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Assessment of Diagnostic Performance of Abbott Real Time PCR for the Detection of Smear Negative Pulmonary Tuberculosis at Ethiopian Public Health Institute, Addis Ababa Ethiopia

By: Million Hailu (BSc, MPH)

Advisors: Adane Bitew (PhD)
Gebreab Teklebirhan (BSc, MSc)
Abebaw Kebede (BSc, MSc)

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Name of investigator	Million Hailu Tesema, BSc, MPH
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Address of investigator	Cell phone: +251 911 93 01 63
	Email: millimht@gmail.com
Name of Advisor(s)	Dr. Adane Bitew (PhD) Gebreab Teklebirhan (BSc,MSc)
Collaborators:	Sample preparation and TB tests: Getu Deriba and Bazezew Yenew Molecular lab tests: Agajie Likie, Kidist Zealiyas and Ajanaw Yizeng Technical Advice/support: Ephram Tesfaye and Yimam Getanah

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Million Hailu, entitled:

Diagnostic Performance of Abbott Real Time for the Detection of Smear Negative Pulmonary Tuberculosis at Ethiopian Public Health Institute, Addis Ababa Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner_____ Signature _____Date _____

Examiner_____ Signature _____Date _____

Advisor: **Dr. Adane Bitew** Signature _____Date _____

Advisor: **Gebreab Teklebirhan** Signature _____Date _____

Chairman of the Department or Graduate Program Coordinator

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LIST OF ABBREVIATIONS

AAU: Addis Ababa University

AFB: Acid Fast Bacilli

AOR: Adjusted Odds Ratio

BAL: Broncho Alveolar Lavage

BCG: Bacille Calmette Guerin

CDC: Center for Disease Control

CI: Confidence Interval

COR: Crude Odds Ratio

CFU: Colony Forming Unit

DNA: Deoxy Ribose Nucleic Acid

DSe: Diagnostic Sensitivity

DSp: Diagnostic Specificity

DST: Drug Susceptibility Test

EPHI: Ethiopian Public Health Institute

HIV: Human Immunodeficiency Virus

IC: Internal Control

INH: Isonicotinic Acid Hydrazide

IS: Insertion Sequence

IQC: Internal Quality Control

IR: Inactivation Reagent

IS: Insertion Sequence

LOD: Lower Detection Limit

LPA: Line Probe Assay

MGIT: Mycobacterium Growth Indicator Test

MTB: Mycobacterium Tuberculosis

NAAT: Nucleic Acid Amplification Tests

NPV: Negative predictive Value

PAB: Protein Antigen B

PCR: Polymerase Chain Reaction

PI: Principal Investigator

PMNC: Polymorpho Nuclear Cells

PPD: Protein Purified Derivate

PPV: Positive Predictive Value

RT: Real Time

RIF: Rifampicin

RNA: Ribonucleic acid

SERO: Scientific Ethical Review Office

SOP: Standard Operating Procedure

SP: Sample Processing

TB: Tuberculosis

TST: Tuberculosis Skin Test

ZN: Ziehl Neelsen

TABLE OF CONTENTS

Contents

ACKNOWLEDGEMENTS i
LIST OF ABBREVIATIONS ii
TABLE OF CONTENTS iv
LIST OF FIGURES vi
LIST OF TABLES vii
ABSTRACT viii
1. INTRODUCTION 1
1.1. Background 1
1.2. Statement of the Problem 3
1.3. Significance of the Study 4
2. LITERATURE REVIEW 5
3. OBJECTIVES 8
3.1. General Objective 8
3.2. Specific Objectives 8
4. MATERIALS AND METHODS 9
5.1. Study Setting 9
5.2. Study Design 9
5.3. Source Population 9
5.4. Study Population 9
5.5. Inclusion and Exclusion Criteria 9
5.6. Study Variables 10
5.7. Sample Size Determination and Sampling Procedure 10
5.8. Data Collection Procedures 11
5.9. Testing Principle and Procedure 11
5.10. Quality Assurance 16
5.11. Data Processing and Analysis 16
5.12. Definition of Terms 16
5.13. Ethical Clearance 17
5.14. Dissemination of the Results 17
6. RESULTS 18
6.1. Socio-demographic Characteristics 18
6.2. Clinical and Related Factors 18
6.3 Diagnostic Performance Characteristics 19
6.4. Verification of IR 21
6.5. Multivariate Analysis of Abbott real time PCR Performance 21
7. DISCUSSION 23

8. STRENGTH AND LIMITATIONS 26
8.1. Strength 26
8.2. Limitation 26
9. CONCLUSION AND RECOMMENDATION 27
9.1. Conclusions 27
9.2. Recommendations 27
10. REFERENCES 29
11. LIST OF ANNEXES 32
11.1. Laboratory Test SOPs 32
11.1. Data Capturing Worksheet 51
10.3. Declaration 52

LIST OF FIGURES

Figure 1: MTB identification at National TB laboratory and Abbott Real Time PCR, May 2018 14

