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Performance Evaluation of the Abbott RealTi*me* MTB and RIF/INH Resistance Assays for the Detection of *Mycobacterium tuberculosis* and Resistance Markers in Respiratory Specimens at Nigist Eleni Mohammed Memorial Hospital, Hosanna, South Ethiopia.

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Abbreviations

AFB	Acid fast bacilli
BSC	Biosafety cabinet
CI	Confidence interval
DST	Drug susceptibility testing
EPHI	Ethiopian Public Health Institute
INH	Isoniazid
IR	Inactivation reagent
LJ	Lowenstein-Jensen
LOD	Limit of detection
MDR TB	Multi-drug resistant TB
MGIT	Mycobacteria Growth Indicator Tube
MTB	*Mycobacterium tuberculosis* complex
NAAT	Nucleic acid amplification test
NALC	N-acetyl-L-cysteine
NPV	Negative predictive value
NTM	Nontuberculosis Mycobacteria
PAB	Protein antigen b
PCR	Polymerase chain reaction
PPV	Positive predictive value
PTB	Pulmonary Tuberculosis
RIF	Rifampicin
RRDR	Rifampicin resistance determining region
RR-TB	Rifampicin-resistant TB
SOP	Standard operational procedures
SR	Sample reagent
TB	Tuberculosis
TTD	Time to detection
USP	Upper strand promoter
WHO	World Health Organization

Abstract

Background: Despite the availability of highly efficacious treatment, TB remains a major global health concern and its control is hampered by lack of early and accurate diagnosis. The Abbott RealTi*m*e MTB and RIF/INH assays are recently launched high-throughput automated nucleic acid amplification tests by Abbott laboratories for the rapid detection of *Mycobacterium tuberculosis* complex (MTB) and genetic markers of resistance to Isoniazid (INH) and Rifampin (RIF) from respiratory specimens. This test could be useful in Ethiopia for early and accurate diagnosis, however as far as our knowledge its performance is not evaluated so far.

Objective: To evaluate the diagnostic performance of the Abbott RealTime MTB and RIF/INH assays for the detection of *M. tuberculosis* and resistance markers from respiratory specimens.

Methods: A cross sectional study was conducted on 112 study subjects presumptive to have pulmonary tuberculosis at Nigist Eleni Mohammed Memorial Hospital, Hossana, South Ethiopia from April to December 2017. Two morning expectorated sputum specimens were collected from each study participant. One sample was tested directly by Xpert MTB/RIF assay and the other was used for smear microscopy, TB culture, Abbott RealTi*m*e MTB and INH/RIF Assays. Data was entered and analyzed using STATA statistical software version 12.1. Sensitivity, specificity, PPV and NPV was calculated using MGIT 960 as a reference. Kappa statistics was applied to test agreement between assays.

Results: For the detection of MTB the Abbott RealTime MTB assay exhibited an overall sensitivity, specificity, PPV and NPV of 92.3%, 93.8%, 85.7% and 96.8% respectively. For Rifampicin resistance MTB (RR-MTB) detection, the Abbott RealTi*m*e MTB RIF/INH assay showed 100% sensitivity, specificity, PPV and NPV while for the detection of INH resistance MTB the sensitivity, specificity, PPV and NPV of the Abbott MTB RIF/INH assay was 81.8%, 100%, 91% and 100% respectively. In this study strong agreement was found between the Abbott RealTime MTB and Xpert/ RIF assays for MTB (k=0.81) and RR-TB detection (k= 1).

Conclusion: The Abbott RealTime MTB and RIF/INH assays revealed high sensitivity and specificity in MTB diagnosis and provide reliable INH and RIF resistance profile. This test has similar diagnostic performance to Xpert RIF/INH assay with the advantages of high-throughput. This test could be a suitable and an alternative for high TB burden countries such as Ethiopia.

Key words: Abbott RealTi*m*e MTB, Abbott RealTi*m*e MTB RIF/INH, Performance

1. Introduction

1.1 Background

Tuberculosis (TB) is the leading killer among communicable diseases caused by the bacillus *Mycobacterium tuberculosis* (MTB). It typically affects the lungs (pulmonary TB) but can also affect other sites (extrapulmonary TB). Accurate and rapid diagnosis of active tuberculosis is required to reduce its transmission and burden (1). Methods of diagnosis that are available now days are AFB staining, radiologic findings, Mycobacterial culture (solid and liquid media) and Nucleic acid amplification tests (NAATs) (2).

NAATs represent a significant advancement in the diagnosis of TB as well for the detection of multidrug-resistant TB (MDR-TB), since drug resistance in *M. tuberculosis* complex ~~(MTB)~~ isolates is mostly the result of random genetic mutations in the target gene for drug (3). This tests allow an advantages of rapid diagnosis, standardized testing, potential for high throughput and fewer requirements for laboratory biosafety (4). This test is endorsed by CDC to be performed on at least one respiratory specimen from each presumptive TB cases and for whom the test result would alter case management or TB control activities (5).

The Abbott m2000™ RealTime system integrates advanced automation with precise and proven performance for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) in human plasma (6). Recently this system integrates the Abbott RealTi*me* MTB (RT MTB) and Abbott RealTi*me* MTB INH/RIF resistance assays which is used for the qualitative detection of MTB DNA and genotypic markers for rifampicin (RIF) and isoniazid (INH) resistance which are the two most important first line MTB drugs directly from specimen (7, 8).

The Abbott RealTi*me* MTB assay

The Abbott RealTi*m*e MTB assay is an in vitro polymerase chain reaction (PCR) assay for the qualitative detection of eight MTB subspecies DNA from smear positive and negative sputum or bronchial alveolar lavage (BAL), as well as from N-acetyl-L-cysteine (NALC) sediments prepared from sputum and BAL. An inactivation procedure is performed to liquefy viscous samples and for MTB viability reduction in specimens before processing using an inactivation reagent (IR) [0.6% sodium hydroxide (w/v), 60% isopropanol (v/v), and 1.8% Tween 20 (v/v)] in a ratio of 3:1(IR: specimen) for safe testing outside Biosafety cabinet (BSC) (9).

The assay includes an automated extraction step, which can manage up to 94 specimens in a single batch using *m2000sp* or using a manual sample preparation protocol, master mix preparation, a real time PCR-based amplification of MTB targets (the insertion sequence 6110 (IS6110) and protein antigen b (PAB)) using DNA polymerase in the presence of dNTPs and magnesium on the Abbott *m*2000*rt* instrument. The presence of MTB amplification products is detected during the annealing/extension step by measuring the real-time fluorescence signal of the MTB IS6110 and PAB probes. Result is reported as “MTB detected” or “MTB not detected” (7).

The Abbott RealTi*me* MTB RIF/INH Resistance assay

The Abbott RealTi*me* MTB RIF/INH Resistance assay is a companion test to the Abbott RealTime MTB assay. It can be executed standalone, or in a reflex mode (samples that are reported as "MTB detected" using Abbott RealTime MTB may be tested in a reflex procedure using Abbott RealTime MTB RIF/INH Resistance for RIF and INH resistance from the remaining sample of Abbott RealTime MTB). A maximum of twenty two specimens and two controls can be analyzed per run. But this assay incorporates the amplification reagent extended feature which allows for the use of amplification reagent pack a total of 4 times and an internal control a total of 2 times stored at -25°c to -15°c up to 90 days for processing fewer samples (8).

The principle of this test is similar to the Abbott RealTime MTB assay except target genes that are amplified are different. Preparation of target DNA is performed using a magnetic microparticle-based technology using the Abbott *m*2000*sp* or a manual sample preparation protocol. The targets of PCR used to detect RIF and INH resistance are amplified by DNA polymerase in the presence of dNTPs and magnesium on the Abbott *m*2000*rt* instrument. Detection of RIF and INH drug resistance is determined through the combined interpretation of 12 different target probe signals. RIF resistant MTB is detected by the use of 8 *rpo*B wild type probes while INH resistant MTB is detected by the use a labeled probe to detect *katG* wild type sequence, a labeled probe to detect the *katG* S315T1 drug resistance mutation associated with high level INH resistance, labeled probe to detect *inhA* upper strand promoter (USP) wild type sequence, and a labeled probe to detect the *inhA* C-15T mutation commonly associated with low level INH resistance (8, 10)

1.2 Statement of the problem

TB is the ninth leading cause of death worldwide and the top cause of death from a single infectious agent, ranking above HIV/AIDS. According to World Health Organization (WHO) global tuberculosis report, in 2016 there were an estimated 1.3 million TB deaths among HIV-negative people and an additional 374, 000 deaths among HIV-positive people. An estimated 10.4 million incident cases with 490,000 million multidrug-resistant TB (MDR-TB) were reported. Ethiopia is one of the 30 high TB burden countries. There were an estimated 182,000 (177 per 100,000 populations) incident cases of TB in Ethiopia in 2016. While an estimated 26,000 deaths (25 per 100,000), 4,000 deaths (3.9 per 100,000 deaths) due to TB in HIV negative and positive individuals respectively were reported during the same period (1).

One of the major challenges in TB control is caused by the lack of early and accurate diagnosis (11). The diagnosis relies on radiologic evaluations, smear microscopy, liquid and solid media culturing and molecular techniques. Chest X-ray is the primary radiologic diagnosis for pulmonary TB, but atypical radiologic findings make the diagnosis not specific (12). Even if smear microscopy is highly specific and most widely used method, it has low sensitivity between 20-80% (13). In addition this test doesn't differentiate between MTB species and it gives no indication of drug susceptibility to guide appropriate treatment (13, 14). Culturing using liquid and solid media is the standard method yet it takes longer period (2 to 8 weeks), is cumbersome and is prone to contamination (5-10%) (12).

Drug susceptibility testing (DST) of MTB can be determined by phenotypic methods through observation of growth or metabolic inhibition of isolates in drug containing and drug free medium or using genotypic methods by detecting mutation of the drug targeting gene. Conventional phenotypic DST remains the gold standard but it is technically demanding, lack reproducibility and it generally takes 2-3 months (15-17).

One of the new real-time based PCR system recently developed is Xpert MTB/RIF assay which is cartridge-based NAAT that simultaneously detects the DNA of MTB and resistance to RIF within 2 hours (4).

The combination of an effective tuberculocidal sample reagent (SR) and the closed configuration of the Xpert MTB/RIF cartridge effectively reduce the risk of infectious-aerosol creation so that it can be used close to point of care (18, 19). This test is endorsed by WHO in 2010 as first line diagnostic test in endemic countries when rapid results are crucial for TB diagnosis in HIV infected patients or for appropriate management of MDR-TB cases (4). In 2013, it has also been recommended for use in children and to diagnose specific forms of extrapulmonary TB (1).

