	12		70.6 (44.1–88.6)
	Smear positive	10	0	10		100 (65.6–100)
Swabs	MTBC negative	6	6	0	100 (51.7–100)	
	MTBC positive	4	1	3		75 (21.9–98.7)
	Smear negative	3	1	2		66.7 (12.5–98.2)
	Smear positive	1	0	1		100 (5.5–100)

LOD (34.8 ± 2.9) compared to samples yielding interpretable results in the RT MTB INH/RIF (28.4 ± 5.3; unpaired Student's *t* test, $P = 0.0001$) (data not shown).

Compared to the results of the phenotypic first-line DST and the resistance patterns obtained by GenoType MTBDR*plus*, the concordance was 99.5% (188/189) confirming the high accuracy of the RT MTB INH/RIF assay. Discordance was found with only one sample (1/189; 0.5%), which tested susceptible by RT MTB INH/RIF but INH resistant by phenotypic first-line DST (Table 3). GenoType MTBDR*plus* performed on the correspondent culture identified both wild-type and mutated *katG* signals, suggesting heteroresistance, i.e., the coexistence of INH-resistant and INH-susceptible MTB organisms.

The handling of the assay was assessed as very easy. Hands-on times for processing 96 samples, including three controls, were estimated to be 60 min for sample prearrangement and inactivation; 45 min for setting up the *m*2000*sp* instrument, i.e., preparing and loading buffers, reagents, and consumables; and 15 min for preparing and loading PCR reagents summing up to about 2 h. The time to result for RT MTB was 9 to 10 h for 96 samples and 7 to 8 h for 48 samples. In the case of a positive RT MTB result, subsequent RT MTB INH/RIF was applied, requiring 15 min of

TABLE 3 Comparison of Abbott RT MTB INH/RIF results with those of phenotypic and genotypic DST

Abbott RT MTB INH/RIF results[a]	No.	Results of phenotypic and genotypic DST (culture)[a]	
		Phenotypic DST	GenoType MTBDR*plus*[b]
Complete results			
rpoB wt, *katG* wt, *inhA* wt	170	RIF s/INH s	*rpoB* wt, *katG* wt, *inhA* wt
rpoB wt, *katG* wt, *inhA* wt	1	RIF s/INH r	*rpoB* wt, *katG* wt and *katG* MUT1 (S315T1), inhA wt
rpoB wt, *katG* wt, *inhA* −15T	3	RIF s/INH r	*rpoB* wt, *katG* wt, *inhA* MUT1 (C15T)
rpoB wt, *katG* 315T1, *inhA* wt	5	RIF s/INH r	*rpoB* wt, *katG* MUT1 (S315T1), *inhA* wt
rpoB Pb4−, *katG* 315T1, *inhA* wt	3	RIF r/INH r	*rpoB* MUT3 (S531L), *katG* MUT1 (S315T1), *inhA* wt
rpoB Pb5−, *katG* 315T1, *inhA* wt	1	RIF r/INH r	*rpoB* Δwt 2/3/4, *katG* MUT1 (S315T1), *inhA* wt
rpoB Pb2−, *katG* 315T1, *inhA* −15T	1	RIF r/INH r	*rpoB* MUT1 (D516V), *katG* MUT1 (S315T1), *inhA* MUT1 (C15T)
rpoB Pb4−, *katG* 315T1, *inhA* −15T	5	RIF r/INH r	*rpoB* MUT3 (S531L), *katG* MUT1 (S315T1), *inhA* MUT1 (C15T)
Below LOD			
rpoB wt (no *katG*, no *inhA* results)	5	RIF s/INH s	*rpoB* wt, *katG* wt, *inhA* wt
rpoB wt (no *katG*, no *inhA* results)	1	RIF s/INH r	*rpoB* wt, *katG* MUT1 (S315T1), *inhA* wt
katG wt, *inhA* wt (no *rpoB* results)	9	RIF s/INH s	*rpoB* wt, *katG* wt, *inhA* wt
(no *rpoB*, no *katG*, no *inhA* results)	28	RIF s/INH s	*rpoB* wt, *katG* wt, *inhA* wt
(no *rpoB*, no *katG*, no *inhA* results)	1	RIF s/INH r	*rpoB* wt, *katG* MUT1 (S315T1), *inhA* wt

[a] s, susceptible; r, resistant; wt, wild type; Δwt, missing signals with GenoType MTBDR*plus* wild-type probes; Pbx−, missing signals with RT MTB wild-type *rpoB* probes.
[b] GenoType MTBDR*plus* was routinely performed with positive cultures; in 59 cases (all smear microscopy positive), the assay was performed directly with the specimen.

hands-on time for PCR set up. Results of RT MTB INH/RIF were available after a total assay time of 3 h.