Figure 2: Abbott Real Time PCR MTB Detection and DST, May 2018 14

Figure 3: National TB Laboratory MTB LPA DST interpretation, May 2018 15

LIST OF TABLES

Table 1: Abbott Real Time PCR MTB Specimen Preparation Reagent Proportion, May 2018 12
Table 2: Abbott real time PCR DST code and interpretation, May 2018 15
Table 3: Basic Socio-demographic characteristics of study participants, Addis Ababa, May 2018..... 18
Table 4: Clinical related factors of study participants, Addis Ababa, May 2018 19
Table 5: Performance characteristics of Abbott real time PCR for the diagnosis of smear negative MTB, Addis Ababa, May 2018 20
Table 6: Performance characteristics of Abbott real time PCR for MTB RIF/INH DST, Addis Ababa, May 2018 20
Table 7: Verification of inactivation reagents for Abbott real time PCR MTB detection and DST, May 2018 21
Table 8: Bi-variate and multi-variate logistic regression of Abbott real time PCR with explanatory variables for the diagnosis of smear negative MTB, Addis Ababa, May 2018 22

ABSTRACT

Background: In 2016, an estimated 10.4 million people developed TB and of these more than 1.6 million died from the disease, 374,000 (22%) of whom were HIV-positive. In most of the high burden of tuberculosis but resource poor countries, microscopy method issued in which acid-fast bacilli are detected in smear using a light microscope. However, smear microscopy is not so reliable. As of other most advanced tests, molecular techniques still not implemented in developing countries including Ethiopia. To improve MTB case detection rate, there is a need to introduce and implement rapid batch testing molecular methods in Ethiopia.

Objective: The objective of this study was to assess the performance characteristics of Abbott real time for the diagnosis smear negative MTB

Methods: Both prospective and retrospective cross-sectional survey was carried out to enroll 127 study participants' records along with left over sputum specimens in Addis Ababa, EPHI national TB/HIV laboratories from March to May 2018. Performance characteristics including sensitivity, specificity and predictive values was calculated by using SPSS version 23 with 95% CI. For all statistical significance tests, the cut-off point was 0.05 and $p < 0.05$ considered as statistically significant association with testing performance.

Results: Diagnostic sensitivity of Abbott real time for the diagnosis of smear negative was 82.9% and specificity exhibited 89.1%. Drug susceptibility testing demonstrated diagnostic specificity of 87.5% and 100% for INH and RIF respectively. Abbott real time performance significantly and negatively associated with month of treatment [Adjusted OR (95%CI) = 0.01 (0.00. 0.41)] for 1st to 6th month, 0.005 (0.00, 0.20) for 7th to 12th months and 0.001 (0.00, 0.90) for the clients on treatment for more than a year

Conclusions: The diagnostic sensitivity of the method for identification of MTB and drug resistance-sensitivity and specificity similar with the previous similar studies. The method exhibited lower specificity for the diagnosis smear negative MTB cases. The finding also revealed the specificity of Abbott real time the lowest as of other molecular methods for the diagnosis of smear negative MTB cases who are on MTB treatment. Therefore, we strongly recommend not to use the method for all follow-up MTB cases except zero month and further investigation should be continued to explain exhaustively the effect of explanatory variables on Abbott MTB performance.

Key words: Abbott real time PCR, MTB, Sensitivity, Specificity

1. INTRODUCTION

1.1.Background

Tuberculosis (TB) remains one of the world's deadliest communicable diseases. In 2016, an estimated 10.4 million people developed TB and of these more than 1.6 million died from the disease, 374,000 (22%) of whom were HIV-positive. The 2016 WHO report also shows that Ethiopia is among the thirty TB burden countries (1).

Mycobacterium tuberculosis (MTB) is a large rod shaped and non- motile bacterium. The length of these rods is 2-4 micrometers and width is 0.2-0.5 um. It is obligate aerobic in nature and for this reason, tuberculosis mainly found in lungs lobes where full aeration is provided. It has a slow generation time of around 15-20 hours. It cannot be classified as either gram positive or gram negative. Nevertheless, it contains murein in the cell wall so stains very weakly with gram staining. These are stained by carbol fuchsin stain and are referred to as acid fast bacilli (2) .

Its cell wall contains peptidoglycan and more than 60% of it made up of lipid. The lipid portion of cell wall comprises of three major components, wax-D, mycolic acids and cord factors. Mycolic acid makes up 50% of the dry weight of the mycobacterial cell envelope. It plays a significant role in virulence of mycobacterium. It prevents attack of the mycobacterium by cationic proteins, lysozyme, and oxygen radicals in the phagocytic granule. Cord factor is an inhibitor of Polymorpho Nuclear Cells (PMNC) migration (2).

The diagnosis and treatment monitoring of tuberculosis conducted in a wide range of laboratory facilities worldwide, using a variety of methods. Several of these methods endorsed by the WHO and significantly improved case detection of TB and management of patients. Culture is the most common method of diagnosis. However, it takes long time of around 2-4 weeks. Sputum induction is an approach, which has a high yield in pulmonary tuberculosis. Most of the patients in pleural tuberculosis do not produce spontaneous sputum so this method can be used for effective diagnosis (3). Sputum induction gives best results when used with smear microscopy and can detect the patients who have negative spontaneous smear microscopy (4). In most of the high burden of tuberculosis but resource poor countries, microscopy method used in which

acid- fast bacilli are detected in smear using a light microscope. The smears are stained with Ziehl-Neelsen (ZN) stain but smear microscopy is not so reliable (5).