Even though the Xpert MTB/RIF is a major advance in TB diagnostic testing it has limitations, firstly it detects the genetic markers of RIF resistance only disregarding INH which is a surrogate marker of MDR, secondly it uses a single gene copy (IS6110) for the detection of MTB missing the MTB species which have zero or deleted copies of IS6110 (20, 21). Lastly but not least this assay is not designed as a high-throughput system (22) which is not suitable for high-throughput laboratories.

Even if TB is totally preventable and curable, it is still the major cause of mortality and morbidity. One of the pillars to the end TB strategy with the vision of a world with zero deaths, disease and suffering due to TB is early detection of TB including DST and further reducing the burden of disease in high TB burden countries. This requires a rapid diagnostic technologies or methods with improved performance. The Abbott RealTime MTB and RIF/ INH assays are recently launched by Abbott molecular diagnostics for MTB and MDR-TB detection on Abbott m2000^{TM} system which is already in use for HIV-1 viral load testing in our country. However these new molecular methods are not evaluated in Ethiopia and most African context and we need to address this research gap to look an alternative fast and reliable molecular diagnostics for TB.

1.3 Significance of the Study

This study provides information about the diagnostic performance of the Abbott RealTime MTB and RIF/INH assays for the detection of MTB and drug resistance TB in sputum samples and will have implication for policy makers and others involved in TB control program in scaling up implementation of these tests in the national TB control program.

2. Literature review

2.1 Performance of Abbott RealTime MTB and MTB RIF/INH Resistance assays

The analytical and clinical performance of Abbott RealTime MTB for detection of MTB in pulmonary specimens of sputum and NALC sediment was studied in USA. The study showed this assay is a sensitive and reliable molecular method for MTB detection. According to this study the clinical sensitivity of the assay was 93% overall; 99% in smear positive and 81% in smear negative specimens. The clinical specificity was 97% and no cross reactivity or carryover were observed (23).

Another study was conducted in USA to evaluate the performance of the Abbott Realtime MTB RIF/INH Resistance assay for the detection of rifampicin and isoniazid resistance MTB in pulmonary specimen. When compared to DST the Abbott RealTime MTB RIF/INH Resistance assay demonstrated a RIF sensitivity of 94.8% (95% CI 88.3-98.3%) and a RIF specificity of 100 (95% CI 97.0-100%). The Abbott RealTime MTB RIF/INH Resistance assay demonstrated an INH sensitivity of 88.3% (95% CI 80.0-94.0%) and an INH specificity of 94.3% (95% CI 88.6-97.7%). The Abbott Realtime MTB RIF/INH Resistance assay demonstrated statistically equivalent clinical sensitivity and specificity as compared to Cephheid GeneXpert MTB/RIF assay and Hain MTBDR*plus* for RIF and INH resistance detection respectively (10).

In Germany a total of 715 clinical specimens using Abbott RT MTB for MTB detection was compared with culture. The overall sensitivity of RT MTB was 92.1%; the sensitivities for smear-positive and smear-negative samples were 100% and 76.4%, respectively. The method showed 100% (412/412) specificity with culture negative specimens and 96% (48/50) specificity with specimens which grew NTM. The RT MTB INH/RIF assay identified ten (4.3%) cases with multi-drug resistance, eight (3.4%) with isoniazid resistance and 171 (72.7%) with no resistance markers for isoniazid or rifampicin. Concordance with resistance patterns obtained by Genotype MTBDRplus and phenotypic DST in MGIT was 100% (22).

Another study was conducted in Spain to evaluate the Abbott real-time PCR assay for the detection of RIF and INH resistance in MTB using 99 specimens. From 72 samples that were phenotypically susceptible to RIF, no *rpoB* mutation was detected by Abbott RealTime MTB RIF/ INH, and of the 27 samples that were resistant to RIF, Abbott RealTime MTB INH/RIF detected resistance mutations in 26 of them. For the RIF resistance mutation, the Abbott RealTime MTB RIF/INH test demonstrated 96.3% (95% CI 87.32%–100%) sensitivity and 100% (95%CI 99.3%–100%) specificity. The kappa coefficient between the Abbott Real-Time MTB RIF/INH Resistance assay and the indirect DST results for RIF was 0.97. For INH, the Abbott RealTime MTB RIF/INH test displayed 78.8% (95%CI 66.8%–90.9%) sensitivity and 100% (95%CI 98.9%–100%) specificity (24).

In China the performance of the newly developed Abbott RealTime MTB assay was compared with that of the Cepheid GeneXpert assay using solid culture as the reference standard. The RealTime MTB assay demonstrated a sensitivity of 100% and a specificity of 84.4%; the GeneXpert assay had a sensitivity of 96.9% and specificity of 89.6%. After the resolution of discordant results by PCR-based molecular method, the sensitivities and specificities of the RealTime MTB and GeneXpert assays were 100% vs. 97% and 90.0% vs. 95.6%, respectively. No significant difference in sensitivity or specificity was found between the RealTime MTB and GeneXpert assays (25).

Another study was conducted in this country to assess the performance of the Abbott RealTime MTB assay for rapid detection of *Mycobacterium tuberculosis* complex in comparison with the Roche Cobas TaqMan MTB assay for 214 respiratory specimens. The overall diagnostic sensitivity was 100% for the Abbott assay and 76.1% for the Cobas assay, while the overall diagnostic specificity for the Abbott and Cobas assay was both 99.3% and in the prospective analysis of the total 520 specimens the overall estimates for sensitivity and specificity of the Abbott assay were both 100% among smear-positive specimens, whereas the smear-negative specimens were 96.7% and 96.1% respectively (26).

Another study was conducted in China using 610 sputum specimens compared with conventional culture results and clinical background as reference standards. The Abbott-RT exhibited an overall sensitivity and specificity of 95.2% and 99.8%, respectively. Genotypic RIF/INH resistance of 178 "MTB detected" specimens was subsequently analyzed by Abbott-RIF/INH. Compared to phenotypic drug susceptibility test results, Abbott-RIF/INH detected resistance genotypic markers in 84.6% MDR-TB, 80% mono-RIF-resistant and 66.7% mono-INH-resistant specimens (27).

A prospective clinical validation study was conducted in Johannesburg, South Africa on 206 individuals (73% HIV positive). Sensitivity and specificity of RT MTB was 82.5% (CI:67.2,92.7) and 93.1%(CI:86.2,97.2) on raw sputum and 77.5% (CI:61.5,89.2) and 95.1% (CI:88.9,98.4) on concentrated sputum respectively, compared to liquid culture. RT MTB correctly identified 17/35 more smear-negative, culture-positive specimens than Xpert MTB/RIF. Both RT MTB and Xpert MTB/RIF displayed sensitivities >70% and specificities >90% in HIV-positive individuals. The drug-resistance results agreed with MTBDR*plus* and drug susceptibility profiles (28).

2.2 Performance of Xpert MTB/RIF assay

According to WHO study the Xpert MTB/RIF has high sensitivity in detecting MTB (88%), with the NPV greater than 98% in setting with a high and a low prevalence of TB. The specificity of Xpert MTB/RIF for detecting TB was very high (99%). The study assumes that false-positive results are linked to the detection of dead MTB bacilli by Xpert MTB/RIF that would not be detected by culture. The PPV of this test is adversely affected in setting with a low prevalence setting that the test should be interpreted with other screenings such as symptom assessment and chest X-ray. The sensitivity, specificity, PPV and NPV of this test for the diagnosis of rifampicin resistance was 95%, >98%, 90% and 98% respectively. And recommends the test result needs to be interpreted appropriately considering the prevalence of the disease and based on risk assessment (4).

Twenty-four publications on the three diagnosis methods were meta-analyzed on the performance of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear negative MTB. The pooled sensitivity and specificity for the detection of smear negative pulmonary tuberculosis for the Genexpert were 67% and 98% respectively. The sensitivity of GeneXpert reduced from 67% to 54% when sub-group analysis of studies with patient HIV prevalence ≥30% was performed. This study concludes the accuracy of the tests for the diagnosis of smear negative TB are extremely variable (29).

A multi country evaluation study was conducted on Xpert/ RIF assay in India, South Africa, Peru and Azerbaijan by FIND on 1710 symptomatic patients. A single direct test on sputum detected 551 of 561 (98.2%) patients with smear-positive TB and 124 of 171 (72.5%) patients with smear-negative TB. The test was specific in 604 of 609 (99.2%) patients without TB, as defined using a combined reference standard of culture and clinical review. Compared with the results of phenotypic drug susceptibility testing, the assay also correctly identified 200 of 205 patients (97.6%) with rifampicin-resistant isolates and 504 of 514 patients (98.1%) with rifampicin-susceptible isolates (19).

A study was conducted in China to evaluate the performance of GeneXpert MTB/RIF in diagnosing pulmonary tuberculosis (TB) using 240 presumptive TB cases. Compared to MGIT culture the sensitivity, specificity, PPV and NPV of the GeneXpert MTB/RIF assay was 84.0% (68/81), 87.8% (129/147), 78.2% (68/87), and 87.2% (129/148) respectively. The agreement for results between Gene Xpert MTB/RIF and the MGIT 960 system was 82.8% and the Kappa value was 0.73 which is satisfactory (30).

In Turkey a study was conducted to compare the efficacy of culture, GeneXpert MTB/RIF device, and Erlich–Ziehl–Neelsen direct microscopic method in the identification of *Mycobacterium tuberculosis.* 927 samples (243 respiratory and 684 nonrespiratory) samples were included in this study. When compared to standard culture, sensitivity, specificity, and positive and negative predictive values of the GeneXpert system for respiratory samples were 100%, 98.7%, 87%, and 100%, respectively (31).

According to the Ethiopia Public Health Institute (EPHI) National Operational Validation Study on GeneXpert MTB/RIF assay the sensitivity, specificity, PPV and NPV of GeneXpert for detecting MDR-TB in new cases were 100% for each whereas in previously treated cases it was 95.8%, 89.7%, 88.5% and 96.3%, respectively. The overall sensitivity, specificity, PPV and NPV of GeneXpert in detecting MDR cases were 96.8%, 92.9%, 90.9%, and 97.5%, respectively. The sensitivity, specificity, PPV and NPV of GeneXpert in smear negative cases were 75.0%, 81.6%, 31.2% and 96.7%, respectively. Where as in smear positive cases, the sensitivity was 88.2% and PPV was 88.2 % (32).

