DIAGNOSTICS

Analytical and clinical performance characteristics of the Abbott RealTime MTB RIF/INH Resistance, an assay for the detection of rifampicin and isoniazid resistant *Mycobacterium tuberculosis* in pulmonary specimens

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**A R T I C L E   I N F O**

**Article history:**
Received 27 April 2016
Accepted 15 September 2016

**Keywords:**
*Mycobacterium*
Rifampicin
Isoniazid
Sensitivity
Specificity
Reflex

**S U M M A R Y**

Clinical management of drug-resistant tuberculosis patients continues to present significant challenges to global health. To tackle these challenges, the Abbott RealTime MTB RIF/INH Resistance assay was developed to accelerate the diagnosis of rifampicin and/or isoniazid resistant tuberculosis to within a day. This article summarizes the performance of the Abbott RealTime MTB RIF/INH Resistance assay; including reliability, analytical sensitivity, and clinical sensitivity/specificity as compared to Cepheid GeneXpert MTB/RIF version 1.0 and Hain MTBDRplus version 2.0. The limit of detection (LOD) of the Abbott RealTime MTB RIF/INH Resistance assay was determined to be 32 colony forming units/milliliter (cfu/mL) using the *Mycobacterium tuberculosis* (MTB) strain H37Rv cell line. For rifampicin resistance detection, the Abbott RealTime MTB RIF/INH Resistance assay demonstrated statistically equivalent clinical sensitivity and specificity as compared to Cepheid GeneXpert MTB/RIF. For isoniazid resistance detection, the assay demonstrated statistically equivalent clinical sensitivity and specificity as compared to Hain MTBDRplus. The performance data presented herein demonstrate that the Abbott RealTime MTB RIF/INH Resistance assay is a sensitive, robust, and reliable test for realtime simultaneous detection of first line anti-tuberculosis antibiotics rifampicin and isoniazid in patient specimens.

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1. Introduction

Drug resistant *Mycobacterium tuberculosis* is a significant issue; in 2013, there were an estimated 480,000 new cases of multiple drug resistant TB (MDR-TB), which is defined as MTB with resistance to rifampicin (RIF) and isoniazid (INH), the two most important first-line anti-TB drugs [1]. For the detection of MDR-TB, both World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) have recommended the use of rapid nucleic acid amplification tests (NAAT) as well as gold standard culture-based drug sensitivity testing (DST) [2–6].

The Abbott RealTime MTB, an assay for the qualitative detection of MTB in pulmonary specimens (sputum, bronchial alveolar lavage (BAL), and their N-acetyl-L-cysteine (NALC)-prepared sediment equivalents) was developed for use on the Abbott m2000 system [7]. Initial data has been published on the MTB inactivation strategy and performance of Abbott RealTime MTB [7–9]. The Abbott RealTime MTB RIF/INH Resistance assay is a companion assay to the Abbott RealTime MTB assay; it can be executed standalone, or in a reflex mode using the remaining eluents from Abbott RealTime MTB. The assay is designed to detect RIF and/or INH resistance in MTB positive samples. This article summarizes the performance of the Abbott RealTime MTB RIF/INH Resistance assay.

2. Materials and methods

The overall workflow for the Abbott RealTime MTB RIF/INH Resistance assay is described in Figure 1.
0.80 mL of IR-treated sample and assay controls are loaded on an preparation reagent that is placed onto the wash steps are completed, the nucleic acids bound to the mi-
the DNA bound microparticles through a series of wash steps. After the sample inactivation process is completed, the samples are removed from the biological safety cabinet for sample preparation.

2.3. Amplification and detection

The Abbott m2000sp instrument is used for amplification and realtime fluorescence detection. Three separate PCR reactions A, B, and C are performed per sample. To detect the IC, each reaction contains a primer pair and labeled probe. Reactions A and B contain a primer pair to produce amplicons for detecting the 81 base pair long RIF resistance determining region (RRDR) of rpoB using eight different dye-labeled probes (Probes 1–4 in reaction A; Probes 5–8 in reaction B) [10,11]. Each RRDR probe is designed to hybridize to a RIF drug sensitive sequence of MTB. Mutation(s) in the RRDR would prevent complete hybridization of the rpoB wild type probe(s) in the respective region, generating an assay result of “rpoB B-,” with an assay interpretation of “RIF R det,” RIF resistance detected. Conversely, for a sample where all 8 rpoB probes are detected, an assay result of “rpoB wt” would accompany an assay interpretation of “RIF R-,” RIF resistance not detected. Reaction C contains two primer pairs to produce amplicons for detecting INH resistance: one primer pair targeting the katG gene and the second primer pair targeting the inhA Upper Strand Promoter (USP) region [10,11]. There are four labeled probes in reaction C: a labeled probe to detect katG wild type sequence, a labeled probe to detect the katG S315T1 drug resistance mutation commonly associated with high level INH resistance, a labeled probe to detect inhA USP wild type sequence, and a labeled probe to detect the inhA C-15T mutation commonly associated with low level INH resistance.