Nucleic acid amplification tests (NAAT) is exponential amplification of specific sequence of nucleic acid. It is becoming a standard of test that helps to increase the sensitivity of the assay especially when only few organisms or nucleic acids present in the sample within 48 hours (6) . NAAT for the detection of MTB can provide faster results as compared to culture and with higher sensitivity as compared to smear microscopy. CDC recommended that at least one respiratory specimen NAAT for each patient with signs and symptoms of pulmonary TB (7). Gene Xpert MTB is cartridge based automated molecular technique, with limited bio-safety requirement, minimal hands on manipulation and deliver results within four hours. It is a real-time PCR that uses *rpoB* probe (6). The sensitivity of Xpert ranged from 70 to 100% in culture positive and around 60% of smear negative clients (8).

Abbott molecular machine currently installed at 14 regional and high load hospital laboratories in big towns of Ethiopia and providing HIV molecular tests after in country evaluation. Abbott *m*2000^{TM} is an automated molecular batch analyzer used for the diagnosis and monitoring of several infectious diseases including MTB. It is also real- time PCR can release batch of 96 test results within a period of 6-8 hours (9). For the diagnosis of MTB, it uses inactivation reagent, fluorescent labeled primers targeting Protein Antigen B (PAB) (10) and Insertion Sequence 6110 (IS6110). Multi copy target to maximize the sensitivity, minimize false negativity due to target gene mutation or deletion (11).

The company summarized the performance characteristics during development of m2000^{TM} RealTime MTB assay by testing both prospective and stored sputum collected from different populations. Abbott real time versus culture sensitivity was about 81% for smear negative MTB with specificity of 97% (9).

1.2.Statement of the Problem

Early diagnosis and treatment of smear negative MTB cases benefit not only the clinical services in the diagnosis and management an individual patient but also reduce the transmission of infection in the community. As of other most advanced tests, molecular techniques are limited to the developed countries and not still implemented in developing countries including Ethiopia for the diagnosis of smear negative MTB. Molecular techniques are most sensitive and specific methods within short turnaround time for the diagnosis of MTB especially smear negative cases. Gene Xpert has been utilized widely for the diagnosis of MTB but relatively expensive. Since it processes few number of cartridges at a time, it is not suitable for high volume health facilities and also as mentioned above it misses significant proportion of smear negative MTB cases.

CDC endorsed that every country need to create access to at least a single molecular laboratory test for each MTB suspected clients. WHO recommends to design and conduct in country evaluation for new test kits and methods. Despite the method along with the machine was recommended for the service, the evaluation not exhaustively conducted elsewhere and not yet done in Ethiopia for the diagnosis of smear negative MTB.

1.3.Significance of the Study

There is a need to introduce and implement rapid batch testing molecular method in Ethiopia to improve TB case detection rates, diagnose and manage at early stages to significantly mitigate the morbidity and mortality from MTB. Effective, efficient and early diagnosis of disease is required to prevent the spread of disease. Abbott Real time is the advanced method for the diagnosis of MTB as compared to other conventional diagnostic methods. In addition, the results can be released within 2 days especially for high load health facilities.

Evaluating Abbott machine for the diagnosis of smear negative MTB by standardizing and controlling factors that significantly affect the performance and operational characteristics, ensures the method and the machine function properly and/or not affected by shipment and installation processes. It also improves further knowledge about test performance and staff competency.

The evaluation report will reveal to policy makers and stakeholders to decide objectively on the use of this technology for the diagnosis of smear negative MTB. The findings of this study will have public health importance for the identification of effective strategies in early diagnosis, prevention and control of the disease in the country by increasing case detection rates from smear negative MTB suspected cases. The findings of this study will be also ground work for the evaluation of other batch testing molecular techniques for the detection MTB in particular and other infectious diseases in particular.

2. LITERATURE REVIEW

Tuberculosis is the leading cause of death in the world which is caused by the infectious bacteria MTB. It mainly affects lungs but can affect other parts also like spine, kidney, brain, heart. It is ranked along with HIV in leading cause of deaths. Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014: Even today in India, two deaths occur every three minutes from TB (12). An estimated 29% of the world's TB cases occur in the African region, where high HIV prevalence has been known to impel the burden of TB cases. Ethiopia is one of the 22 high disease burden countries and TB remains one of the leading causes of mortality (1, 12).

Diagnostics methods of MTB continue to evolve (13). Older technologies utilized in new ways are also making inroads in diagnostics (14) and in 2016 there were more than 50 diagnostic methods developed for the diagnosis of tuberculosis (15). Acid-fast stains widely used in developing countries but lack sensitivity and a large number of bacilli (10^4-10^6/ml) are required for a positive stain. The sensitivity of conventional light microscopy ranges from 32 to 94% (16) and Immunocompromised individuals often present with lower bacterial loads making detection by smear difficult. The sensitivity of fluorescent microscopy increased to 52 to 97% as compared to light microscope (5) and the results are unaffected by HIV status of client (17).