The diagnostic yield of Xpert MTB/RIF assay in comparison with smear microscopy and culture was assessed in HIV positive adults eligible for ART at selected health centers in a region of Ethiopia. TB was diagnosed in 145/812 participants (17.9%) with bacteriological confirmation in 137 (16.9%). Among bacteriologically confirmed cases, 31 were smear positive (22.6%), 96 were Xpert-positive (70.1%) and 123 were culture positive (89.8%). The Xpert MTB/RIF assay increased TB detection rate by 64 cases (47.4%) compared with smear microscopy (33).

Assefa D. *et al* conducted a cross-sectional study on patients with presumptive of pulmonary TB to evaluate the performance of Xpert MTB/RIF assay for detection of MTBC in Ethiopia. A total of 227 specimens were analyzed. Overall 25.5% (58/227) samples were positive for MTBC by MGIT and/or LJ media of which 36.2 % (21/58) and 65.5% (35/58) were positive by AFB smear microscopy and Xpert MTB/RIF respectively. The sensitivity, specificity, positive and negative predictive value of Xpert MTB/RIF assay were 65.5%, 96.3 and 88.6% respectively. Eighteen (31.0%, 18/58) more cases, which were smear microscopy negative, were detected by Xpert MTB/RIF assay (34).

3. Objective of the study

3.1 General Objective

- ✓ To evaluate the diagnostic performance of the Abbott RealTime MTB and RIF/INH assays for the detection of MTB and resistance markers in respiratory specimens at Nigist Eleni Mohammed Memorial Hospital, Hossana, South Ethiopia.

3.2 Specific Objectives

- ✓ To determine the diagnostic sensitivity, specificity, positive and negative predictive values of Abbott RealTime MTB assay in detecting MTB.
- ✓ To determine the performance of Abbott RealTi*m*e MTB RIF/INH assay in detecting INH and RIF resistance TB.
- ✓ To compare the performance of the Abbott RealTime MTB and RIF/INH assays with Xpert MTB/RIF assay in detecting MTB and RR-TB.

3.3 Hypothesis

- ✓ There is no diagnostic performance difference between the Abbott Real time MTB and RIF/INH assays with the Xpert MTB/RIF assay in detecting MTB and RR-TB.

4. Materials and Methods

4.1 Study Area

The study was conducted at Nigist Eleni Mohammed Memorial Hospital which is found in Hosanna, the capital of Hadiya zone, Southern Nations, Nationalities and People's Region (SNNPR) of Ethiopia. The town is located 232 km south of the capital city Addis Ababa and 194 km far from the regional capital, Hawassa. It's the only hospital in Hadiya zone which serves more than 1,506, 733 peoples since1984. The hospital has 250 beds capacity served by 20 GP (MD), 2 surgeons, 2 Gynecologist, 1 pediatrician, 1 Emergency surgeon and 120 nurses (including midwifes). Approximately 6,ooo TB patients are served by this hospital per year.

4.2 Study Design and Period

A cross sectional study was conducted from April to December 2017, at Nigist Eleni Mohammed Memorial Hospital, Hossana, South Ethiopia.

4.3 Population

4.3.1 Source population

All patients who visited Nigist Eleni Mohammed Memorial Hospital during the study period.

4.3.2 Study population

Consecutive adults presented to the study area during the study period with presumptive pulmonary TB that fulfilled the inclusion criteria were included.

4.4 Inclusion and Exclusion criteria

4.4.1 Inclusion criteria

- ✓ Patients with age of ≥18 years.
- ✓ Patients with signs and symptoms suggestive of pulmonary TB.
- ✓ Provision of informed consent to participate in the study.

4.4.2 Exclusion criteria

- ✓ Patients those unable to produce sputum or with insufficient volume
- ✓ Participants on anti- tuberculosis treatment

4.5 Study variables

4.5.1 Dependent variables

- ✓ Sensitivity
- ✓ Specificity
- ✓ Positive predictive values
- ✓ Negative predictive values
- ✓ Cohen's kappa

4.5.2 Independent variables

- ✓ AFB smear status
- ✓ Previous TB treatment
- ✓ Time to detection in MGIT 960

4.6 Sampling procedure and sample size

4.6.1 Sample size determination

For evaluation of qualitative test performance Clinical and Laboratory Standard Institute (CLSI) 2008 guideline recommends testing should continue until at least 50 positive and 50 negative specimens are obtained with the comparative method (35). In this study a total of 92 samples were included for the performance evaluation of the Abbott RealTime MTB assay and 44 positive samples for the RIF/INH assay.

4.6.2 Sampling method

- ✓ **Sampling Method for the performance evaluation of the Abbott MTB assay**

Non-probability, convenience sampling technique was applied until the achievement of the expected sample size within the given study period. Two morning expectorated sputum samples having 2-3ml were collected from each patient (36). One sample was tested on Xpert

assay and the other sample was used for AFB, TB culture and the Abbott RT assay.

- ✓ **Sampling Method for the performance evaluation of the Abbott RealTime MTB RIF/INH assay**

Non-probability, convenience sampling technique was applied until the achievement of the expected sample size within the given study period. All samples that were positive by Xpert MTB/RIF assay and MGIT 960 were included in the study until the achievement of expected sample size.

4.6.3 Data collection procedure

A clinical and laboratory request form was used to extract medical history and socio demographic information results of the participants after informed consent was collected. Data log book was used to record all information of the patients enrolled in the study in a consecutive order.

4.6.4 Laboratory analyses

The first collected fresh sputum specimen was tested by the Xpert MTB/RIF assay at Nigist Eleni Mohammed Memorial Hospital. The other specimen collected on the study participant second visit was transported to ICL on the same day of collection and processed the next day. The leftover NALC treated sediment of sputum after TB culture and AFB smear was stored at –25°C to –15°C for later analysis using the RealTime MTB assays. DST was performed with MGIT 960 liquid culture media as a reference.

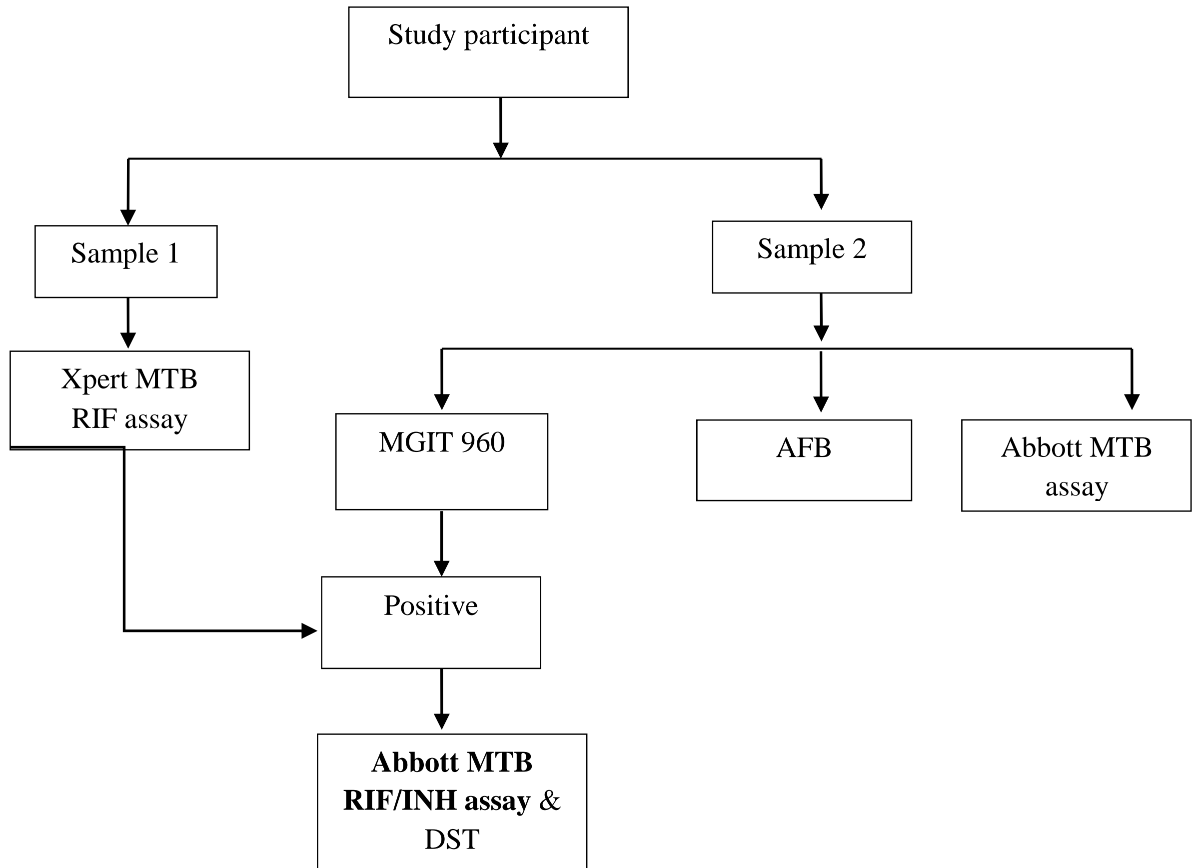

Fig. 4.1 Flowchart for laboratory investigation

4.6.4.1 Sample collection, storage and transportation

One sputum sample for Xpert MTB/RIF assay was collected on the patient first visit to the hospital, the other specimen used for smear, TB culture and the Abbott RealTime assay was collected on the patient second visit to the hospital. Specimens were collected with 50ml falcon tubes after consent form was obtained. The tubes were checked for proper labeling and stored at 2-8ºc until its transport to ICL. Specimens were transported on the same day of collection placed in leak proof biohazard bag with sealed lids and absorbent material in a cooler with chilled ice packs (38).

4.6.4.2 Xpert MTB/RIF assay

The sample reagent (2ml) supplied with the test was added in a 2:1 ratio to the sputum (1ml). The mixture was mixed vigorously and incubated at room temperature for 15 minutes. Two ml of the reagent sample mix was then transferred to an Xpert MTB/RIF cartridge using a pasteur pipette and the cartridge was loaded onto Xpert MTB/RIF instrument after appropriate patient data is filled into the software (37).

4.6.4.3 RealTime MTB assay

Inactivation reagent (0.4 M NaOH, 60% Isopropanol and 0.18% Tween-20) which inactivate the MTB viability was added at ratio 3:1 to N-Acetyl-L-Cysteine (NALC)-treated sediments of sputum and incubated for at least 1 hour and maximum 24 hours under BSC. 46 IR-treated specimens, 2 assay controls (Abbott RealTi*m*e MTB negative and positive controls) and sample preparation reagents were loaded onto an *m2000sp* instrument for DNA extraction. At the end of sample preparation the amplification master mix was loaded onto an *m2000sp* which dispenses 25 mL aliquots of the master mix followed by 25 mL aliquots of the extracted eluates to a 96-well optical reaction plate. The plate was sealed manually and transferred to the *m2000rt* for amplification and detection. Results were reported as “MTB not detected” or “MTB detected” (7).