2.4. Assay controls

A minimum of one replicate of the Negative Control and one replicate of the Positive Control are used to determine run validity. The positive control consists of plasmid DNA containing five DNA sequences of three target regions (rpoB RRDR wildtype, katG wildtype, inhA USP wildtype, katG S315T1 mutation, and inhA C-15T USP mutation). The IC is formulated with a synthetic DNA construct containing a pumpkin hydroxypyruvate reductase (HPR) sequence and is retaining a pumpkin hydroxypyruvate reductase (HPR) sequence and is containing a primer pair and labeled probe. Reactions A and B contain a primer pair to produce amplicons for detecting the 81 base pair long RIF resistance determining region (RRDR) of rpoB using eight different dye-labeled probes (Probes 1–4 in reaction A; Probes 5–8 in reaction B) [10,11]. Each RRDR probe is designed to hybridize to a RIF drug sensitive sequence of MTB. Mutation(s) in the RRDR would prevent complete hybridization of the rpoB wild type probe(s) in the respective region, generating an assay result of “rpoB B-,” with an assay interpretation of “RIF R det,” RIF resistance detected. Conversely, for a sample where all 8 rpoB probes are detected, an assay result of “rpoB wt” would accompany an assay interpretation of “RIF R-,” RIF resistance not detected. Reaction C contains two primer pairs to produce amplicons for detecting INH resistance: one primer pair targeting the katG gene and the second primer pair targeting the inhA Upper Strand Promoter (USP) region [10,11]. There are four labeled probes in reaction C: a labeled probe to detect katG wild type sequence, a labeled probe to detect the katG S315T1 drug resistance mutation commonly associated with high level INH resistance, a labeled probe to detect inhA USP wild type sequence, and a labeled probe to detect the inhA C-15T mutation commonly associated with low level INH resistance.

2.1. Sample inactivation

A sample inactivation procedure is required prior to automated or manual sample processing. Approximately three volumes of inactivation reagent (IR) [in 500 mL of IR: 20 mL 10 M NaOH, 300 mL isopropanol, 0.9 mL Tween-20, 179.1 mL Molecular Biology Grade water] are added to each volume of sample (unprocessed sputum or BAL, or re-suspended NALC-prepared sediment of sputum or BAL) [9]. The sample inactivation procedure should occur in a class II biological safety cabinet. The sample/IR mixture is vortexed twice for 20–30 s during the first hour of incubation. The validated incubation time for the sample mixture is between 1 and 48 h. Once the sample inactivation process is completed, the samples are removed from the biological safety cabinet for sample preparation.

2.2. Sample preparation

The Abbott m2000sp instrument is used for automated sample processing. Prior to initiating an automated sample extraction protocol on the instrument, an internal control (IC) is added to a sample preparation reagent that is placed onto the m2000sp. A minimum of 0.80 mL of IR-treated sample and assay controls are loaded on an m2000sp where DNA is isolated from samples using guanidinium thiocyanate magnetic microparticle technology to capture nucleic acids. Unbound sample and cellular components are removed from the DNA bound microparticles through a series of wash steps. After the wash steps are completed, the nucleic acids bound to the microparticles are eluted and the 250 µL of eluent are transferred to a 96 deep well plate. The m2000sp instrument is used to create three different amplification mastermixes A, B, and C. Each mastermix contains AmpliTag Gold Polymerase, a magnesium chloride activa-
tion reagent, and oligonucleotide reagent (A, B, C) containing primers, probes, and dNTPs. The m2000sp mixes and dispenses 25 µL aliquots of mastermixes A, B, and C, followed by 25 µL of the extracted sample eluates to each mastermix in a 96 well optical reaction plate. The plate is manually sealed with an optical adhesive film and transferred to an Abbott m2000rt for realtime PCR. As an alternative to the automated m2000sp, sample preparation, mastermix preparation, and PCR plate setup can be performed manually.