The Tuberculosis Skin Test (TST) has been in used for the diagnosis of tuberculosis infection. TST is a protein–purified derivate (PPD) method which results from a culture filtrate of tubercle bacilli containing over 200 antigens common both in Bacilli Chalmette-Guerin vaccine (BCG) and in most non tuberculosis bacteria. Therefore, the specificity and accuracy of this test is low. Furthermore, it takes around 48-72 hours to read TST after initial administration. It can produce false results as error can be made in performing and reading of results (18).

Interferon release assay is the alternatives of tuberculin skin tests and more sensitive and less time consuming (19). These are blood tests which measure *ex vivo release* of interferon-γ by T lymphocytes which are stimulated by antigens specific for *MTB (20)*. Fluorescence-activated cell sorting technique is used in suspects with negative AFB sputum smears. In this method BAL cells or sputum cells are immune phenotyped. But the frequency of region of difference

MTB specific T cells is too low in the sputum to be used as stimulants for flow cytometry, cultures and other immune based assays (21).

The newly identified culture approach that could minimize the time for MTB detection on culture and drug susceptibility testing (DST). This is known as MGIT 960 method which decreases the turnaround time of DST to 27 days as compared to solid culture method which takes around 70 days (22). There are also immunoassay methods, which allow the identification of MTB complex from culture by detection of *Mtb*-specific antigens. These tests are relatively rapid requiring only minutes to perform after growth of the organism but have variable performance (23, 24).

Genotype MTB method is designed to detect directly from clinical specimens. This assay is performed in three parts consisting of an RNA isolation and capture step, followed by an isothermal amplification. Evaluation of this assay showed sensitivity and specificity ranging from 80.5-97% to 75-100% respectively when compared to culture (25, 26). In general, the sensitivity of these line assays is lower when tested directly on clinical specimens as compared to their sensitivity when used on culture isolates. However, with a turn-around time of about 2 days compared to at least 6 weeks to obtain growth in culture, these assays, with their high specificity, provide information that is directly useful for clinical management of patients (27).

Polymerase Chain Reaction (PCR) conjugated with smear microscopy to diagnose the disease in smear-negative results. This proved better technique by detecting two targets *devR and* IS*6110* sequences in the sample. These can prove a cost effective process for rapid diagnosis of smear negative tuberculosis (28). In this approach, an experiment was carried out with PCR that was specific for *devR* and IS *6110* sequences. The sensitivity was 87.5% for the combined PCR results (29).

Unlike PCR assays previously mentioned for differentiation of members of MTB complex, a real-time PCR assay showed the ability to differentiate among members of MTB complex directly from clinical specimens. The assay was able to detect 94% and 97% of positive BACTEC MGIT-960 bottles (27). Gene Xpert assay detected 71% of the confirmed TB cases where culture was positive (30). All 715 study specimens were initially subjected to routine TB NAAT using FT MTB. Direct comparison of RT MTB and FT MTB results showed concordance

in 696 of 715 (97.3%) samples. Four smear-negative MTBC cases tested negative by RT MTB but positive by FT MTB, while 15 tested positive by RT MTB but negative by FT MTB, including the two discrepant positive samples growing NTM. RT MTB showed statistically insignificant trends toward lower specificity (99.6% versus 100%; chi-square test, $P = 0.15$) but higher sensitivity (92.1% versus 88.5%; $P = 1.7$) than FT MTB, particularly with smear-negative samples (76.2% versus 65.5%; $P = 0.13$) (9).

The overall costs pretest in South Africa were $3.40 for fluorescence smear microscopy, $2.25 for Ziehl-Neelson light microscopy, $12.16 for MGIT culture, $26.19 for DST using MGIT- and $14.93 for Xpert, $23.46 for MTBDR*plus* (31). The high cost of Xpert that leads to lack of major impact on TB morbidity and mortality (32).

The study done on TB suspected patients' samples collected from Russia, South Africa, Uganda, USA and Vietnam split tested by Abbott RealTime and one aliquot smear and culture. The overall sensitivity of Abbott RealTime MTB during development compared to culture has shown that 93% and the specificity was reported 97% Compared to the results of the phenotypic first-line DST and the resistance patterns obtained by GenoType MTBDR*plus*, the concordance was 99.5% (188/189) confirming the high accuracy of the RT MTB INH/RIF assay. Discordance was found with only one sample (1/189; 0.5%), which tested susceptible by RT MTB INH/RIF but INH resistant by phenotypic first-line DST. GenoType MTBDR*plus* performed on the correspondent culture identified both wild-type and mutated *katG* signals, suggesting heteroresistance, i.e., the coexistence of INH-resistant and INH-susceptible MTB organisms (9).