4.6.4.4 RealTime MTB RIF/INH assay

Specimens were inactivated as previously described in RealTime MTB assay. IR-treated samples (22 MTB positive samples), assay controls (Abbott RealTime MTB RIF/INH positive and MTB negative controls) and sample preparation reagents were loaded on an *m2000sp*. After the wash steps are completed, the nucleic acids bound to the microparticles are eluted and the 250 ml of eluent are transferred to a 96 deep well plate. The *m2000sp* mixes and dispenses 25 ml aliquots of master mixes A, B, and C, followed by 25 ml of the extracted sample eluates to each master mix in a 96 well optical reaction plate. The plate is manually sealed with an optical adhesive film and transferred to an Abbott *m2000rt* for amplification and detection.

Results for RIF resistance detection were reported as RIF R^-, RIF R det, RIF indet or Below LOD; if Mutation(s) not detected in the *rpo*B Rifampicin resistance determining region (RRDR), Mutation(s) detected in the *rpo*B RRDR, RIF resistance could not be determined due to insufficient signal(s) from target probe or Target probe signals could not be detected from *rpo*B respectively. Results for INH resistance detection were reported as INH R^-, INH High R, INH Low R, INH R*, INH Indet or Below LOD; if Mutation(s) not detected in the *kat*G and *inhA* upper promoter regions, *katG* 315T1 mutation detected in the *kat*G region, *inhA*–15T mutation detected in the *inhA* upper promoter region, either *kat*G/ *inhA* Wild-Type or mutant gene probes were not detected, INH resistance could not be determined due to insufficient signal(s) from target probe(s) or Target probe signals could not be detected from *kat*G, and *inhA* upper promoter regions respectively (8).

4.6.4.5 MTB culture and identification of MTB

MycoPrep solutions in a volume equal to the specimen was added which allows its digestion and decontamination. This mixture was incubated for 15-20 minutes with vortexing for 15-20 seconds. Phosphate buffer (pH 6.8) was added up to the top ring on the centrifuge tube and then centrifuged at a speed of 3000 g for 15-20 minutes in a refrigerated centrifuge. The supernatant was decanted into a suitable container and the sediment was resuspended using 2ml PBS. 0.8 ml of MGIT growth supplement/PANTA and 0.5 ml of a well-mixed processed/concentrated specimen was added to the appropriately labeled MGIT tube and loaded to the instrument. MGIT tubes were entered into a MGIT 960 instrument where they were incubated and monitored for increasing fluorescence every 60 minutes. The instrument reports a tube negative if it remains negative for six weeks (42 days) (38).

MGIT positive tubes were inoculated on blood agar plate (BAP) and smear were prepared for Ziehl-Neelsen staining. Tubes were visually inspected for contamination (turbidity) as MTB appears granular that settles at the bottom of the tube, tubes that appeared turbid with growth on BAP at 35+/- 1˚c within 24-48 hours of incubation were reported as “tube contaminated”.

If the smear is negative for AFB and the tube does not appear to be contaminated, it's re-entered into the instrument for further monitoring. AFB smears were repeated after 1-3 days. For AFB positive tubes ID were performed using SD Bioline immunochromatography (ICT) test kit (39).

4.6.4.6 First line drug Susceptibility testing for MTB

For each appropriately labeled MGIT tube 800 µl SIRE Supplement, 100 µl of drug concentrations (0.1µg/ml for INH labeled tube, 1.0 µg/ml for RIF labeled tube) and 500 µl organism suspension was added. All tubes were mixed gently and loaded into MGIT 960 instrument using DST carrier. The instrument continuously monitors the tubes for increased florescence. Analysis of florescence in drug containing tube compare to the florescence of the growth control tube was used by instrument to determine the susceptibility result. Results were interpreted automatically and reported as susceptible or resistant. (38).

4.7 Quality assurance

4.7.1 Pre analytical

Samples were collected, stored and transported according to the SOP. All patient information collected during the study period was checked for its clarity and completeness in a regular basis.

4.7.2 Analytical

All laboratory tests were performed by well-trained laboratory personnel according to the SOP of laboratory to ensure the reliability and validity of test result. Each new shipment or lot number of staining reagents (Carbol Fuschin, Methylene Blue or 3% Acid Alcohol) were QC tested before use using a positive and negative QC slides. Start and end control were included in every run of MTB culturing. In addition *M. tuberculosis* H37Rv strain (ATCC 27294) that is susceptible to all first line anti -TB drugs were run when a batch of DST was set up or as every 6th isolate. (40). A negative and a positive control were included in each test order to evaluate run validity of the Abbott assays.

4.7.3 Post analytical

All laboratory results were recorded on a logbook during the study period. The collected data were analyzed and interpreted accordingly after it's checked for its completeness, accuracy and clarity on a regular base, data's which were incomplete were discarded.

4.8 Data analysis and interpretation

Statistical analysis was performed using STATA statistical software version 12.1. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using MGIT 960 as the reference method and results were interpreted based on 95% confidence interval. Categorical variables were compared using McNemar test while categorical variables were compared with continuous variables using an independent unpaired *t*-test. Statistical significant was taken at p-value <0.05. Kappa values were interpreted as follows; from 0.01–0.20 slight agreement; from 0.21– 0.40 fair agreement; 0.41–0.60 moderate agreement; 0.61–0.80 substantial agreement and 0.81–0.99 perfect agreement (41).

4.9 Ethical considerations

The study was approved by Research and Ethics Review Committee of the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences; Addis Ababa University. The purpose of the study was clearly explained for each study participant. Written consent was obtained from the study participants prior to enrolment. All results were kept confidential; the participants were not identified by their name; instead appropriate coding system was used. The results were provided to the study participants and those in need of medical attention were communicated to respective physicians and laboratory personnel.

4.10 Dissemination of result

The finding of this study was disseminated to the department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences; Addis Ababa University and Nigist Eleni Mohammed Memorial Hospital. Whenever necessary the research will be presented to other concerned bodies like professional associations and it will be submitted to peer reviewed journals for publication.

4.11 Definition of Terms

MDR-TB - TB that does not respond to at least isoniazid and rifampicin, the two most powerful anti-TB drugs.

Sensitivity-The percentage (number fraction multiplied by 100) of subjects with the target condition (as determined by the diagnostic accuracy criteria) whose values are positive.

Specificity-the percentage (number fraction multiplied by 100) of subjects without the target condition (as determined by the diagnostic accuracy criteria) whose test values are negative

Predictive value of a positive result/positive predictive value (PPV)- the percentage (number fraction multiplied by 100) of subjects with a positive test result who have the target condition (as determined by the diagnostic accuracy criteria)

- For drug resistance (the proportion of cases diagnosed as rifampicin/isoniazid-resistant that are truly resistant).

Predictive value of a negative result/negative predictive value (NPV)- the percentage (number fraction multiplied by 100) of subjects with a negative test result who don’t have the target condition (as determined by the diagnostic accuracy criteria)

- For drug resistance (the proportion of cases diagnosed as rifampicin/isoniazid-susceptible that are truly susceptible).

5. Results

5.1 Socio demographic and diagnostic characteristics

A total of 122 subjects were recruited, but 10 were excluded from the study. Of these samples 3 were excluded due to insufficient sample volumes while 7 (5.7%) were excluded due to contaminated culture result. A total of 112 study participants were included in the analysis. Among these 57 (50.9%) were females and 55 (49.1%) were males. The study participant's age ranged from 18 to 75 years old with the average age ± SD (38±13.9) (Table 5.1).

Table 5.1: Sociodemographic characteristics of study participants at Nigist Eleni Mohammed Memorial Hospital from April to September 2017 (n=112)

Variables		**Frequency (%)**
Sex		
	F	57 (50.9 %)
	M	55 (49.1%)
Age		
	18-27	37 (33.0)
	28-37	24 (21.4)
	38-47	28 (25.0)
	48-57	12 (10.7)
	≥58	11 (9.8)

When specimens were classified by smear status 77 (68.7%) were AFB negative while 35 (31.2%) were positive. Out of smear negative specimens 64 (83.1%) were culture negative and 13 (16.9%) were culture positive. One (2.8%) of smear positive specimen was found to be culture negative, while the rest 34 (97.1%) were positive. On MGIT the median time to detection (TTD) of *M .tuberculosis* was 12.3 days (±SD 3.9 days), 10 (±SD 3.1 days) for smear positive and 15.7 (±SD 3 days) for smear-negative cases. Out of 44 MTB positive subjects 19(43.2%) had previous TB treatment history (Table 5.2).

Table 5.2: Diagnostic characteristics of study participants at Nigist Eleni Mohammed Memorial Hospital from April to September 2017 (n=112)

Diagnostic characteristics	Frequency (%)
AFB results	
Neg	77 (68.7)
Scanty	1 (0.89)
1+	6 (5.3)
2+	5 (4.5)
3+	23 (20.5)
Treatment history	
Previously diagnosed with TB	19 (16.9)
New cases	28(25.0)
Bacteriological classification	
SNCN	64 (57.1)
SPCN	1 (0.89)
SPCP	34 (30.3)
SNCP	13 (11.6)

SNCN-Smear negative, culture negative, SPCP-Smear positive, culture positive
SNCP-Smear negative, culture positive, SPCN-Smear positive, culture negative

5.2 Performance of Abbott RealTime MTB assay for detection of MTB

A total of 92 samples were tested by Abbott MTB assay, of this 1 (0.92%) invalid result was excluded from analysis. Out of 91 valid results 18(19.8%) were smear and culture positive (SPCP), 8 (8.8%) were smear negative and culture positive (SNCP), 64(70.3%) were smear and culture negative (SNCN) while 1(1.1%) was smear positive and culture negative (SPCN).

The Abbott RealTime MTB assay was positive in 31(34.1%) and negative in 60 (65.9%) specimens. This assay identified MTB correctly in 24(92.3%) out of 26 bacteriological confirmed TB cases. Overall using MGIT liquid culture media as a reference the sensitivity of this assay were 94.4% (95% CI 70.6-99.7%), 87.5% (95% CI, 46.7-99.3%) and 92.3% (95% CI 73.4-98.6%) from smear positive, smear negative and all samples respectively.

