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ORIGINAL RESEARCH

Performance of the Abbott RealTime MTB RIF/INH resistance assay when used to test *Mycobacterium tuberculosis* specimens from Bangladesh

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Introduction: The Abbott RealTime MTB RIF/INH Resistance Assay (RT MTB RIF/INH) is an assay for the detection of rifampicin (RIF)- and/or isoniazid (INH)-resistant *Mycobacterium tuberculosis* (MTB). The assay can be used to test sputum, bronchial alveolar lavage, and N-Acetyl-L-Cysteine (NALC)/NaOH pellets prepared from these samples. The assay can be used in direct testing mode, or in reflex mode following a MTB positive result produced by its companion assay, Abbott RT MTB.

Methods: In this study, the direct testing mode was used to test paired sputum and NALC/NaOH pellets prepared from sputum collected from Bangladesh TB patients. One hundred and thirty two paired samples were tested.

Results: The RT MTB RIF/INH inhibition rate was 0%. One hundred and twenty-two paired samples had results above the assay limit of detection and were analyzed by comparing with results from phenotypic drug sensitivity testing, GeneXpert MTB/RIF (Xpert), and MTBDR plus (Hain). RT MTB RIF/INH results were in good agreement with those of GeneXpert and Hain.

Conclusion: The ability of this assay to detect RIF and INH resistance may contribute to the global control of multidrug resistant tuberculosis.

Keywords: tuberculosis, rifampicin, isoniazid, resistance

Introduction

Mycobacterium tuberculosis (MTB) remains a significant disease with 10.4 million new cases of tuberculosis (TB) reported in 2015.[1] Anti-TB therapy is effective in at least 85% of cases when the causative strain of MTB is sensitive to the four drugs (rifampicin [RIF], isoniazid [INH], ethambutol, and pyrazinamide) that constitute front-line therapy.[1] Treatment with front-line therapy is less effective when MTB is resistant to one or more of these drugs.[1] It is therefore important to detect MTB resistance to one or more of these front-line drugs so that therapy can be modified appropriately.[1] Detection of drug-resistant MTB is often performed using the accurate but slow (up to 12 weeks) culture-based (phenotypic) drug sensitivity testing (DST).[1] In response to the relative length of time to generate a phenotypic DST result, more rapid nucleic acid amplification tests (NAAT) have been developed.[2] The Cepheid GeneXpert MTB/RIF (Xpert) assay detects mutations associated with RIF resistance while Hain MTBDR Plus (Hain) detects mutations associated with RIF and INH resistance.[3–6]

Abbott RealTime MTB RIF/INH Resistance (RT MTB RIF/INH) detects mutations associated with resistance to RIF and INH.[7] RT MTB RIF/INH is a companion assay to Abbott RealTime MTB (RT MTB).[8,9] It can be used in direct testing mode to test

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respiratory specimens (sputum, bronchial alveolar lavage, or N-Acetyl-L-Cysteine (NALC)/NaOH sediments prepared from sputum or bronchial alveolar lavage) or used in reflex mode following a positive RT MTB result.[7] The performance of RT MTB RIF/INH has been studied in in Germany, Hong Kong, and South Africa in reflex mode, following an RT MTB positive result.[10–12]

The intent of this study was to review the performance of RT MTB RIF/INH when used in direct testing mode with MTB culture-positive samples from Bangladesh for which phenotypic DST, Xpert, and Hain results had been generated. The inclusion of DST, as well as Xpert and MTBDR plus results for this patient population provides a measure of performance for RT MTB RIF/INH against phenotypic DST and Xpert and Hain assays.

Methods

Samples

Three sputum specimens were collected from 132 subjects suspected of having active TB. Specimens were collected under the supervision of the National TB Reference Lab Institutional Review Board and Intercenter Agreement. All subjects enrolled in this study provided written informed consent. The three sputum specimens were collected from each patient over a maximum period of 5 days, with at least 5 hours between collections. Collection was performed at the National Reference Laboratory in Dhaka, Bangladesh. Specimens were collected following Institutional Review Board approval. The HIV status of each subject was not determined.

Testing

At the National Reference Laboratory, the first sputum sample from each patient was subjected to NALC/NaOH decontamination. The resulting NALC/NaOH pellet was tested using three methods: 1) fluorescent microscopy using the World Health Organization (WHO) scoring system, 2) mycobacterial growth indicator tubes liquid culture with a maximum incubation time of 6 weeks and Lowenstein–Jensen (L–J) solid culture for a maximum incubation time of 8 weeks, and 3) Hain. If culture was positive for MTB, DST was performed using the L–J proportion method using an RIF concentration of 40.0 mcg/mL and an INH concentration of 0.2 mcg/mL. The second sputum from each patient was tested using Xpert. For each subject, if drug resistance results were produced by DST, Hain, and Xpert, the third sputum and residual NALC/NaOH pellet was sent to Abbott Molecular where they were tested using RT MTB RIF/INH.