2.5. Optional reflex testing

The Abbott RealTime MTB RIF/INH Resistance assay can be performed in standalone mode starting with sample inactivation/ preparation and going through amplification and detection, or it
can be run in reflex mode following Abbott RealTime MTB. In reflex mode, the Abbott RealTime MTB RIF/INH Resistance uses remaining eluents from Abbott RealTime MTB. Samples identified as “MTB detected” by Abbott RealTime MTB can be reflex tested using Abbott RealTime MTB RIF/INH Resistance. For samples to be reflex tested, the Abbott RealTime MTB RIF/INH Resistance Positive Control must have been processed through sample preparation, alongside the controls for Abbott RealTime MTB.

2.6. Panels and clinical specimens

2.6.1. MTB complex subspecies detection and samples with identified drug resistant mutations and heteroresistance

Twenty MTB complex DNA samples were tested including Mycobacterium africanum 25420, M. africanum 35711, Mycobacterium bovis 35735, M. bovis 19274, M. bovis BCG 35746, M. bovis BCG 35747D, Mycobacterium canetti, Mycobacterium caprae BAA-824D, Mycobacterium microti 11152, M. microti 19422, Mycobacterium pinnipedii BAA-688D, MTB 25177D-5 (H37Ra), MTB 25618D-5 (H37Rv), MTB BAA-2236D, MTB BAA-2237D, MTB 27294D, MTB BAA-2234D, MTB BAA-823D, MTB BAA-2235D [13]. Nineteen of these DNA samples were obtained from the American Type Culture Collection (ATCC, Manassas, VA) while one (Mycobacterium canetti) was provided by Ibis Biosciences (Carlsbad, CA). The DNA concentrations of the samples were determined using the PicoGreen® NanoDrop method with the exception of three samples where such measurements could not be obtained. These three samples were diluted at a sample to water ratio of 1:600 and tested directly. Heteroresistance detection was studied using MTB wild type and mutation panel members that included major mutations rpoB 5531L (TGC-TTG), katG S315T1 (AGC-ACC), and inhA C-15T.

2.6.2. Limit of detection (LOD)

A RIF/INH drug sensitive MTB H37Rv panel targeted to 1 × 10^5 cfu/mL was prepared by Zeptometrix (Buffalo, NY). Three one mL aliquots of the Zeptometrix panel were combined and centrifuged at 3000 ×g for 15 min to remove free MTB DNA in the supernatant. The cell pellet was re-suspended in TE buffer and then added to a pool of homogenized MTB–negative sputum, obtained from Northwestern University Memorial Hospital (Chicago, IL), to make the following diluted MTB panel-members: 80 cfu/mL, 50 cfu/mL, 25 cfu/mL, 10 cfu/mL, 5 cfu/mL, 1 cfu/mL, 0.5 cfu/mL, and 0.1 cfu/mL. The same LOD panel members were used to test the LOD performance of Abbott RealTime MTB [7].

2.6.3. Analytical specificity

Analytical specificity panel members (n = 80) were collected as follows: 69 mycobacterial and other microorganism species were obtained from ATCC, eight bacterial isolates were cultured at Abbott Molecular, and Cytomegalovirus, Herpes Simplex virus 1, and Varicella-zoster virus were obtained from Advanced Biotechnology Inc. (Columbia, MD).

2.6.4. Potentially interfering substances

The following materials were tested: blood, DNA from human cells, gastric acid, hypertonic saline, physiologic saline, culture media, NALC pellet material, five anti-TB medications (Isoniazid, Rifampicin, Streptomycin, Pyrazinamide, Ethambutol), and bovine mucus.

2.6.5. Carryover

Carryover studies were performed using drug sensitive MTB and drug resistant MTB mutant plasmid panel members.

2.6.6. Reproducibility

Reproducibility studies were performed using an MTB strain H37Rv cell line at a concentration less than or equal to three times the Limit of Detection in pooled MTB negative sputum, and one MTB negative panel member in pooled MTB negative sputum MTB negative sputum was obtained from Northwestern University Memorial Hospital (Chicago, IL).

2.6.7. Clinical specimens

MTB positive specimens (unknown HIV status) were collected from patients in Russia and Bangladesh by Discovery Life Sciences (Los Osos, CA) and Sage Bio Networks Ltd. (Dhaka, Bangladesh) at the National TB Reference Lab. All patient specimens were collected under collection countries National TB Reference Lab Institutional Review Board (IRB) and Intercenter Agreement (ICA). Samples were consecutively collected and stored at −20°C for approximately 6 months until tested. For all specimens smear, culture, and comparator testing was performed near the collection site, while Abbott RealTime MTB RIF/INH Resistance assay testing was performed at Abbott Molecular. For discrepant results/interpretations between Abbott RealTime MTB RIF/INH Resistance and DST, bi-directional sequencing was performed for the respective PCR products from reactions A, B, and C by Abbott Diagnostic Division using Life Technology BigDye 3.1 and POP-7 and Applied Biosystems 3130xL DNA Sequencer/Analyzer.