MTB was not detected in 61 out of 65 (93.8%) MTB negative specimens, while 4(6.2%) positives were reported from culture negative cases with an average CN of 34.78. The specificity, PPV and NPV were 93.8% (95% CI 93.8 84.2-98.0%), 85.7% (95% CI 66.4-95.3%) and 96.8% (95% CI 88.0-99.4%) respectively**.**

Out of 91 specimens processed with Xpert MTB/RIF assay 23 (25.3%) were positive and 68 (74.7%) were negative for MTB. The diagnostic sensitivities of this test were 94.4% (95% CI 70.6-99.7%), 50 % (95% CI 17.4-82.5%) and 80.8% (95% CI 60.0-92.6%) from smear positive, smear negative and all samples respectively. The specificity, PPV and NPV were 96.9% (95% CI 88.3-99.5%), 91.3% (95% CI 70.5-98.5%) and 92.6 (95% CI 82.9-97.1%) respectively.

The McNemar's test results showed that the two assays had no statistically significant difference in diagnostic performance from smear negative and positive specimens (p=0.125,1.00) as well as from new and previously treated cases ($p>0.05$). Almost perfect agreement (k=0.81) was observed between the Abbott RealTime MTB and Xpert/ RIF assay for MTB detection.

Table 5.3: Performance of Abbott RealTime MTB and Xpert MTB/RIF assays for MTB detection using MGIT liquid culture as a reference

Assay	Smear Result	% Performance (95% CI)			
		Sensitivity	**Specificity**	**PPV**	**NPV**
Abbott	Positive	94.4(70.6-99.7)	N/A	94.4(70.6-99.7)	N/A
	Negative	87.5(46.7-99.3)	95.3(86.0-98.8)	70 (35.3-91.9)	98.4(90.2-99.9)
	All	92.3 (73.4-98.6)	93.8 (84.2-98.0)	85.7 (66.4-95.3)	96.8 (88.0-99.4)
Xpert	Positive	94.4(70.6-99.7)	N/A	100(77.1-100)	N/A
	Negative	50(17.4-82.5)	96.9(88.2-99.4)	66.7(24.1-94.0)	93.9(84.4-98.0)
	All	80.8 (60.0-92.6)	96.9 (88.3-99.5)	91.3 (70.5-98.5)	92.6 (82.9-97.1)

CI-Confidence Interval, PPV-Positive Predictive Value, NPV-Negative Predictive Value N/A-Not applicable

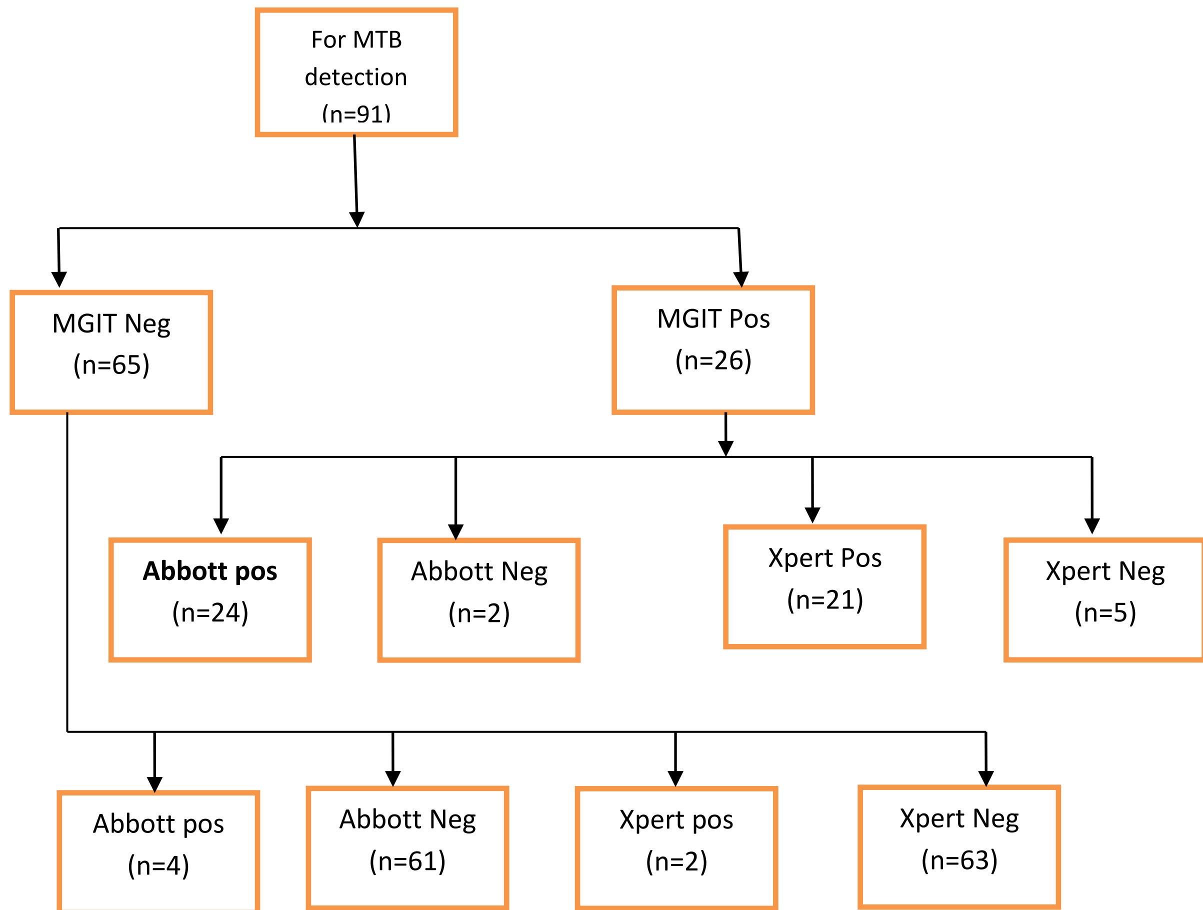

Fig 5.1 Summary of results

5.3 Performance of Abbott MTB RIF/INH assay

A total of 44 MTB positive sediments prepared from sputum samples were processed by Abbott RIF/INH assay for the qualitative detection of RIF and INH resistance MTB. Of this 13 (29.5%) results were reported as "Below LOD" due to the target probe signals could not be detected from one or more resistance detection genes. Only 31 valid results were compared with indirect DST, this comprises 5 MDR, 6 INH mono-resistance, 1 RIF mono-resistance and 19 pan-susceptible TB specimens.

Using Abbott RT MTB RIF/INH assay mutation(s) was detected in the *kat*G and/or *inhA* upper promoter regions in 9 (81.8%) out of 11 INH resistance samples. Two (18.2%) INH resistance isolate was reported as mutation(s) not detected in the *kat*G and *inhA* upper promoter regions, which indicate INH susceptibility. In all of 20 INH susceptible samples no mutation were detected in INH resistance determining regions. Of 6 RIF resistance samples mutation(s) was detected in the *rpo*B RRDR of all (100%) (in all cases probe 4 were missed) and no mutation was detected in all (25) RIF susceptible samples. The sensitivity, specificity, PPV and NPV for the detection of INH TB resistance was 81.8%, 100%, 91% and 100% respectively with (k=0.85). The sensitivity, specificity, PPV and NPV for the detection of RR-TB were 100% with (k=1).

Xpert MTB/RIF assay detect mutations in all RIF resistance TB (RR-TB) and no mutation was detected in all RIF susceptible MTB isolates. The kappa coefficient between the Abbott RealTime MTB RIF/INH Resistance assay and Xpert MTB/RIF assay for the detection of RR-TB was 1.0 (Table 5.4, Table 5.5).

Table 5.4: Comparison of Abbott RT MTB INH/RIF and Xpert MTB/RIF assays for the detection of MDR-TB using phenotypic DST as a reference (n=31)

Phenotypic DST	**Abbott RealTime MTB RIF/INH Resistance assay**		**Xpert MTB/RIF assay**	
	Susceptible	**Resistance**	**Susceptible**	**Resistance**
Isoniazid				
Susceptible (n=20)	20 (100%)	0 (0%)	N/A	N/A
Resistance (n=11)	2 (18.2%)	9 (81.8%)	N/A	N/A
Rifampicin				
Susceptible (n=25)	25 (100%)	0 (0%)	25 (100%)	0 (0%)
Resistance (n=6)	0 (0%)	6(100%)	0 (0%)	6 (100%)

N/A –Not applicable DST- Drug susceptibility testing

Table 5.5: Comparison between Abbott RIF/INH resistance detection with phenotypic DST (n=44)

AFB smear Result	No of Specimens (n=44)	Abbott RIF/INH resistance detection			MGIT 960	
		rpoB	***KatG***	***inhA***	**RIF**	**INH**
Pos [n=31]	18	*rpo*B wt[a]	*KatG* wt[b]	*inhA* wt[c]	S	S
	1	*rpo*B wt	*Kat*G wt	*inhA* wt	S	R
	2	*rpo*B-[d]	*kat*G wt	*inhA* wt	S	S
	5	*rpo*B wt	*kat*G 315T1[e]	*inhA* wt	S	R
	3	*rpo*B Pb4-[f]	*kat*G 315T1	*inhA* wt	R	R
	1	*rpo*B Pb4-	*kat*G wt	*inhA* wt	R	S
	1	*rpo*B Pb4-	*kat*G-[g]	*inhA* wt	R	R
Neg [n=13]	1	*rpo*B wt	*Kat*G wt	*inhA* wt	S	S
	3	*rpo*B-[h]	*Kat*G wt	*inhA* wt	S	S
	1	*rpo*B wt	*Kat*G wt	*inhA* wt	S	R
	1	*rpo*B Pb4-	*Kat*G315T1	*inhA* wt	R	R
	7	*rpo*B-	*kat*G-	*inhA*-[i]	S	S

a. Mutation(s) not detected in the *rpo*B rifampicin resistance determining region (RRDR),
b. Mutation(s) not detected in the *kat*G
c. Mutation(s) not detected in the *inhA* upper promoter regions
d. Target probe signals could not be detected from *rpo*B (Below LOD)
e. *kat*G 315T1 mutations detected in the *kat*G (high level resistance)
f. Mutation(s) detected in the *rpo*B rifampicin resistance determining region(probe 4 missing)
g. Target probe signals could not be detected from *Kat* G region (Below LOD)
h. Target probe signals could not be detected from *rpo*B region (Below LOD)
i. Target probe signals could not be detected from *inhA* upper promoter region (Below LOD)

6. Discussion

So far 16 Abbott m2000™ systems are available in Ethiopia which are well established for quantitative detection of HIV, Hepatitis B and Hepatitis C viruses, and qualitative detection of HPV and *C.trachomatis* and *N.gonorrhoeae.* Now this platform is available for the qualitative detection of MTB and resistance detection of INH & RIF resistance MTB from pulmonary samples. Since this platform is already in use with many trained personnel and quality procedures are already in place using the system for TB diagnosis may support the TB control strategy.

