Published in final edited form as:

Int J Tuberc Lung Dis. 2015 June ; 19(6): 676–678. doi:10.5588/ijtld.14.0867.

A Comparison of the Xpert MTB/RIF and MTBDR_{plus} 2 Assays in Georgia

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Abstract

Few studies have directly compared the performance of rapid molecular diagnostic tests for tuberculosis (TB). We found that commercially available molecular diagnostic tests, the Xpert MTB/RIF and GenoType MTBDR_{plus}, both provided timely and accurate results as compared to conventional phenotypic tests in the detection of TB and rifampicin resistance.

Keywords

tuberculosis; diagnosis; molecular; drug-resistance; multidrug resistance

INTRODUCTION

Multi-drug-resistant tuberculosis (MDR-TB) (resistance to isoniazid [INH] and rifampicin [RIF]) presents a major obstacle to achieving Global TB control. The WHO recently indicated, that global efforts are “off track” in managing MDR-TB and that < – 25% of patients have their disease and associated drug-resistance detected (1). Promising rapid molecular diagnostic tests have been developed in response to low MDR-TB detection rates and WHO has endorsed the line-probe assay (LPA), (Genotype MTBDR_{plus}, Hain Lifescience), and the Xpert MTB/RIF (Cepheid) assay for clinical use. Comparison of these two molecular technologies is crucial in order to validate the accuracy and feasibility of implementation of each test in local laboratory settings and to help National TB Programs optimize allocation of limited resources (2, 3). Within this context, we compared the performance of the Xpert MTB/RIF and MTBDR_{plus} in a setting with high rates of MDR-TB.

METHODS

The study was conducted at the National Reference Laboratory (NRL) of the National Center for Tuberculosis and Lung Diseases (NCTLD) in Tbilisi, Georgia. The Institutional Ethics Committee approved the study. Over a five-month period in 2013, all TB suspects

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with a positive acid-fast bacillus (AFB) smear microscopy as a part of standard care had a sputum culture performed along with the MTBDR_{plus} and Xpert MTB/RIF.

AFB positive sputum specimens were split into three portions and the following tests were performed: AFB culture, Xpert MTB/RIF and Genotype MTBDR_{plus} (version 2.0) LPA. The processed specimen was inoculated onto Löwenstein-Jensen (LJ) based solid medium and/or the BACTECTM MGITTM 960 broth culture system (BD, Sparks), as previously described (4). First-line DST was performed on all cultures positive for *Mycobacterium tuberculosis* using either the absolute concentration method on LJ medium (INH 0.2 mg/ml, RIF 40 mg/ml) or in 7H9 broth with the BACTEC MGIT 960 system (INH 0.1 mg/ml, RIF 1 mg/ml) (4). A 500- μ l portion of decontaminated sample was used to perform the Genotype MTBDR_{plus} assay according to the manufacturer's instructions (5). The Xpert MTB/RIF test was performed on the portion of the clinical sample using G4 cartridges as per manufacturer's instruction. All sample results data were entered into an online database and analyzed using SAS, version 9.3.

RESULTS

382 AFB smear-positive sputum samples from TB suspects were included. Among these, 264 (69%) were from new TB cases, and 128 (31%) retreatment TB cases. Overall, 357 (94%) samples were culture-positive for *M. tuberculosis* by either solid or liquid culture; 20 (5%) were culture-negative and 5 (1%) had contaminated cultures. Among the 357 culture-positive samples 346 (97%) and 336 (94%) were detected by the Xpert MTB/RIF and Genotype MTBDR_{plus} assay, respectively. The Xpert MTB/RIF failed 12 (3.1%) times while there were no MTBDR_{plus} failures. If Xpert MTB/RIF failures were not included in analysis the sensitivity of the assay was 100% when compared to culture (figure 1).

The Xpert and LPA showed good sensitivity (87% and 83%, respectively) and excellent specificity (both 99%) in detecting rifampin resistance. The overall agreement between the Xpert ($k=0.89$) and LPA ($k=0.88$) with culture-based DST in the detection of RIF resistance was excellent. Additional advantage of LPA was detection of INH resistance. The sensitivity and specificity in detecting INH resistance was 83 (76–90) and 100 (99–100), respectively.

Median time to *M. tuberculosis* detection was substantially less with the both the MTBDR_{plus} (5 days) and Xpert (2 days) assays as compared to solid culture (33 days) and liquid culture (9 days) (Table 1).

DISCUSSION

The sensitivity of both molecular assays was excellent and in-line with previous reports (2). The Xpert and MTBDR_{plus} identified *M. tuberculosis* complex DNA in 11 (55%) and 10 (50%), respectively, of the 20 samples with a negative culture. This phenomenon (positive molecular test and negative culture) has been described previously and has been attributed to either the amplification of DNA released from nonviable bacilli, laboratory cross contamination, or a transcription error that has failed to indicate that the sample was a treatment follow-up sample rather than a diagnostic sample (6). However, in some cases the positive molecular test may in fact be a true positive result as indicated by two study patients

with negative baseline cultures and a positive molecular test who had a follow up culture positive for *M. tuberculosis*.