3. OBJECTIVES

3.1. General Objective

- To assess the performance characteristics of Abbott real time PCR for the diagnosis of smear negative TB at EPHI, national HIV and TB laboratories

3.2. Specific Objectives

- To estimate the diagnostic sensitivity and specificity of Abbott Real Time PCR for the diagnosis of smear negative MTB
- To compare the predictive values of Abbott Real Time PCR with the available culture diagnostic methods for the identification of smear negative MTB
- To identify factors that associated with the performance characteristics of Abbott Real Time PCR for the diagnosis of smear negative MTB

4. MATERIALS AND METHODS

5.1. Study Setting

The study was conducted in Addis Ababa, Ethiopian Public Health Institute (EPH) national TB and HIV laboratories. Both public and private health facilities from Addis Ababa and surrounding region refer specimen for MTB culture and drug resistance services. Every month, an average of 120 specimens stored in temperature monitored freezers at national TB laboratory after culture inoculation that transported from different geographical areas and population groups. Demographic, specimen related and other clinical information documented in lab request forms at referring health facilities and transcribed to national TB laboratory registration log book.

5.2.Study Design

Both prospective and retrospective cross-sectional study design was employed to determine the performance characteristics of Abbott real time for the diagnosis MTB March to May 2017.

5.3.Source Population

The source population for this study was smear negative clients suspected for MTB and their specimen sent for TB culture.

5.4.Study Population

The study population was clients who suspected for MTB and the specimens sent to national laboratory during the study period. From the study population, those who fulfilled the inclusion criteria were the study subjects.

5.5.Inclusion and Exclusion Criteria

Specimens collected, stored at 2-8ºC up to 7 days and -25 to – 15 ºC up to 28 days prior to the study, and adequate for the required tests was included in the study. Insufficient specimens and, specimens that have no basic laboratory and demographic information was excluded from the study.

5.6.Study Variables

Dependent Variables

- Abbott MTB Performance (both detection and DST)

Independent Variables

- Demographic factors including region, age and sex
- Clinical related factors: HIV status, TB classification, previous treatment, diagnosis/ follow-up and follow-up month

5.7.Sample Size Determination and Sampling Procedure

For Specific objective 1 and 2:

Sample size calculation formula for population proportion

$n = \frac{z^2 * p * q}{d^2}$, where;

n= Sample size

z= alpha risk express in z-score

Standard score at level of significance of 0.05 (z) is 1.96

d= margin of error (5%, 0.05)

p= proportion

q = 1 – p

As shown below, sample size (n) calculated by taking Dse (n_1) of 93% and Dsp (n_2) of 95% used to estimate sample size to be included in the study (9).

$$n_1 = \frac{z^2 * p\,(DSe) * q}{d^2} = \frac{(1.96)^2 * 0.93 * 0.07}{0.05^2} = 100$$

$$n_2 = \frac{z^2 * p\,(DSp) * q}{d^2} = \frac{(1.96)^2 * 0.97 * 0.03}{0.05^2} = 44$$

Therefore, $n = (n_1 + n_2) = 144$

Sampling procedure: Convenient sampling method used and all stored and newly received specimens that fulfilled the inclusion criteria were included in the study

5.8. Data Collection Procedures

A structured worksheet was used to collect all necessary information including demographic, clinical information and lab results from national TB laboratory and molecular testing results was recorded at national HIV laboratory. Laboratory methods including culture, DST and Abbott real time PCR results documents on the worksheet (Annex 10.2)

5.9.Testing Principle and Procedure

The Abbott RealTime MTB assay consists of two reagent kits:

- Abbott RealTime MTB Amplification Reagent Kit
- Abbott RealTime MTB Control Kit

The Abbott RealTime MTB assay uses PCR to generate amplified product from the DNA genome of MTB.

Specimen Types

Smear negative specimens of sputum (induced or expectorated) broncho alveolar lavage (BAL) samples, or N-Acetyl-L- Cysteine (NALC)-treated sediments of sputum and BAL samples was used with the Abbott RealTime MTB assay. A sample inactivation step was performed to reduce the infection risk associated with clinical specimens that may contain MTB (9).

Specimen material directly in contact with IR for at least 1 hour was considered to have a reduced infection risk. However, this inactivation procedure may not completely eliminate the infection risk. Other potential sources of contamination not treated by this IR should be treated as potential sources of Tb infection. The Reduction of Infection Risk procedure as detailed in the package insert was followed.

Sample Preparation

The purpose of sample preparation is to prepare target DNA for PCR amplification. Preparation of target DNA was performed using a magnetic micro particle-based technology (Abbott *m*Sample Preparation System*DNA*). It was performed using an Abbott *m*2000*sp* automated sample preparation or using a manual sample preparation protocol. An internal control (IC), positive control, and negative control was processed from the start of sample preparation to demonstrate that the process has proceeded correctly. Minimizing the transfer of visible particles in the IR-treated samples during this step.