In this study the diagnostic sensitivity of the Abbott RealTime MTB assay for the detection of MTB in smear positive samples was high (94.4%). Similar studies reported high sensitivities in USA by Tang *et al*. (23), and in China by Chen *et al*. (26). The sensitivity of the Abbott RealTime MTB assay for the detection of MTB was comparatively high (87.5%) among SNCP specimens. Higher sensitivity results were reported in China (100%) (25, 26). The reason for higher sensitivity results in this studies could be caused because discrepancies were resolved using clinical symptoms, medical history & 16S–23S ITS sequencing. But moderately lower result was reported in Germany 76.2% (22) which might be caused by longer storage of specimen at -30°c before analysis. MTB DNA could be reduced by longer storage of specimens accompanied by lower bacillary nature of specimen.

Although limited samples were included, higher sensitivity was reported with the Abbott RealTime MTB than the Xpert MTB/RIF assay in SNCP cases (87 vs 50%). In general in this study the Abbott RealTime MTB assay detects 37.8% (3/8) more SNCP cases than Xpert MTB/RIF assay. Similarly Scott L *et al*. reported 48% (17/35) more SNCP cases in high TB and HIV co infected patients. (28). But a study conducted by Wang SF *et al*. in China reported similar sensitivities (100%) between the two assays for the detection of SNCP TB (25) which might be caused by solid culture media was used as the reference method which is less sensitive.

Higher sensitivity of the Abbott RealTime MTB assay when compared to Xpert MTB/RIF assay from SNCP cases in our study might be due to the Abbott RealTime MTB assay uses two target genes for the detection of MTB, multi copy insertion sequence 6110 (IS6110) and the single copy PAB gene. Studies have shown diagnostic performance of PCR for TB can be improved with the use of more than one target gene (43-45) this is because there are strains of MTB lacking IS6110 (21,46,47). The other possible cause could be the difference in samples. Concentrated sputum samples after TB culture were used for Abbott MTB RIF/INH assay while raw sputum specimens were processed for the Xpert MTB/RIF assay.

The Specificity of the Abbott RealTime MTB assay in this study was 93.8% (95% CI 85.9-98.3), relatively similar to other studies which shows specificity between 95.1 -100% (22, 23, 25). Lower specificity (93.8%) could be caused by the detection of latent TB with low bacterial load, which is culture negative specimen or false negative culture results due to the effect of decontamination procedures, rather than false positive results that decrease the specificity. A third explanation may be these specimens were collected from patients with retreatment cases. In those cases specimens may contain non-viable bacteria which are detected by molecular tests targeting DNA but not grow on culture (23). Almost perfect agreement was found between the Abbott RealTime MTB and Xpert/ RIF assays for MTB detection (k= 0.81) similar to a study conducted in China (k=0.89) (25).

With Abbott RealTime MTB testing one (1.1%) specimen result gave *m2000* error code, while high (29.5%) invalid results were reported using Abbott MTB RIF/INH assay for resistance detection as a result of the target probe signals could not be detected from INH and RIF resistance determining genes in contrast to the Xpert MTB/RIF, 1(2.2%) indeterminate RIF resistance result. This is relatively similar to a study in South Africa (33%) by Scott L *et al*. (28) and in China (30.9%) by Tam KK-G *et al.* (27) specimens that could not generate result as DNA concentration was below the LOD.

In our study 76.9% (10/13) of indeterminate results were from SNCP samples ($p<0.05$), suggesting the "Below LOD" report may be due to paucibacillary nature of samples. This suggestion is supported with time to detection (TTD) on MGIT 960 liquid media by the finding of significantly higher mean TTD for MTB in MGIT 960 with samples classified as below the LOD (14.62±3.4) compared to samples with valid result in Abbott RealTime MTB RIF/INH assay (11.15±3.6) ($p=0.007$).

One of the limitations of this assay was higher invalid results reported for the detection of genetic mutations associated with drug resistance. The manufacturer claim of LOD is lower (17 CFU/ml) for Abbott RealTime MTB assay than for Abbott RealTime MTB RIF/INH Resistance assay (60 CFU/ml). Lower LOD for Abbott RealTime MTB is required since Abbott RealTi*m*e MTB targets *IS6110*, which is present in multiple copies per genome of MTB as compared to resistance determining targets (*rpoB*, *katG*, *inhA*) of Abbott RealTi*m*e RIF/INH Resistance that are single copy per genome (10).

In this study 9 out of 11 phenotypically INH resistance specimens carried mutation at *Kat*G 315T codon while, in two INH phenotypically resistance specimens, mutation(s) was not detected with the Abbott RealTime MTB RIF/INH assay resulting with 81.8% sensitivity. This proportion is relatively similar to the study conducted by Ruiz P *et al*. (78.8 %) and Tam KK *et al*., who reported 84% sensitivities (24, 27). Discordant INH resistance pattern between DST and this assay could be as a result of INH resistance is linked with multiple genes and appears more complex (48). Mutations in the *katG* and the *inhA* genes are associated with only approximately about 80% of INH-resistant MTB isolates (49). Other genes such as *ahpC, kasA*, *oxyR*-*ahpC* and *furA*-*katG* which are not detected with this test are also related with INH resistance (48).

For RR-TB the Abbott RealTime assay showed no discrepant result as compared to the indirect DST. This is similar to a study conducted in South Africa (28). Lower sensitivity was reported for the detection of rifampicin resistance TB by a study by Tam KK-G *et al*. in China (27). Lower sensitivity in this study might be caused by larger sample size with the greater variety of RIF associated *rpo*B mutation patterns were included. Similarly no discordant result

was found for the Xpert MTB/RIF assay for RR-TB detection comparable to a study by WHO (4) and FIND (19).

The Abbott RealTime MTB RIF/INH Resistance assay had statistically equivalent sensitivity and specificity compared to Xpert MTB/RIF for detection of RIF resistance with (k=1). Similar study by Kostera J *etal*., (10) reported statistically equivalent diagnostic performance between the two assays.

Detecting both RIF and INH resistance at the same time, the Abbott RealTime MTB RIF/INH assay has an advantage over Xpert MTB/RIF assay to detect INH mono-resistance MTB. In this study three (6.8%) INH mono-resistance were-detected which were disregarded by Xpert MTB/RIF assay. Even though the treatment outcome for these patients could not be anticipated; INH mono-resistance TB have been associated with treatment failure (50,51), so early identification of this cases may help to reduce poor treatment outcome.

The inactivation procedure should be performed under a biosafety cabinet. After the proper inactivation procedure samples can be safely handled outside a biosafety cabinet for sample preparation and amplification by Abbott RT system. The biosafety of the IR reagent was evaluated in one study which showed effective inactivation of *M. tuberculosis* in clinical specimens when treated with IR at a sample to-IR ratio of 1:3 for 60 min (9).

7. Strength and limitation of the study

Strength

- To the best of our knowledge, this is the first attempt to evaluate the performance of Abbott Realtime PCR for the detection of MTB and resistance markers in our country.
- MGIT liquid media is used as a reference for MTB detection and phenotypic DST which is recommended by WHO.

Limitation

- The sample numbers used in this study were small which result in wider confidence intervals.
- Clinical backgrounds of the study participants such as CXR and sign and symptoms were not included in this study which may be important to resolve discrepant cases.

8. Conclusion

The Abbott RealTime MTB assay has high sensitivity and specificity in detecting MTB from smear positive and negative respiratory specimens. In addition the Abbott RealTime MTB RIF/INH assay provides reliable drug resistance profile directly from sputum specimen. Interestingly this test has similar diagnostic performance to Xpert RIF/INH assay with the advantages of high-throughput and simultaneously diagnosis of INH and RIF resistance MTB. This study suggests that the Abbott assay may be a promising method for the diagnosis of TB in high TB burden countries such as Ethiopia.

9. Recommendations

- The Abbott RealTime assay needs to be considered in Ethiopia for the detection of MTB and resistance profile for RIF and INH.
- A national study with larger sample size that includes patient's clinical data as a reference standard should be designed and implemented by FMOH.
- Further study on the diagnostic performance of Abbott MTB RIF/INH assay should be done using MTB positive samples with a variety of mutation pattern.

10. References

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11. Annexs

Annex I. Participant information sheet and consent form

I. Participant information sheet (English Version)

Principal Investigator: Bezalem Tesfaye. Addis Ababa University School of Allied Health Sciences Department of Medical Laboratory Sciences

Title: Performance Evaluation of the Abbott RealTime MTB and RIF/INH Resistance assays for the Detection of *Mycobacterium tuberculosis* and Resistance markers in respiratory specimens at Nigist Eleni Mohammed Memorial Hospital, Hosanna, South Ethiopia.

Purpose: To evaluate the diagnostic performance of the Abbott RealTime MTB and the Abbott MTB RIF/INH Assays for the detection of MTB and its resistance pattern.

Procedures to be carried on: you are invited to participate in the study after giving your consent by giving sputum samples.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

Benefits of the study: There will be no financial or other direct benefit to you. But the result of the study will play a role in the TB control program. There will be no compensation for using your sputum.

Confidentiality of your information: The results of the lab findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

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Participant Consent form (English version)

I undersigned to confirm that, as I give consent to participate after a clear understanding of the objectives and conditions of the study and give permission for my sputum sample to be used in the current research project.