DNA sequencing

Sanger DNA bidirectional sequencing was performed on samples that had discrepant results between DST and the RT MTB RIF/INH assay.

Data analysis

DST was considered to be the gold standard for determination of RIF and INH drug resistance. The Exact test was used to calculate the 95% CI.

Results

Samples from 132 subjects were included in the study. All 132 subjects had smear, culture, DST, Xpert, and Hain results. RT MTB RIF/INH was used to test all 132 paired NALC/NaOH pellets and sputum samples. The percent of valid RT MTB RIF/INH results was 100% (264/264); no assay inhibition was observed. The percentage of RT MTB RIF/INH results that were above the assay limit of detection (LOD) was for 96.2% (127/132) NALC/NaOH pellets and 94.7% (125/132) for sputum samples. Table 1 shows the RT MTB RIF/INH data by microscopy result.

A total of 122 subjects had RT MTB RIF/INH results for both NALC/NaOH pellets and sputum. One hundred percent (122/122) of these paired RT MTB RIF/INH results were in agreement. The 122 RT MTB RIF/INH results were compared against those of the gold standard, DST. To ensure that the same data set was analyzed, the same 122 subjects were analyzed for Xpert and Hain versus DST.

The comparative data for RIF resistance detection are shown in Table 2.

The probe detection patterns were available for both RT MTB RIF/INH and Xpert (but not for Hain RIF or INH responses), thus allowing a comparison of these patterns. The RT MTB RIF/INH uses eight probes to detect RIF resistance, while Xpert uses five probes (A–E).[7,13] 28 samples had RT

Table 1 Percentage of samples with RT MTB RIF/INH results greater than assay LOD

Microscopy status	RT MTB RIF/INH resistance results		RT MTB RIF/INH below LOD results	
	NALC/NaOH pellets	Sputum	NALC/NaOH pellets	Sputum
Positive	98.2 (110/112)	96.4 (108/112)	1.8 (2/112)	3.6 (4/112)
Negative	85.0 (17/20)	85.0 (17/20)	15.0 (3/20)	15.0 (3/20)
Total	96.2 (127/132)	94.7 (125/132)	3.8 (5/132)	5.3 (7/132)

Abbreviations: RT MTB RIF/INH, RealTime MTB RIF/INH Resistance Assay; MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; INH, isoniazid; LOD, limit of detection.

Table 2 RIF assay results of Hain, Xpert, and RT MTB RIF/INH compared against DST

Assay results	DST	
Hain	**Resistant**	**Sensitive**
RIF resistant	27	1
RIF sensitive	5	89
Hain RIF sensitivity =84.4% (27/32) [95% CI 67.2%–94.7%]		
Hain RIF specificity =98.9% (89/90) [95% CI 94.0%–100.0%]		
Hain RIF PPV =96.4% (27/28) [95% CI 81.7%–99.9%]		
Hain RIF NPV =94.7% (89/94) [95% CI 88.0%–98.3%]		
Xpert		
RIF resistant	28	0
RIF sensitive	4	90
Xpert RIF sensitivity =87.5% (28/32) [95% CI 71.0%–96.5%]		
Xpert RIF specificity =100% (90/90) [95% CI 96.0%–100.0%]		
Xpert RIF PPV =100.0% (28/28) [95% CI 87.7%–100.0%]		
Xpert RIF NPV =95.7% (90/94) [95% CI 89.5%–98.8%]		
RT MTB RIF/INH		
RIF resistant	28	0
RIF sensitive	4	90
RT MTB RIF/INH RIF sensitivity =87.5% (28/32) [95% CI 71.0%–96.5%]		
RT MTB RIF/INH RIF specificity =100% (90/90) [95% CI 96.0%–100.0%]		
RT MTB RIF/INH RIF PPV =100.0% (28/28) [95% CI 87.7%–100.0%]		
RT MTB RIF/INH RIF NPV =95.7% (90/94) [95% CI 89.5%–98.8%]		

Abbreviations: RT MTB RIF/INH, RealTime MTB RIF/INH Resistance Assay; MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; INH, isoniazid; DST, drug sensitivity testing; PPV, positive predictive value; NPV, negative predictive value.

Table 3 Comparison of RT MTB RIF/INH and Xpert RIF probe responses

		Number of samples with Xpert RIF probe responses				
		A	**B**	**C**	**D**	**E**
Number of samples with RT MTB RIF/ INH probe responses	1					
	2		1			
	3					
	4					19
	5	3	3			
	6					
	7				2	
	8					

Abbreviations: RT MTB RIF/INH, RealTime MTB RIF/INH Resistance Assay; MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; INH, isoniazid.