3. Results

3.1. MTB complex subspecies detection and detection of resistance mutations and heteroresistance

Each of 20 different purified DNAs representing eight different species (M. africanum, M. bovis, M. bovis BCG, M. canetti, M. caprae, M. microti, M. pinnipedii, and M. tuberculosis) of the MTB complex...
was tested four times at 600 genomes/reaction. All the tested DNAs from the 20 MTB complex species were detected by Abbott Real-Time MTB RIF/INH Resistance (i.e. gave assay interpretations). 18 of 20 strains tested had assay results (rpoB wt, katG wt, inhA wt) and interpretations of RIF and INH drug sensitive (RIF R-; INH R-). A “rpoB wt,” “katG wt,” or “inhA wt” assay result correlates to detectable signal from the respective assay reaction wild type probes, corresponding to a respective drug sensitive assay interpretation. Strains _M. bovis_ BCG ATCC 35747D and MTB ATCC 35822D had assay results (rpoB wt, katG-, inhA wt) and interpretations of RIF drug sensitive and possible INH resistance (RIF R-; INH R*). For these samples where all 8 rpoB probes are detected, an assay result of “rpoB wt” would accompany an assay interpretation of “RIF R-,” RIF resistance not detected. A “katG-” result correlates to the absence of detectable signal from both _katG_ wildtype probe and 315T1 mutation probe (may indicate presence of non-315T1 mutation or deletion). A “inhA wt” assay result correlates to no mutation in _inhA_ USP probe binding region, therefore the _inhA_ wild type probe signal would be detected resulting in an assay interpretation of “RIF R-; INH R*”. Both strains (ATCC 35747D and ATCC 35822D) have confirmed _katG_ deletions supporting the correct assay results and interpretations [14,15]. Additional MTB panel sets consisting of wild type panel member and resistance panel members covering major resistance mutations (_rpoB_ S31L, S531L, S536Y, S516V, S511P; _katG_ S315T1, S315T1, S315N, S315I; _inhA_ USP -15T, -8A, -8C, -17T) were tested, generating correct Abbott RealTime MTB RIF/INH Resistance results and interpretations (data not shown). Heteroresistant mutation detection by the Abbott RealTime MTB RIF/INH Resistance assay was studied with mixed panels using a wild type panel and a mutation panel. The mutation panel included major mutations _rpoB_ S531L (TCG-TTG), _katG_ S315T1 (AGC-ACC), and _inhA_ C-15T. The following mixed panel testing scheme was used: 100% WT:0% MT (1.0E4copies/ml:0copies/ml), 90% WT:10% MT (9.0E4copies/ml:1.0E4copies/ml), 70% WT:30% MT (7.0E4copies/ml:3.0E4copies/ml), 50% WT:50% MT (5.0E4copies/ml:5.0E4copies/ml), 30% WT:70% MT (3.0E4copies/ml:7.0E4copies/ml), 10% WT:90% MT (1.0E4copies/ml:9.0E4copies/ml), and 0% WT:100% MT (0copies/ml:1.0E4copies/ml). The results of this study demonstrated that a sample must contain at least a 70% mutation composition for the mutations to be detected with the correct Abbott RealTime MTB RIF/INH Resistance assay result (rpoB Pb4-, _katG_ 315T1, _inhA_ wt) and the correct interpretation (RIF R det; INH High R). 3.2. Limit of detection (LOD) 20 replicates of each panel member (80 cfu/ml, 50 cfu/ml, 25 cfu/ml, 10 cfu/ml, 5 cfu/ml, 1 cfu/ml, 0.5 cfu/ml, and 0.1 cfu/ml) were tested across eight assay runs. The study was conducted using one lot of Abbott RealTime MTB RIF/INH Resistance assay and control reagents on two instrument systems. A sample was counted as detected if the correct result and interpretation was produced. The detection rate was calculated for each target concentration (Table 1). A Probit analysis of the data determined that the concentration of MTB detected with 95% probability was 32 cfu/ml (95% CI 16–183 cfu/ml). The claimed LOD of the Abbott RealTime MTB RIF/INH Resistance assay is 60 cfu/ml in pooled homogenized MTB negative sputum using the MTB H37Rv strain. 