- Thaw assay controls amplification reagents at 2-8ºC or 15 to 30ºC
- Vortex each control 3 times for 2 to 3 seconds each before use
- Ensure the bubbles and foam not created
- By using a calibrated precision pipettes add 180 µL of IC to the bottle of mlysis DNA buffer
- Add 25 µL of distilled water to 200 µL 95% ethanol to mLysis buffer and IC reagent
- Gently invert the container to homogenize the solution

  Sample Preparation Reagents and Internal Control Requirements

Table 1: Abbott Real Time PCR MTB Specimen Preparation Reagent Proportion, May 2018

Reagent	For 1 to 48 samples	For 49 to 96 samples
Micro particles DNA	1 bottle	1 bottle
Lysis DNA	1 bottle	2 bottles
Wash 1 DNA	1 bottle	2 bottles
Wash 2 DNA	1 bottle	2 bottles
Elusion buffer	1 bottle	2 bottles

- Place the negative and positive control and patient specimen into the Abbott 2000sp sample rack
- Place 5 mL reaction vessels into the Abbott 2000sp and 1mL subsystem carrier. Put 96 deep well plate on the Abbott 2000SPand then run

Amplification and Detection

- Thaw set of reagents (1 for each 24 samples) and remove vial caps

- Load amplification reagent pack and master mix on the Abbott 2000sp after sample preparation completed
- Switch on and initialize the Abbott 2000rt

Purified sample DNA and master mix was added to a 96-well PCR plate using an Abbott *m*2000*sp* instrument or manually. After addition, each plate was 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*). (9).

The presence of MTB amplification products is detected during the annealing and 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; Quasar IC), thus allowing the amplification products of MTB and IC to be simultaneously detected in the same reaction (Annex 10.1).

Drug Susceptibility

Resistance to RIF was detected by using eight *rpoB* wild-type probes, and resistance to INH was detected by using wild-type and mutants (315T1) *katG* probes as well as wild-type and mutants (-15T) *inhA* promoter probes.

Identify amplification and detection reagent

- Activation reagent (Reagent 1): clean bottle, teal cap
- MTB RIF/INH resistance amplification reagent A (reagent 2): black bottle, orange cap
- MTB RIF/INH resistance amplification reagent B (reagent 2): black bottle, white cap
- MTB RIF/INH resistance amplification reagent C (reagent 2): black bottle, teal cap
- DNA polymerase (reagent 3): clean bottle, white cap

- Gently vortex and load on SP and initialize RT

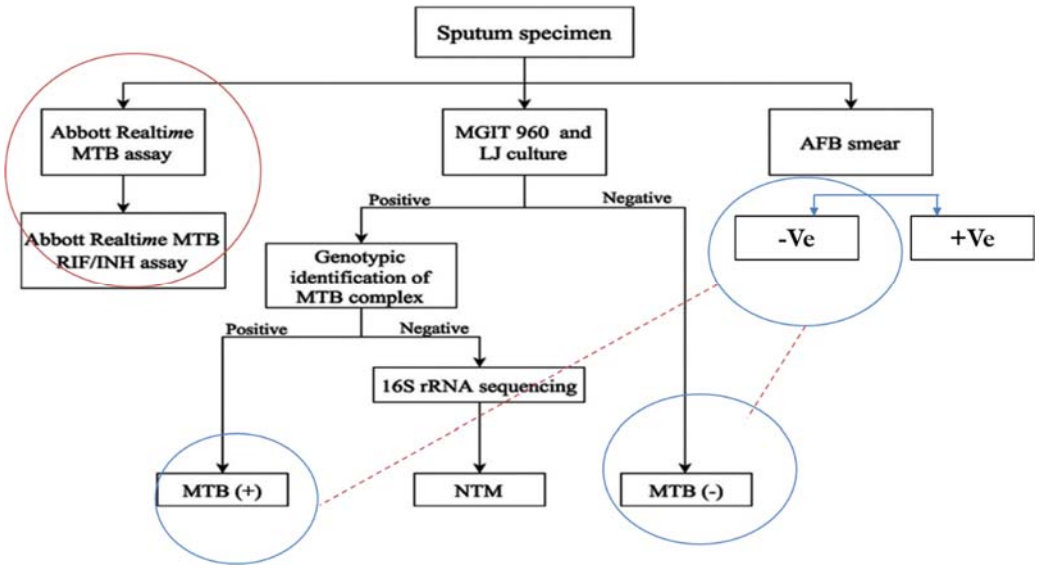

Figure 1: MTB identification at National TB laboratory and Abbott Real Time PCR, May 2018