MTB RIF/INH and Xpert RIF responses. The comparative data are shown in Table 3.

The comparative data for INH resistance detection are shown in Table 4.

Sanger sequencing of the potential five false-positive INH results by RT MTB RIF/INH detected INH resistance mutations (two samples had positive results for KatG 315T1, while three samples had positive results for inhA). If sequencing is correct, this would increase the INH specificity of RT MTB RIF/INH to 100%.

Table 4 INH assay results of Hain and RT MTB RIF/INH compared against DST

Assay results	DST	
Hain	**Resistant**	**Sensitive**
INH resistant	26	0
INH sensitive	6	90
Hain INH sensitivity =81.3% (26/32) [95% CI 63.6%–92.8%]		
Hain INH specificity =100% (90/90) [95% CI 96.0%–100.0%]		
Hain INH PPV =100.0% (26/26) [95% CI 86.8%–100.0%]		
Hain INH NPV =93.8% (90/96) [95% CI 86.9%–97.7%]		
RT MTB RIF/INH	**Resistant**	**Sensitive**
INH resistant	28	5
INH sensitive	4	85
RT MTB RIF/INH INH sensitivity =87.5% (28/32) [95% CI 71.0%–96.5%]		
RT MTB RIF/INH INH specificity =94.4% (85/90) [95% CI 87.5%–98.2%]		
RT MTB RIF/INH INH PPV =84.8% (28/33) [95% CI 68.1%–94.9%]		
RT MTB RIF/INH INH NPV =95.5% (85/89) [95% CI 88.9%–98.8%]		

Abbreviations: RT MTB RIF/INH, RealTime MTB RIF/INH Resistance Assay; MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; INH, isoniazid; DST, drug sensitivity testing; PPV, positive predictive value; NPV, negative predictive value.

Analysis of the 122 Bangladeshi subjects, for whom RT MTB RIF/INH results were generated, using phenotypic DST showed that 71.3% (87/122) had RIF- and INH-sensitive MTB, 22.9% (28/122) had RIF- and INH-resistant MTB, 3.3% (4/122) had RIF-resistant and INH-sensitive MTB, and 2.5% (3/122) had RIF-sensitive and INH-resistant MTB.

Discussion

This study assessed the performance of RT MTB RIF/INH when used to test paired sputum and NALC/NAOH pellets of sputum collected from Bangladeshi TB subjects. The direct testing mode was used in contrast to the reflex mode used in other evaluations of RT MTB RIF/INH.[10–12] The level of invalid RT MTB RIF/INH results was 0% in both sputum and NALC/NaOH specimens. This is consistent with the other evaluations of RT MTB RIF/INH where the percent of invalid RT MTB RIF/INH results was negligible.[10–12] This suggests that the RT MTB RIF/INH sample preparation protocol was well optimized to process respiratory specimens (sputum, NALC/NaOH pellets of sputum). This is the first evaluation where paired sputum and NALC/NaOH pellets prepared from sputum were evaluated. One hundred percent of the paired results were in agreement, suggesting that the RT MTB RIF/INH assay has good reproducibility across sample types. As was seen in the other evaluations, some samples tested using RT MTB RIF/INH had results of "Below LOD."[10–12] In another study, the LOD of RT MTB RIF/INH was determined to be 60 cfu/mL in sputum when used to test a quantitated culture of MTB H37Rv diluted in

pooled sputum.[7] In this study, "Below LOD" results were more frequent in smear negative-specimens (15%) than in specimens with smear-positive results (1.8%–3.6%). There was not a large difference in the frequency of "Below LOD" results when paired sputum (5.3%) and NALC/NaOH (3.8%) samples were tested suggesting that the RT MTB RIF/INH has similar sensitivity in these two sample types. Similar rates of "Below LOD" RT MTB RIF/INH results were observed in two other studies.[10,11]

In this paper, we report on the sensitivity and specificity vs phenotypic DST of RT MTB RIF/INH, Hain, and Xpert as regards detection of RIF and INH resistance. For RIF resistance, RT MTB RIF/INH and Xpert had 87.5% sensitivity and 100% specificity for detection of RIF resistance. Hain, by contrast, had 84.4% sensitivity and 98.9% specificity. For INH resistance, RT MTB RIF/INH and Hain had a sensitivity of 87.5% and 81.3%, respectively. The INH specificity of RT MTB RIF/INH for INH detection at 94.4% was lower than that of Hain at 100%. Sanger sequencing detected INH resistance mutations in the false-positive INH RT MTB RIF/INH assay results. If true, this would increase the INH specificity of RT MTB RIF/INH to 100%. We decided to report the RT MTB RIF/INH INH specificity without benefit of the sequencing information. This is because DST is the gold standard and sequencing of discrepant samples is for information only. In general, these data confirm previous observations that showed similar performance between RT MTB RIF/INH and other NAAT for detection of RIF and INH resistance.[7,10–12]

An analysis of the RIF probe response patterns for both RT MTB RIF/INH and Xpert was performed. This analysis showed that the majority of RIF-resistant mutations (19/28) were in RT MTB RIF/INH RIF probe 4 and Xpert probe RIF E. The remaining nine samples reacted with three RT MTB RIF/INH RIF probes (2, 5, and 7) and three Xpert RIF probes (A, B, and D). This analysis is useful in that it will allow correspondence to be generated between RT MTB RIF/INH and Xpert RIF RIF responses. A similar analysis was not performed between RT MTB RIF/INH and Hain because the Hain RIF and INH probe results were not available.