3.3. Analytical specificity Each of the 80 potential cross-reactants was tested in both a MTB drug resistant sample and a MTB drug sensitive sample. Nucleic acid from cultured microorganisms at a target concentration of 1 × 10^4 cfu/ml or purified nucleic acid from each microorganism or virus at a targeted concentration of 1 × 10^2  to 1 × 10^7 copies or genomes/ml were added to samples that contained 1000 cfu/ml MTB RIF/INH drug resistant cells (rpoB S531L, _katG_ S315T1, _inhA_ wt) or 1000 cfu/ml MTB RIF/INH drug sensitive cells (rpoB wt, _katG_ wt, _inhA_ wt). All samples gave the expected assay results and interpretations (“rpoB Pb4-, _katG_ 315T1, _inhA_ wt” result with “RIF R det; INH High R” interpretation for RIF/INH drug resistant cells or “rpoB wt, _katG_ wt, _inhA_ wt” result and “RIF R-; INH R*” interpretation for RIF/INH drug sensitive cells) when tested in the presence of potential cross-reactors (Table 2). 3.4. Potentially interfering substances Performance susceptibility of the Abbott RealTime MTB RIF/INH Resistance assay to interfering substances found in respiratory specimens (mucus, blood, DNA from human cells, gastric acid, hypertonic saline, physiological saline, culture media, NALC pellet material and five anti-TB medications [isoniazid, Rifampicin, Streptomycin, Pyrazinamide, Ethambutol]) were evaluated. All samples, 1000 cfu/ml (12.5 cfu/25 µl eluate in 50 µl PCR reaction) MTB RIF/INH drug resistant cells (rpoB S531L, _katG_ S315T1, _inhA_ wt) and 1000 cfu/ml (12.5 cfu/25 µl eluate in 50 µl PCR reaction) MTB RIF/INH drug sensitive cells (rpoB wt, _katG_ wt, _inhA_ wt), gave the expected assay results and interpretations (“rpoB Pb4-, _katG_ 315T1, _inhA_ wt” result with “RIF R det; INH High R” interpretation for RIF/INH drug resistant cells or “rpoB wt, _katG_ wt, _inhA_ wt” result and “RIF R-; INH R*” result for RIF/INH drug sensitive cells) when tested in the presence of potential cross-reactors (Table 3). 3.5. Carryover Potential sample carryover within the Abbott RealTime MTB RIF/INH Resistance assay was evaluated by testing five runs consisting of 55 high concentration (1 × 10^4 copies/ml) RIF/INH drug sensitive MTB wild type plasmid samples interspersed with 55 low concentration (1 × 10^3 copies/ml) RIF/INH drug resistant MTB mutant plasmid samples and five runs consisting of 55 high concentration (1 × 10^4 copies/ml) RIF/INH drug sensitive MTB mutant plasmid samples interspersed with 55 low concentration (1 × 10^3 copies/ml) RIF/INH drug sensitive wild type plasmid samples. All samples, MTB RIF/INH wild type plasmid samples (rpoB wt, _katG_ wt, _inhA_ wt) and MTB RIF/INH mutant plasmid samples (rpoB S531L, _katG_ S315T1, _inhA_ C-15T), gave the expected assay results and interpretations (“rpoB wt, _katG_ wt, _inhA_ wt” result and “RIF R-; INH R*” for wild type plasmid samples or “rpoB Pb4-, _katG_ 315T1, _inhA_ -15T” result with “RIF R det; INH High R” for drug resistant mutant plasmid samples), with no detectable carryover from high concentration samples to low concentration samples. 3.6. Reproducibility The reproducibility of Abbott RealTime MTB RIF/INH Resistance was evaluated by testing a panel consisting of one positive panel