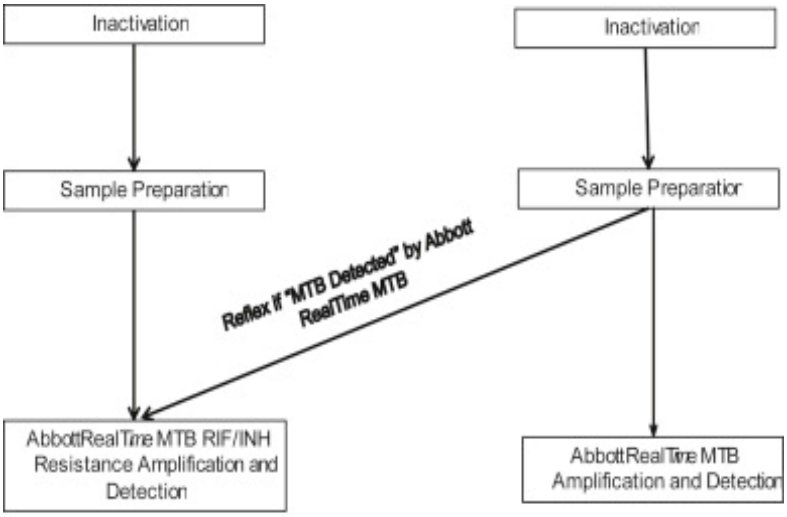

Figure 2: Abbott Real Time PCR MTB Detection and DST, May 2018

Drug Susceptibility Test Interpretation

Table 2: Abbott real time PCR DST code and interpretation, May 2018

Result Column	Interpretation column
rpoB wt: katG wt: inhA wt	*RIF R-: INH R-*
rpoB pbx-: katG 3151: inhA-15T	*RIF R det: INH R det-H*
rpoB pbx-: katG 3151: inhA wt	*RIF R det: INH R det-H*
rpoB pbx-: katG 3151: inhA intermediate	*RIF R det: INH R det-H*
rpoB pbx-: katG 3151: inhA-	*RIF R det: INH R det-H*
rpoB pbx-: katG wt: inhA-15T	*RIF R det: INH R det-L*
rpoB pbx-: katG -: inhA-15T	*RIF R det: INH R det-L*
rpoB pbx-: katG intermediate: inhA-15T	*RIF R det: INH R det-L*
rpoB pbx-: katG -: inhA wt	*RIF R det: INH R det*
rpoB pbx-: katG wt: inhA-	*RIF R det: INH R det*
rpoB intermediate: katG wt -: inhA wt	*RIF R det: INH R -*
rpoB wt-: katG wt: inhA-	*RIF R-: INH R indet*
rpoB pbx-: katG -: inhAwt	*RIF R det: INH R indet*
rpoB indeterminate: katG indeterminate: inhA wt	*RIF R indet: INH R indet*
rpoB indeterminate: katGwt: inhA indeterminate	*RIF R indet: INH R indet*
rpoB indeterminate: katG indeterminate: inhA indeterminate	*RIF R indet: INH R indet*
rpoB wt: katG -: inhA -	*Below LOD*
rpoB -: katG -: inhA wt	*Below LOD*
rpoB -: katG wt: inhA -	*Below LOD*
rpoB -: katG -: inhA -	*Below LOD*

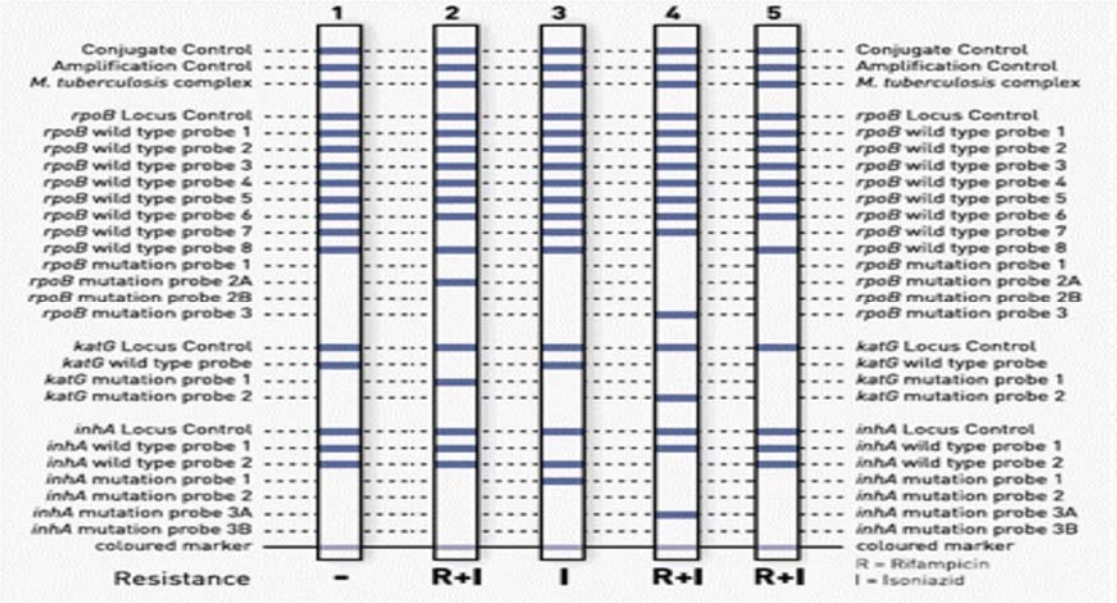

Figure 3: National TB Laboratory MTB LPA DST interpretation, May 2018

5.10. Quality Assurance

The pre-analytical, analytical and post-analytical quality was assured by using different approaches. Trained laboratory professionals assigned at molecular testing. Internal quality control was analyzed along with study specimens. The supervision was continued from the starting up to end of testing and data collection dates. Double data entry was done both hard and soft copy data cleaning was made before analysis. The third controller was verifying microscopic disagreement.