Per WHO guidelines, TB subjects infected with RIF- and INH-sensitive disease should be treated with front-line therapy, while those infected with RIF- and INH-resistant, or RIF-resistant and INH-sensitive TB, should be treated with second-line therapy.[1,14] Rapid NAAT that detect RIF resistance are very useful for such patients. However, this type of NAAT would not have detected the 2.5% of patients in this population with RIF-sensitive but INH-resistant disease. Rapid detection of RIF-sensitive, INH-resistant patients is useful because efficacy of front-line therapy is reduced in such patients.[15] NAAT that detect both RIF and INH resistance, such as RT MTB RIF/INH, therefore may complement phenotypic DST for the detection of resistance that impacts front-line therapy.[2–6] Given that DST is the accurate but slow gold standard for detection of drug-resistant MTB, more rapid NAAT are being widely implemented. The level of implementation of NAAT varies by country. The RT MTB RIF/INH assay is an alternative NAAT to the two WHO-endorsed NAAT. It may prove useful in certain testing situations, in particular those that benefit from centralized, automated batch testing.

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Author contribution

All authors contributed toward data analysis, drafting, and critically revising the paper, and agree to be accountable for all aspects of the work.

Disclosure

All authors are employees of Abbott Molecular, the company which makes the RT MTB RIF/INH product. The authors report no other conflicts of interest in this work.

References

1. World Health Organization (WHO). *Global Tuberculosis Report 2016*. Geneva: World Health Organization; 2016.
2. UNITAID. *Tuberculosis Diagnostics Technology Landscape 2017*. Geneva: UNITAID; 2017.
3. World Health Organization. *Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System*. Geneva: World Health Organization; 2011.
4. World Health Organization. *Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children*. Geneva: World Health Organization; 2013.
5. World Health Organization. *Molecular Line Probe Assays for Rapid Screening of Patients at Risk for Multi-drug Resistant Tuberculosis (MDR-TB)*. Geneva: World Health Organization; 2008.
6. World Health Organization. *The Use of Molecular Line Probe Assay for the Detection of Resistance to Isoniazid and Rifampicin: Policy Update*. Geneva: World Health Organization; 2016.
7. Kostera J, Leckie G, Tang N, et al. Analytical and clinical performance characteristic of the Abbott RealTi*m*e MTB RIF/INH Resistance, an assay for the detection of rifampicin and isoniazid resistant *Mycobacterium tuberculosis* in pulmonary specimens. *Tuberculosis*. 2016;101:137–143.
8. Tang N, Frank A, Pahalawatta V, et al. Analytical and clinical performance of Abbott RealTi*m*e MTB, an assay for detection of *Mycobacterium tuberculosis* in pulmonary specimens. *Tuberculosis*. 2015;95:613–619.

9. Qi C, Wallis C, Pahalawatta V, et al. Evaluation of the efficiency of the sample inactivation reagent in the Abbott RealTi*me* MTB assay for inactivation of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2015;53:3001–3002.
10. Hofman-Thiel S, Molodtsov N, Anonenka U, Hoffman H. 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. *J Clin Microbiol*. 2016;54:3022–3027.
11. Tam KK, Leung KS, To SW, et al. Direct detection of *Mycobacterium tuberculosis* and drug resistance in respiratory specimen using Abbott RealTi*me* MTB detection and RIF/INH resistance assay. *Diagn Microbiol Infect Dis*. 2017;89(2):118–124.
12. Scott L, David A, Noble L, et al. Performance of the Abbott RealTi*me* MTB and MTB RIF/INH assays in a Setting of high Tuberculosis and HIV coinfection in South Africa. *J Clin Microbiol*. 2017;55(8): 2491–2501.
13. Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol*. 2010;48:229–237.
14. World Health Organization. *WHO Treatment Guidelines for Drug-Resistant Tuberculosis*. Geneva: World Health Organization; 2016.
15. Gegia M, Cohen T, Kalandadze I, Vashakidze L, Furin J. Outcomes among tuberculosis patients with isoniazid resistance in Georgia, 2007–2009. *Int J Tuberc Lung Dis*. 2012;16:812–816.

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