<table>
<thead>
<tr>
<th>MTB target concentration CFU/ml</th>
<th>Number tested</th>
<th>Number detected</th>
<th>Percent detected</th>
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<tbody>
<tr>
<td>80</td>
<td>19</td>
<td>19</td>
<td>100%</td>
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<td>50</td>
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<td>10</td>
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<td>80%</td>
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<td>4</td>
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<td>1</td>
<td>20</td>
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<td>15%</td>
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<td>0.5</td>
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* One replicate of the 80 CFU/ml Panel Member was excluded from the data analysis due to an Internal Control invalid result.
member (MTB H37Rv) at a concentration between two to three times the assay LOD in pooled culture negative sputum, and one MTB-negative panel member in pooled culture negative sputum.

The panels were tested by four operators using two lots of Abbott MTB-negative panel member in pooled culture negative sputum, and one member (MTB H37Rv) at a concentration between two to three times the assay LOD in pooled culture negative sputum, and one member (MTB H37Rv) at a concentration between two to three times the assay LOD in pooled culture negative sputum.

Table 2

<table>
<thead>
<tr>
<th>Potential cross reactors tested.</th>
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<tr>
<td>Mycobacterium abscessus</td>
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<td>Mycobacterium avium</td>
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<td>Mycobacterium haemolyticum</td>
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<td>Mycobacterium kansasii</td>
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<td>Mycobacterium ulcerans</td>
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<td>Mycobacterium ulcerophagesens</td>
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<td>Mycobacterium pinnaceolata</td>
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<td>Mycobacterium macculatum</td>
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<td>Mycobacterium parvum</td>
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<td>Mycobacterium retinitisflavum</td>
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<td>Mycobacterium pseudotuberculosis</td>
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3.7. Clinical sensitivity and specificity

Clinical performance characteristics of the Abbott RealTime MTB RIF/INH Resistance assay were evaluated by testing MTB positive patient specimens from non-US populations. Acid-Fast Bacilli (AFB) smear testing was performed for all patient specimens. MTB suspect patient specimens, AFB smear positive or negative, were processed by NALC 2% NaOH method prior to culture using Lowenstein-Jensen medium. RIF and INH susceptibility test (DST) was performed on culture positive patient specimens. RIF and INH susceptibility was determined by solid Lowenstein-Jensen medium drug susceptibility tests using proportion method with rifampicin concentration of 40.0 mcg/ml and isoniazid concentration of 0.2 mcg/ml. RIF results were obtained for Abbott and Cepheid GeneXpert MTB/RIF for 216 (40 AFB smear negative or scanty/176 AFB smear positive), culture positive specimens with DST information. All testing between Cepheid GeneXpert MTB/RIF version 1.0 and Abbott RealTime MTB RIF/INH Resistance was blinded and decoded. Bi-directional DNA sequencing was performed for clinical specimens with discrepant results/interpretations between Abbott RealTime MTB RIF/INH Resistance and DST. The Abbott RealTime MTB RIF/INH Resistance assay demonstrated a RIF sensitivity of 94.8% (91/96) with 95% CI (88.3–98.3%) for 99.6% (159/160) for samples prepared manually.

MTB drug susceptibility tests using proportion method with rifampicin concentration of 40.0 mcg/ml and isoniazid concentration of 0.2 mcg/ml. RIF results were obtained for Abbott and Cepheid GeneXpert MTB/RIF for 216 (40 AFB smear negative or scanty/176 AFB smear positive), culture positive specimens with DST information. All testing between Cepheid GeneXpert MTB/RIF version 1.0 and Abbott RealTime MTB RIF/INH Resistance was blinded and decoded. Bi-directional DNA sequencing was performed for clinical specimens with discrepant results/interpretations between Abbott RealTime MTB RIF/INH Resistance and DST. The Abbott RealTime MTB RIF/INH Resistance assay demonstrated a RIF sensitivity of 94.8% (91/96) with 95% CI (88.3–98.3%) for 99.6% (159/160) for samples prepared manually. The Abbott RealTime MTB RIF/INH Resistance assay demonstrated an INH sensitivity of 95.8% (92/96) with 95% CI (89.7–98.9%) and a RIF specificity of 100% (120/120) with 95% CI (97.0–100%). The rpoB amplicons of the five samples with negative Abbott RealTime MTB RIF/INH Resistance assay RIF results vs. DST were analyzed using bi-directional DNA sequencing; four of the samples did not contain any mutations in the rpoB amplicon probe-binding region, while one sample contained a mutation S531L (TGG to TGG) in rpoB amplicon probe-binding region that Abbott RealTime MTB RIF/INH Resistance assay INH results, but negative by DST, were confirmed by wild type in the katG and inhA USP target regions. One sample contained a mutation S531L (TGG to TGG) in rpoB amplicon probe-binding region that Abbott RealTime MTB RIF/INH Resistance assay INH results, but negative by DST, were confirmed by wild type in the katG and inhA USP target regions. One sample contained a mutation S531L (TGG to TGG) in rpoB amplicon probe-binding region that Abbott RealTime MTB RIF/INH Resistance assay INH results, but negative by DST, were confirmed by wild type in the katG and inhA USP target regions. One sample contained a mutation S531L (TGG to TGG) in rpoB amplicon probe-binding region that Abbott RealTime MTB RIF/INH Resistance assay INH results, but negative by DST, were confirmed by wild type in the katG and inhA USP target regions. One sample contained a mutation S531L (TGG to TGG) in rpoB amplicon probe-binding region that Abbott RealTime MTB RIF/INH Resistance assay INH results, but negative by DST, were confirmed by wild type in the katG and inhA USP target regions.
Table 3
Potential interfering substances tested.