DISCUSSION

The Abbott RT MTB in combination with the add-on resistance assay, RT MTB INH/RIF, is a novel fully automated real-time PCR system for high-throughput diagnostics of TB, including resistance markers for INH and RIF. The device will most likely be a great contribution in the metropolitan areas of high-prevalence countries, which would have the need of high-capacity assays. Xpert MTB/RIF is an excellent test for low numbers of samples in decentralized laboratories, particularly on the district level (9). However, due to the module technique, handling large series of samples becomes challenging (14). In comparison, the automated RT MTB platform based on a barcode-controlled 96-well microformat allows easy and safe handling of large sample numbers with extremely low risk of sample mix-up or mislabeling. With intensive funding from international donors, the price of the Xpert MTB/RIF has been cut down to US$9.98 per cartridge in developing countries (21). So far, similar prices are not negotiated with Abbott. However, the Abbott molecular platform and the RT MTB assay are included in a Global Fund framework agreement aimed at cost reduction for an expanded assay menu combining TB and virus diagnostics (22). Thus, RT MTB may become a cost-efficient alternative for health centers in regions with high burdens of TB and HIV.

In four studies so far, the RT MTB assay has been assessed with sputum samples (17, 23–25). The aims of the present study were to evaluate for the first time the performance of the RT MTB INH/RIF assay, to assess the RT MTB with extrapulmonary specimens, and to compare the RT MTB head to head with the FT MTB assay, which is widely used in low-prevalence Central European countries.

The sensitivity of the RT MTB with smear-negative respiratory specimens (76.2%) was in the most upper levels of what has been reported from other commercial NAATs, ranging between 49% and 77.7%. In our direct head-to-head comparison, RT MTB seemed to demonstrate a sensitivity superior to that of FT MTB, although differences were statistically not significant. Both assays use the same multicopy target, IS*6110*, suggesting that the better sensitivity of RT MTB may be attributed to more efficient DNA extraction (17). Additionally, the sensitivity of RT MTB may benefit from the inclusion of the *pab* gene as a second target. Sharma et al. (26) reported an increase in sensitivity with a multicopy PCR targeting both IS*6110* and *pab* compared to that of a PCR amplifying only IS*6110*. With the use of a second target, RT MTB also circumvents the known problem of false-negative results due to MTB strains harboring only single or zero copies of IS*6110*, which are mostly endemic in Southeast Asia (27).

Whereas we recorded 100% specificity for RT MTB with culture-negative samples, the specificity was 96% with the specimens growing NTM due to two (4.2%) samples that yielded discrepant positive RT MTB. Both samples were recovered from patients with repeated isolation of NTM and known severe NTM disease in association with long-standing chronic lung diseases. In the patients' medical histories, there was no evidence of TB, suggesting that discrepant positive results may be related to unspecific signals of at least one RT MTB target. The FT MTB assay, which only targets the IS*6110* element, showed 100% specificity with the same NTM samples as well as in a previous evaluation study (6). We can only speculate that the potential cross-reactivity of RT MTB with NTM may be attributed to the *pab* gene.

The assessment of RT MTB with extrapulmonary specimens recorded excellent positivity rates with tissue (100%) and body fluids (81.5%), confirming the high sensitivity of RT MTB for extrapulmonary TB. However, the numbers of different types of specimens were too low to allow subgroup analyses. Thus, assuming a trend of RT MTB to higher sensitivity with pleural or cerebrospinal fluids would be speculative.

Our study was the first evaluation of the RT MTB INH/RIF assay. The resistance markers identified by RT MTB INH/RIF were in nearly full (99.5%) concordance with those from the GenoType MTBDR*plus* and phenotypic DST. Only one specimen yielded discrepancies. It was recovered from a TB patient with INH heteroresistance, i.e., both wild-type and *katG* S315T-mutated MTBC bacteria were present in the culture. The spectrum of encountered *rpoB* mutations among study isolates encompassed the most frequent mutations, S531L and D516V, as well as rare mutations, L511P and D516G. This certainly does not reflect the full capacity of the RT MTB INH/RIF assay for the detection of resistance markers, which is being addressed in a follow-up study with a broader spectrum of resistance patterns and *rpoB*, *katG*, and *inhA* mutations. RT MTB INH/RIF yielded complete resistance profiles for 81.1% (189/233) of RT MTB-positive samples and for about one-third (23/64; 35.9%) of smear-negative samples. The rates of incomplete or indeterminate results with paucibacillary specimens may have been negatively biased by the frozen storage of our study specimens before testing, which may have had a negative effect on the multiplex PCR.

In conclusion, our study demonstrated that RT MTB detects MTBC with high sensitivity and specificity in extra pulmonary specimens and that the add-on resistance assay, RT MTB INH/RIF, reliably indicates resistance markers. Additional strengths of this system are the comparably high sensitivity with paucibacillary specimens, its ability to detect INH resistance in addition to RIF, and its high-throughput capacities. We see the impact of the test in large laboratory centers, in particular in central reference laboratories or in interdisciplinary diagnostic centers analyzing both HIV and TB samples. This remains to be demonstrated in specific multicenter studies.

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