Name of Participant: ___________________
Signature of Participant: _______________
Date: ______________________

Name of data collector____________________________
Signature of data collector: _________________________
Date: ______________________

I. Participant information sheet (Amharic version)

ጥናቱን የምታጠናው ፡ ቤዛዓለም ተስፋዬ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጥናቱ አላማ ፡ አበት ሪል ታይም ኤም ቲቢ እና ኤም ቲቢ ሪፋምፒሲን አይሶናይዝድ የተባለው መሳሪያ በአክታ ውስጥ ቲቢን እና የተላመደ ቲቢን የመመርመር ብቃቱን ማወቅ

በጥናቱ ወቅት ከእርስዎ የሚጠበቀው ፡ በጥናቱ ለመሳተፍ ፈቃደኛ መሆንና አክታ ናሙና መስጠት ነው።

የጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም ፡ በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገር ግን ከጥናቱ የሚገኘው ውጤት ለቲቢ ቁጥጥር ስራ ይጠቅማል::

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት ፡ በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ሊይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም። በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም።

የመረጃ ሚስጥራዊ አጠባበቅ ፡ የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙለ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል።

ቤዛዓለም ተስፋዬ

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ስልክ--0920738007

የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

አዲስ አበባ ዩኒቨርሲቲ

ስልክ +251 0112 75 51 70

II. Participant Consent form (Amharic version)

የስምምነት ውል

እኔ ከዚህ በታች የፈረምኩት የጥናቱ ተሳታፊ የጥናቱን አላማ በሚገባ በመገንዘብ የአክታ ናሙና

በመስጠት በጥናቱ ላይ

ለመሳተፍ ተስማምቼያለሁ፡ ፡

የተሳታፊው ስም፡______________________________

ፊ ር ማ: ___________________________________

ቀን ______________________________________

መረጃውን የሰበሰበው ግለሰብ ስም: ___________________

ፊ ር ማ: _____________________________________

ቀን: ____

Annex II. Standard Operative Procedures

2.1 Specimen digestion, decontamination and inoculation

Principle

Specimens for tubercle bacilli isolation do usually contain associated flora which has to be eliminated before inoculation of the specimen onto culture media. The mucolytic agent NALC-(NaOH) is used for specimen digestion and decontamination. Specimen is incubated for 15 minutes at 20-25°C then PBS is added to prevent the killing effect of NAOH. A relative centrifugal force of 3,000g for 15 minute is adequate to sediment mycobacteria. This sediment is used to inoculate to MGIT (Mycobacteria Growth Indicator Tube) consists of liquid broth medium that is known to yield better recovery and faster growth of mycobacteria.

Procedure

1. If specimen is not collected in a 50 ml centrifuge tube, transfer it to a 50 ml centrifuge tube with a screw cap.
2. Carefully uncap Falcon tube and add an equal volume of NALC-NaOH reagent.
3. Using a vortex, mix tube contents until specimen is liquefied (≈30 seconds per tube). The tube should be inverted to ensure NALC-NaOH reagent contacts all inner surfaces of tube and cap.
4. Dilute the digested-decontaminated specimen to the to the 50 ml mark with Phosphate Buffer. Re-tighten tube caps and mix tube contents by swirling or inversion.
5. Centrifuged at 3000g for15 minutes at 4°C using aerosol-free sealed centrifuge cups.
6. Allow tubes to sit for 5 minutes to settle aerosols formed during centrifugation.
7. Carefully decant the supernatant from each falcon tube into a suitable splash-proof container containing mycobactericidal disinfectant and ensuring the sediment is not lost during the decanting process.
8. Using a transfer pipette spread one drop of concentrated sediment onto a labeled slide.
9. Resuspend remainder of spun sediment in 2ml of fresh PBS with pipette.

Specimen inoculation on MGIT 960

Principle

The MGIT contains 7.0 ml of modified Middlebrook 7H9 broth base. An enrichment, MGIT OADC (Oleic acid, Albumin, Dextrose and Catalase) or MGIT 960 Growth Supplement, is added to make the medium complete. Addition of the MGIT PANTA is necessary to suppress contamination. In addition to Middlebrook 7H9 liquid media, the MGIT tube contains an oxygen-quenched fluorochrome, tris 4, 7-diphenyl-1, 10-phenonthroline ruthenium chloride pentahydrate, embedded in silicone at the bottom of the tube. During bacterial growth within the tube, the free oxygen is utilized and is replaced with carbon dioxide. With depletion of free oxygen, the fluorochrome is no longer inhibited, resulting in fluorescence within the MGIT tube when visualized under UV light. MGIT tubes may be incubated at 37ºC and read manually under a UV light or entered into a MGIT 960 instrument where they are incubated and monitored for increasing fluorescence every 60 minutes.

Procedure

1. Reconstitute MGIT PANTA with 15.0 ml MGIT growth supplement
2. Mix until completely dissolved. Add 0.8 ml of this enrichment to each MGIT tube
3. Label MGIT tubes with specimen number
4. Inoculate 0.5 ml of the resuspended specimen to the labeled MGIT tube
5. Transfer remaining sediment to labeled Nunc cryo tube for freezer storage.
6. Remove processing waste materials for autoclaving before discard. Swab BSC with 70% ethanol
7. Load inoculated MGIT tube to MGIT960 automated culture system.

2.2 MTB identification

Principle

MGIT positive tubes should be confirmed for the presence of MTB since growth of any bacteria utilizing oxygen is detected by MGIT 960 system as positive. If a MGIT tube broth is heavily turbid, contamination is presumptive even if the AFB smear is positive.

The sterility of MGIT positive cultures is checked by inoculating on blood agar plates. Any growth within 48 hours is considered as contaminated tube. Ziehl Neelsen smears is used to determine the presence of acid fast bacilli and serpentine cords that indicate the presence of mycobacteria species.

2.2.1 AFB smears from a positive MGIT tube

1. Add a drop albumin serum to properly labeled slide
2. Mix the broth by vortexing and then by using a sterile pipette, remove and aliquot.
3. Place 1-2 drops on the slide and spread over a small area (approx. 1½ x 1 cm).
4. Heat-fix the smear by passing it over a flame a few times or by using an electric warmer at 65℃ -70℃ for 2 hours to overnight
5. Stain the smear with Ziehl-Neelsen,
6. Examine using 100X microscope for the presence of serpentine cords, loose cords, or individual scattered acid fast bacilli**.** MTB appears as serpentine cords

2.2.2 MPT64 Antigen Detection

Principle

The SD Bioline TB Ag MPT64 Rapid test is an immunochromatographic method which can detect MPT64 antigens produced by *M. tuberculosis* complex from AFB positive liquid or solid culture media. MPT64 is a specific antigen that differentiates the M. tuberculosis complex from the mycobacteria other than tuberculosis (MOTT) species. The pink band in the 'C' region confirmed the test validity. An additional pink band in the 'T' region is interpreted as positive for the MPT64 Ag. Only the pink band in the 'C' region and no band in the 'T' region indicate negative for the MPT 64 antigen. No band in 'C' region was interpreted as an invalid test.

Procedure

1. Vortex the positive MGIT tube well
2. Label the cassette with the specimen ID number.
3. Add Liquid culture (0.1 ml) onto the sample well.
4. Incubate the test cassettes strips for 15-30 minutes at room temperature and read result

2.3 First line drug (SIRE) Susceptibility testing for MTB

Materials and reagents

- BACTEC MGIT 960 SIRE Kit
- MGIT tube
- Normal saline

Principle

The BACTEC MGIT 960 system is a rapid qualitative method for measuring the susceptibility of M. *tuberculosis* to critical concentrations of the drugs (SIRE). Susceptibility testing is a 4-13 day test. The test is based on growth of the M. *tuberculosis* strain in drug containing tube compared with to the drug free tube (Growth control). The instrument continuously monitors the tubes for increased florescence. Analysis of florescence in drug containing tube compare to the florescence of the growth control tube is used by instrument to determine the susceptibility result. Results are interpreted automatically and reported as susceptible and resistant. An isolate is resistance if 1% or more of the population grows in the presence of the critical drug concentration.

Specimen Type

Pure (sub cultured) - 1-5 days old colony

Procedure

1. Accurately transfer 0.8 SIRE Supplement to each tube.
2. Accurately transfer 100µl of reconstituted drug solution to the corresponding tube.
3. Aseptically pipet 0.1 mL of the organism suspension into 10 mL of sterile saline to prepare the 1:100 Growth Control suspension. Mix the Growth Control suspension thoroughly.
4. Inoculate 0.5 mL of the 1:100 Growth Control suspension into the **MGIT** tube labeled “GC.”
5. Aseptically pipet 0.5 mL of the organism suspension (undiluted for 1-2 day colony or 1:5 diluted for 3-5 days colony) into each of the drug tubes

2.4 Xpert MTB/RIF assay

Principle

The Xpert MTB/RIF Assay is an automated *in vitro* diagnostic test using nested real-time PCR for the qualitative detection of MTB-complex and RIF resistance. The primers in this test amplify a portion of the *rpoB* gene containing the 81 base pair core region. The probes are designed to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance. This assay can be performed on Cepheid GeneXpert® Instrument Systems.

Procedure

1. Label each Xpert MTB/RIF Assay cartridge with the sample ID.
2. Transfer at least 0.5 mL of the total resuspended sediment to a conical, screw-capped tube for the Xpert MTB/RIF Assay using a transfer pipette. Alternatively, the entire sediment can be processed in the original tube.
3. Using a transfer pipette, transfer 1.5 mL of Sample Reagent to 0.5 mL of resuspended sediment. For larger volumes of sediment, add Sample Reagent equal to three times or two times (for raw sputum sample) the volume of the resuspended sediment.
4. Recap the tube and shake vigorously 10 to 20 times or vortex for at least 10 seconds.
5. Incubate sample for a total of 15 minutes at 20-30°C.
6. Using the provided transfer pipette, aspirate the liquefied sample close to the line on the pipette.
7. Dispensing the sample slowly to minimize the risk of aerosol formation, transfer the Sample Reagent-treated sample into the sample chamber of the Xpert MTB/RIF cartridge.
8. Close the cartridge lid firmly. Remaining Sample Reagent-treated sample can be kept for up to four hours at 2–8 ºC in case retesting is required.
9. Start the test according to the GeneXpert manual

2.5 Abbott RealTime MTB assay

Intended use

The Abbott RealTi*m*e MTB assay is an in vitro polymerase chain reaction (PCR) for the qualitative detection of MTB complex DNA in smear positive or smear negative specimens of sputum or bronchial alveolar lavage and N-acetyl-L-cysteine (NALC)-prepared sediments prepared from sputum and bronchial alveolar lavage collected from individuals presumptive of having tuberculosis (TB).

Principle

Sample Preparation

The pu*rpo*se of sample preparation is to prepare target DNA for PCR amplification. Preparation of target DNA is performed using a magnetic microparticle-based technology (Abbott *m*Sample Preparation System*DNA*). This can be performed using an Abbott *m*2000*sp* for automated sample preparation or using a manual sample preparation protocol. An internal control (IC), positive control, and negative control are processed from the start of sample preparation to demonstrate that the process has proceeded correctly.

Amplification

Purified sample DNA and master mix are added to a 96-well PCR plate using an Abbott *m*2000*sp* instrument or manually. After addition, each plate is sealed and transferred to an Abbott *m*2000*rt* where PCR amplification is performed using DNA polymerase. The targets of PCR are IC and 2 different MTB targets, the insertion sequence 6110 (IS6110) and protein antigen b (PAB).