<table>
<thead>
<tr>
<th>Potentially interfering substance</th>
<th>Specimen source</th>
<th>Concentration/percentage</th>
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<tbody>
<tr>
<td>Mucus</td>
<td>Sputum</td>
<td>Mucin 5% (w/v)</td>
</tr>
<tr>
<td>Blood</td>
<td>Sputum or BAL</td>
<td>5% (v/v)</td>
</tr>
<tr>
<td>DNA from human cells</td>
<td>Sputum, BAL, NALC</td>
<td>1 x 10^6 cells/mL</td>
</tr>
<tr>
<td>Gastric acid</td>
<td>Sputum/BAL</td>
<td>pH 3–4 HCl in water, neutralized to pH 6.8 with sodium bicarbonate</td>
</tr>
<tr>
<td>Hypertonic saline used to induce sputum</td>
<td>Sputum</td>
<td>NaCl (5% w/v)</td>
</tr>
<tr>
<td>Physiologic saline used to collect BAL</td>
<td>BAL</td>
<td>NaCl (0.9% w/v)</td>
</tr>
<tr>
<td>Culture media</td>
<td>MTB culture</td>
<td>100%</td>
</tr>
<tr>
<td>Material used to re-suspend NALC pellets</td>
<td>NALC pellets</td>
<td>0.067 M phosphate, pH 6.8</td>
</tr>
<tr>
<td>Isoniazid (anti-TB medication)</td>
<td>Sputum or BAL</td>
<td>90 mg/mL</td>
</tr>
<tr>
<td>Rifampicin/rifampin (anti-TB medication)</td>
<td>Sputum or BAL</td>
<td>120 μg/mL</td>
</tr>
<tr>
<td>Streptomycin (Anti-TB medication)</td>
<td>Sputum or BAL</td>
<td>400 μg/mL</td>
</tr>
<tr>
<td>Pyrazinamide (anti-TB medication)</td>
<td>Sputum or BAL</td>
<td>500 μg/mL</td>
</tr>
<tr>
<td>Ethambutol (anti-TB medication)</td>
<td>Sputum or BAL</td>
<td>60 μg/mL</td>
</tr>
</tbody>
</table>

Table 4
Abbott RealTime MTB RIF/INH Resistance RIF clinical sensitivity and specificity.

<table>
<thead>
<tr>
<th>Abbott RealTime MTB RIF/INH Resistance</th>
<th>Drug susceptibility (DST)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIF resistant</td>
</tr>
<tr>
<td>RIF Resistance Detected</td>
<td>91</td>
</tr>
<tr>
<td>RIF Resistance Not Detected</td>
<td>5*</td>
</tr>
</tbody>
</table>

N = 216. Sensitivity 94.8% (91/96), Specificity 100% (120/120).

Note: In the same sample population, a RIF comparator assay demonstrated a RIF sensitivity of 95.8% ([92/96], [95% CI 89.7–98.9%]) and a RIF specificity of 100% ([120/120], [95% CI 97.0–100%]).

* By sequencing of the assay amplicons, four samples were wild type and were corroborated by bi-directional sequencing. One sample contained a mutation in the assay probe binding region.

Table 5
Abbott RealTime MTB RIF/INH Resistance INH clinical sensitivity and specificity.

<table>
<thead>
<tr>
<th>Abbott RealTime MTB RIF/INH Resistance</th>
<th>Drug susceptibility (DST)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>INH resistant</td>
</tr>
<tr>
<td>INH Resistance Detected</td>
<td>83</td>
</tr>
<tr>
<td>INH Resistance Not Detected</td>
<td>11*</td>
</tr>
</tbody>
</table>

N = 217. Sensitivity 88.3% (83/94), Specificity 94.3% (116/123).

Note: In the same sample population, an INH comparator assay demonstrated an INH sensitivity of 87.2% ([82/94], [95% CI 78.8–93.2%]) and a specificity of 96.7% ([119/123], [95% CI 91.9–99.1%]).

* Per sequencing of the amplicons, ten samples were wild type and were corroborated by bi-directional sequencing. One sample contained a mutation in the assay probe binding region.

4. Discussion

The Abbott RealTime MTB RIF/INH Resistance assay was designed as a standalone assay to detect RIF and INH resistant MTB with the additional capability to reflex test MTB positive specimens by Abbott RealTime MTB [7–9]. The Abbott RealTime RIF/INH Resistance assay uses the same sample inactivation and sample preparation protocols as Abbott RealTime MTB to test sputum, BAL, and NALC sediments of sputum and BAL. In the reflex mode, sample eluents with an assay interpretation of “MTB detected” using Abbott RealTime MTB, can be reflex-tested using Abbott RealTime MTB RIF/INH Resistance. The reflex-testing can be performed using a manual PCR plate set-up, or automated m2000sp PCR plate set-up. A workflow advantage of the reflex mode is that only samples positive by Abbott RealTime MTB need to be tested by Abbott RealTime RIF/INH Resistance, thus reducing assay test time as well as cost.