In comparison to other studies, the sensitivity of RIF resistance detection was lower than in South Africa and higher than in India highlighting the fact that genetic mutations conferring phenotypic resistance and hence the performance of molecular assays may vary in different settings (3, 6). One of the most important and obvious reasons for the use of molecular tests is significantly reduced turnaround detection time. Earlier time to detection can result in earlier time to appropriate treatment and improved clinical outcomes (7).

The main distinguishing factor between the Xpert MTB/RIF and MTBDR*plus* assays is that the MTBDR*plus* assay can detect INH resistance. We found that 32% of *M. tuberculosis* isolates were resistant to INH by DST including 38 with INH mono-resistance and 78 with resistance to INH and RIF. Studies have shown less than optimal treatment outcomes, longer treatment durations, and progression to MDR-TB among patients with INH mono-resistance arguing for the evaluation of new regimens (8).

The MTBDR*plus* targets the mutations in *katG* and *inhA* genes associated with INH resistance and thus have the ability to detect low versus high level INH resistance which may be important if high dose INH treatment is found to be useful for low-level INH resistance (9). A barrier to MTBDR*plus* implementation is the requirement for well-trained staff and suitably equipped laboratories. Some additional factors influencing test implementation are represented in a table 2. Further studies comparing the cost effectiveness of each assay and also clinical trials evaluating alternative regimens for INH-mono-resistance TB would decide which assay to use in specific settings.

CONCLUSIONS

We found the Xpert MTB/RIF and GenoType MTBDR*plus* assays both perform well in detecting TB and rifampin resistance and had substantially shorter turnaround time as compared to culture. Implementation of either the molecular assay can decrease time to detection of drug-resistant TB disease and offers great promise in improving MDR-TB care and prevention.

Acknowledgments

Funding: This work was supported in part by the Global Fund to Fight AIDS, Tuberculosis and Malaria Fund (GEO-T-GPIC); the U.S. National Institutes of Health (NIH), Fogarty International Center (FIC) [D43TW007124], National Institute of Allergy and Immunology (NIAID) [K23 AI103044], National Center for Advancing Translational Sciences (NCATS)/Atlanta Clinical and Translational Science Institute [UL1TR000454], and the Emory Global Health Institute. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Figure 1.
Sputum Culture, Xpert TB/RIF, and MTBDRplus Assays Results

Table 1Time to results for Sputum Culture, Xpert MTB/RIF and MTBDR*plus* Assays (n=382)

Test	Time to Result [#] (Days)			
	Mean	SD	Median	IQR
Solid Culture (N=155)	36.7	12.9	33.0	27–41
Liquid Culture (N=227)	11.6	11.3	9.0	7–11
Xpert TB/RIF	3.3	2.3	2.0	2–4
MTBDR <i>plus</i>	5.5	2.4	5.0	3–7

* SD, standard deviation; IQR, interquartile range

[#] Time to result: time from sample collection to time to recorded results

Table 2Comparative characterization of Xpert MTB/RIF and GenoType MTBDR_{plus} (ver.2) tests

		GenoType MTBDR _{plus}	Xpert MTB/RIF
Infrastructure	Space requirements	High (three separate rooms)	Limited (one bench)
	Biosafety requirements	Advanced BSL2*	None
Test performance space	Temperature control	RT~	15–30°C
	Storage space temperature control	Needed (+4; –20)	Room temperature (2–28)
	Power supply backup	Required (generator)	Required (UPS [“])
Equipment	Manufacturer	Twincubator (Hain Lifescience))	Xpert Machine (Cepheid)
	Additional equipment	Thermocycler	None required
		Water bath	
		Refrigerator (+4)	
		Refrigerator (–20)	
		Thermoblock	
Maintenance	Annual Calibration	Suggested	Required
	Software support	None	None
	Service guarantee	None	1 year warrantee provided
	Service available on-site	No	No
HR[#]	Staff qualification	MBB or higher	Laboratory technician
	Training needs	Advanced	Basic
Test turnaround time	Hands-on work	3–4 h	15 min
	Procedure duration	7–8 h	2 h
	Result submission	Second day	Same day
Test interpretation	MTBC identification	Yes	Yes
	<i>M.Tuberculosis</i> speciation	No	No
	INH resistance detected	Yes	No
	Rif resistance detected	Yes	Yes
	MDR-TB [^] detected	Yes	No
Quality control	Internal	Available	Available
	External	Not available	Not available
Interpretation		Manual	Automated

* BSL-biosafety level;

HR-human resources;

“ UPS-uninterruptible power supply;

[^] MDR-TB- resistance to at least isoniazid [INH] and rifampicin [RIF]

~ Only on Hybridization step.