Detection

The presence of MTB amplification products is detected during the annealing/extension step by measuring the real-time fluorescence signal of the MTB IS6110 probe and PAB probe. The presence of IC amplification products is detected by measuring the real-time fluorescence signal of the IC probe.

The MTB and IC probes are single-stranded DNA oligonucleotides consisting of the target-specific binding sequence, a fluorescent moiety covalently linked to the 5′ end of the probe, and a quenching moiety covalently linked to the 3′ end of the probe. In the absence of the MTB or IC target sequences, probe fluorescence is quenched. In the presence of MTB or IC target sequences, the MTB or IC probes specifically bind to their complementary sequences in the targets during the annealing/extension step, allowing fluorescent emission and detection. The MTB probes are labeled with different fluorescent dyes (FAM™ for IS6110 and PAB; QuasarR for IC), thus allowing the amplification products of MTB and IC to be simultaneously detected in the same reaction.

Specimen types

- Smear positive or smear negative specimens of sputum (induced or expectorated),
- Smear positive of smear negative specimens of bronchoalveolar lavage (BAL) samples
- N-Acetyl-LCysteine (NALC)-treated sediments of sputum
- N-Acetyl-LCysteine (NALC)-treated sediments of BAL

Materials required

- Abbott 96-Deep-Well Plate
- Abbott Optical Adhesive Cover
- Abbott Adhesive Cover Applicator
- 200 µL and 1000 µL Disposable Tips for Abbott *m*2000*sp*
- 95 to 100% Ethanol
- pipettes capable of delivering 20 µL to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- 500 mL Polypropylene or glass container
- 10 M NaOH
- Isopropanol
- Purified water for IR buffer
- Tween-20
- Molecular Biology Grade Water (DNase/RNAse Free)*
- 1.7 mL molecular biology grade microcentrifuge tubes*

Procedure

Preparation of IR:

Material Volume Required for 500 mL

10M NaOH	20 mL
Purified water	179.1 mL
Isopropanol	300 mL
Tween-20	0.9 mL

1. Add 179.1 mL of water to an empty polypropylene or glass container
2. Add 0.9 mL of Tween-20 to the container.
3. Add 20 mL of 10M NaOH to the container.
4. Add 300 mL of isopropanol to the container.
5. Mix the components by inversion 20 times. Store at ambient temperatures for up to 1month.

Reduction of MTB Infection Risk Procedure:

1. If frozen, thaw specimens at 15 to 30°C.
2. Estimate the volume of specimen to be inactivated.
3. Gently invert IR to mix the components and add IR at a ratio of 1:3 (eg, 1 mL specimen + 3 mL IR) (the allowable specimen volume is 0.3 to 10 mL).
4. Invert the container to ensure contact between the IR and the specimen. Vortex the mixture for 20 to 30 seconds then incubate the mixture at ambient temperature for at least 1 hour and no more than 24 hours. Vortex the mixture one final time for 20 to 30 seconds at 20 to 30 minutes into the incubation period.

Assay procedure

1. Before use, vortex IR-treated samples for 3 to 5 seconds. Using a pipette, transfer the IR-treated samples to the reaction vessels.
2. Thaw assay controls, IC, and amplification reagents at 2 to 8°C or 15 to 30°C.
    - Once thawed, IC can be stored closed at 2 to 8°C for up to 14 days prior to use.
    - Once thawed, controls can be stored at 2 to 8°C for up to 24 hours prior to use.

3. Vortex each control 3 times for 2 to 3 seconds each time before use

4. Gently invert the Abbott *m*Sample Preparation System *DNA* bottles to ensure a homogeneous solution.

5. Vortex the IC vial 3 times for 2 to 3 seconds each time before use.

Add 180 µL of IC to 1 bottle of *m*Lysis *DNA* buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming. Each bottle of *m*Lysis *DNA* buffer supports up to 48 sample preparations.

6. Add 180 µL of IC to a second bottle of *m*Lysis*DNA* buffer for 49 to 96 samples.

7. Add 25 mL of USP grade 190 to 200 proof ethanol (95 to 100%) to the *m*Lysis *DNA* buffer + IC reagent bottle.

- For 49 to 96 samples, add 25 mL of ethanol to a second bottle of *m*Lysis *DNA* buffer + IC. Gently invert to ensure a homogeneous solution.

9. Add 70 mL USP grade 190 to 200 proof ethanol (95 to 100% ethanol) to *m*Wash 2 *DNA* bottle. Do not use ethanol that contains denaturants. Each bottle of *m*Wash 2 *DNA* supports up to 48 reactions. Gently invert to ensure a homogeneous solution.

10. Place the negative and positive control and the patient specimens into the Abbott *m*2000*sp* sample rack.

11. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.

12. Load the carrier racks containing the Abbott *m*Sample Preparation System *DNA* reagents and the Abbott 96-Deep-Well Plate on the Abbott *m*2000*sp* worktable .

13. From the Run Sample Extraction screen, select and initiate the sample extraction.

14. Load the amplification reagent pack and master mix vial (if needed) on the Abbott *m*2000*sp* worktable after sample preparation is completed.

15. Select the appropriate deep-well plate from the Run Master Mix Addition screen that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol.

16. Switch on and initialize the Abbott *m*2000*rt* in the Amplification Area.

17. Place the Abbott 96-Well Optical Reaction Plate into the Abbott Splash-Free Support Base after the Abbott *m*2000*sp* instrument has completed addition of samples and master mix.

18. Seal the Abbott 96-Well Optical Reaction Plate

19. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument.

20. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* and initiate the Abbott RealTi*m*e protocol as described in the Abbott *m*2000*rt* Operations Manual.

21. After the Abbott *m*2000*rt* instrument has completed the amplification and detection protocol, remove the Abbot 96-Well Optical ReactionPlate and dispose

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2.6 The Abbott RealTime MTB RIF/INH Resistance assay

Principle

The Abbott RealTi*m*e MTB RIF/INH Resistance assay is an in vitro polymerase chain reaction (PCR) assay for the qualitative detection of Rifampicin (RIF) and Isoniazid (INH) resistance in MTB positive samples.

A sample inactivated is performed to reduce the infection risk associated with clinical specimens that may contain MTB. Target DNA is prepared for PCR amplification using a magnetic microparticle-based technology using an Abbott *m*2000*sp* for automated or a manual sample preparation protocol then amplification is performed using Abbott *m*2000*rt*. Targets in the *rpo*B, *kat*G genes, the *inhA* upper promoter region, and the IC target are amplified by DNA polymerase in the presence of dNTPs and magnesium during the amplification/detection reaction on the Abbott *m*2000*rt* instrument.

Rifampicin resistant MTB is detected by the use of 8 *rpo*B wild type probes. Isoniazid resistant MTB is detected by the use of wild type and mutant *kat*G and *inhA* probes. The MTB RIF/INH Resistance and IC probes are each labeled with a different fluorescent moiety. This allows for the simultaneous detection of MTB RIF and INH drug resistant amplification products and IC-specific amplified products within the same reaction. In the absence of the MTB or IC target sequences, the probe fluorescence is quenched. In the presence of MTB or IC target sequences, the MTB or IC probes specifically bind to their complementary sequences in the targets during the annealing/extension step, allowing fluorescent emission and detection.

The Abbott RealTi*me* MTB RIF/INH Resistance assay has an optional Reflex feature if used in conjunction with Abbott RealTi*me* MTB. Samples that are dispositioned as "MTB detected" using Abbott RealTi*me* MTB may be tested in a Reflex mode using Abbott RealTi*me* MTB RIF/INH Resistance

Note- Specimen, Specimen storage, Reagent and Materials, Reagent storage, Reduction of MTB Infection Risk Procedure, Sample Preparation, Amplification and detection procedure are similar as Abbott RT MTB assay except the master mix reagent and positive controls.

Procedure is similar to the Abbott RealTime MTB assay except

- 22 samples and 2 controls are analyzed per run
- Use the Abbott RealTi*me* MTB RIF/INH Resistance assay positive control.
- Use the Abbott RealTi*me* MTB RIF/INH Resistance assay master mixes.
- From the Run Sample Extraction screen, select and initiate the sample extraction protocol for Abbott RealTi*me* MTB RIF/INH Resistance assay. Similarly to the Abbott *m*2000*rt* initiate the protocol for Abbott RealTi*me* MTB RIF/INH Resistance assay.

Annex-III: Laboratory Requesting and Reporting Form

1. PATIENT IDENTIFICATION:

- Patient code no ________Age (Yrs.): ________________Sex (M/F): _____________
- Referring Health Facility: __________________ Co-infection: _______________

2. TB DISEASE TYPE & TREATMENT HISTORY:

- Site: Pulmonary____________ Extra pulmonary (specify):_________________
- Registration Group: New___ Relapse____ Treatment after loss to follow-up___
  Treatment after failure of first treatment__ Treatment after failure of retreatment__
- Previous TB drug use: New___ First line___Second line___MDR-TB contact___

3. REQUEST FOR TESTING AT TB LABORATORY:

- Reason: Diagnosis__ If diagnosis, presumptive TB___DR___ Follow up___
  If follow up, at ___ months during treatment, Follow up at ___ months after treatment
- Specimen: Sputum ____Other (Specify): ______
- Date specimen collected: ____/____/____ (Ethiopian Calendar)
- Requested tests: Microscopy__ Xpert MTB/RIF test__ Culture Phenotypic DST __
- Person requesting examination:
  Name:_______________________Date__________________

4. LABORATORY RESULT: Xpert MTB/RIF test result

- Sample Number: ________ Date specimen collected: ____/___/___ (Ethiopian Calendar)
- Date of result: ____/___/__
- Result
  M. tuberculosis: Detected_____ Not detected_____ Invalid / No result / Error (Repeat Test) _____
  Rifampicin resistance: Detected____Not detected____ Indeterminate result___

Examined by (name and signature): __________________

Annex-IV: Laboratory data (Culture)

Lab serial no	Patient ID	Date sample Collected	AFB Result	Culture Result			
				Positive	Negative	Conta	NTM

Drug susceptibility test (DST) results

Lab serial no	Patient ID	Date sample Collected	Date sample Processed	Result	
				RIF	INH

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Bezalem Tesfaye (B.Sc.)

Signature:__________________

Date of submission:__________________

This thesis has been submitted with our approval as advisors.

Advisor: Kassu Desta (MSc, PhD candidate)

Signature: ____________________

Date: ____________________

Place: Addis Ababa, Ethiopia.

Advisor: Kirubel Eshetu (MSc)

Signature: ____________________

Date: ____________________

Place: Addis Ababa, Ethiopia.