Abbott RealTime MTB RIF/INH Resistance uses 8 target probes to detect the 81 base pair RRDR region of rpoB associated with ~95–98% of RIF resistance, uses 4 target probes to detect the katG and inhA USP regions associated with ~70–84% of INH drug resistance [10,11]. Abbott RealTime MTB RIF/INH Resistance, like Cepheid GeneXpert and Hain MTBDRplus, will not detect mutations out of the assay amplicon regions (e.g., rare rpoB mutations V146F and I572F, which are outside the 81 bp RRDR) [18].

This study assessed the reliability, analytical sensitivity, and clinical sensitivity and specificity of the Abbott RealTime MTB RIF/INH Resistance assay. Reliability was assessed by confirming that the assay detected 20 DNAs representative of the most common sub-species of the MTB complex [13], by demonstrating that the assay did not cross-react with 80 potentially cross-reactive micro-organisms, by producing correct results when challenged with potentially interfering substances and by no-detectable carryover from a high positive drug resistant sample to a low positive drug sensitive sample, or vice-versa. The analytical sensitivity of the assay was evaluated by testing MTB strain H37Rv. The study demonstrated that the analytical sensitivity of Abbott RealTime...
MTB RIF/INH Resistance was 32 cfu/mL with a label claim of 60 cfu/mL. The analytical sensitivity of Abbott RealTime MTB was 2.45 cfu/mL with a label claim of 17 cfu/mL using the same panel [7]. The higher sensitivity of Abbott RealTime MTB as compared to Abbott RealTime MTB RIF/INH Resistance is because Abbott RealTime MTB targets IS6110, which is present in multiple copies per genome of MTB as compared to the targets (RRDR of rpoB, katG, inhA) of Abbott RealTime RIF/INH Resistance that are single copy per genome [16,17].

The clinical sensitivity and specificity of the Abbott RealTime MTB RIF/INH Resistance assay was assessed by comparing it to DST information for all patient specimens. DST was selected as the comparator since it is continues to be considered the gold standard for identifying drug resistance patterns in patient specimens [2–6]. Furthermore, the interpretations and results from the Hain and Cepheid assays were also compared to DST for patient specimens. Sequencing of the rpoB, katG, and inhA USP target regions was performed for samples with discrepant result between the Abbott RealTime MTB RIF/INH Resistance assay, Cepheid GeneXpert, Hain MTBDRplus, and DST. The Abbott RealTime MTB RIF/INH Resistance assay had statistically equivalent sensitivity and specificity compared to Cepheid GeneXpert for detection of RIF resistance. Detecting both RIF and INH Resistance simultaneously, the Abbott RealTime MTB RIF/INH Resistance assay has an advantage over Cepheid GeneXpert MTB/RIF that fails to detect INH mono-resistance, often linked to poor treatment outcome in RIF-susceptible but INH-resistant patients [19–21]. Additionally, the Abbott RealTime MTB RIF/INH Resistance assay demonstrated statistically equivalent sensitivity and specificity as compared to Hain MTBDRplus for INH resistance detection.

The Abbott RealTime MTB RIF/INH Resistance is performed using the m2000™ system analyzer, a system that is capable of running multiple assays including Abbott RealTime HIV, thus enabling efficient management of MTB-HIV co-infected patients. Additionally, the assay delivers an automated solution to simultaneously diagnosis rifampicin and/or isoniazid resistant MTB with statistically equivalent sensitivity and specificity as compared to Cepheid GeneXpert MTB/RIF and Hain MTBDRplus. The Abbott RealTime MTB RIF/INH Resistance assay will provide many benefits for patients worldwide, including rapid diagnosis to administer appropriate efficacious treatment quickly, deterring the transmission of drug resistant MTB.

Acknowledgements

The authors acknowledge the following colleagues at Abbott Molecular for their assistance during the development of the Abbott RealTime MTB RIF/INH Resistance assay; Vihanga Pahalawatta, Andrea Frank, Sandip Patel, Alexis Ergang, Daniel Egeland, Arthur Martinez, Salah Benchehida, Anke Coblenz-Korte, Dana Robinson, Xiaomao Wu, John DeVore, Tracy Miller, Cheng Li, Joann Sustachek, Clifford Chan, Brian Diedrich, David Lealy, Robert Bilkovski and Priscilla Swanson of Abbott Diagnostic Division.

Funding: None.

Competing interests: None declared.

Ethical approval: Not required.

References