5.11. Data Processing and Analysis

Crude data from worksheets was entered by using SPSS version 23. After data cleaning; frequencies, proportions and summary statistics was done and was used to describe the study population in relation to the detection of the methods. Culture results was inserted as gold standard (at top row) and Abbott Real Time MTB results was inserted in the first column (method under evaluation) and performance characteristics was calculated. The performance characteristics including sensitivity, specificity and predictive values was calculated with 95% CI. Performance characteristics was inserted as dependent variable, specimen and clinical related factors, independent variables, was added in to regression model. Bivariate analysis was used to calculate the crude OR with 95% CI to assess the association between Abbott performance characteristics with clinical and specimen related factors. Multivariate logistic regression was employed to calculate AOR for selected variables which had statistically significant associations with dependent variable by filling the model. For all statistical significance tests, the cut-off point was 0.05 and $p < 0.05$ considered as statistically significant association with Abbott MTB performance.

For all statistical significance tests, the cut-off point was 0.05 and $p < 0.05$ considered as statistically significant association with testing performance.

5.12. Definition of Terms

Sensitivity*:* is the ability of the assay under evaluation to detect correctly that contains MTB (reference assays positive).

Specificity*:* is the ability of the assay under evaluation to detect correctly that do not contain MTB

Positive predictive value (PPV): is the probability that when the test is positive that the specimen does contain MTB

Negative predictive value (NPV): is the probability that when the test is negative that a specimen does not contain MTB

Gold Standard: A standard method for determining a sample's true MTB status i.e. culture.

5.13. Ethical Clearance

Ethical clearance gained from Addis Ababa university school of Medical Laboratory Technology. Waiver of consent requested and permission letter was obtained from EPHI IRB. The study was conducted by using leftover samples and confidentiality of the results was assured by keeping the documents secured area. The name of the client was not appeared on the data collection template and after termination of the project all worksheets will destroyed. Therefore, there was no relation to any individual

5.14. Dissemination of the Results

The finding of the study will be presented to, EPHI and Federal Ministry of Health (FMoH). It was also will be presented and disseminated to all stakeholders, public and concerned bodies through presentation in different professional association meetings and conferences. The final paper was sent to an international peer reviewed journal for publication.

6. RESULTS

6.1. Socio-demographic Characteristics

A total of 127 (88.2%) participants were enrolled in the study. The total number of participants varies in the tables due to missing values and all the percentages were calculated by using valid denominators. As presented in table 1, the participants were predominantly of males (56.7%), an age group of 25 to 34 years (38.7%), from Addis Ababa city Administration (64.6%). The age of the respondents was between 9 and 75 with mean age (± SD) of 31.8 (± 12.6) years.

Table 3: Basic Socio-demographic characteristics of study participants, Addis Ababa, May 2018

Variables (n=127)		Frequency	Percentage (%)
Sex (n=126)			
	Female	54	42.5
	Male	72	56.7
Age (n=124)			
	<= 24	34	27.4
	25-34	48	38.7
	35-44	24	19.4
	45+	18	14.5
Region (n=127)			
	Addis Ababa	82	64.6
	Oromiya	33	26.0
	BG /Gambella/ SNNPR	12	9.4

Sample size (n) varies due to missing values

6.2. Clinical and Related Factors

As shown in table 2, the study participants were predominantly new TB cases (53.0%), with follow-up period of first to sixth months (30.4%). The average follow-up period was (± SD) of 1.61 (± 1.06) months. Around 18.5% and 16% of the study participants were HIV positives and on second line TB treatment respectively.

Table 4: Clinical related factors of study participants, Addis Ababa, May 2018

Variables (n=127)	Frequency	Percentage (%)
HIV Status (n=124)		
Negative	64	51.6
Positive	23	18.5
Unknown	37	29.8
TB Classification (n=100)		
New case	53	53.0
Relapse	12	12.0
Failure	35	35.0
Previous Treatment (n=97)		
No	41	42.3
First line	41	42.3
Second line	15	15.5
Diagnosis/Follow-up (n=120)		
Diagnosis	17	14.2
Follow-up	103	85.8
Follow-up month (n=102)		
0^{th} month	18	17.6
1^{st} to 6^{th} month	31	30.4
7^{th} to 12^{th} month	26	25.5
> 12^{th} month	27	26.5

Sample size (n) varies due to missing values.

6.3 Diagnostic Performance Characteristics

As presented in table 3, in this study from the total enrolled 35 (27.7%) of them were MTB positive by the gold standard technique. Diagnostic sensitivity (DSe) of Abbott real time for the diagnosis of smear negative was 82.9% [95% CI: (70.4, 95.3)] and Diagnostic specificity (DSp) exhibited 89.1% [95% CI: (82.8, 95.5)]. The percentage of positive and negative predictive values was found to be 74.4% [95% CI: (60.7, 88.1)] and 93.2% [95% CI: (87.9, 98.5)] respectively. Twenty-two of the positive samples tested for (RIF/INH) drug resistance test.

Drug susceptibility testing demonstrated diagnostic specificity of 87.5% and 100% for INH and RIF respectively. The diagnostic sensitivity of Abbott Real Time was 92.3% (12/13) for both of the above mentioned first line drugs (see table 6 below).

Table 5: Performance characteristics of Abbott real time PCR for the diagnosis of smear negative MTB, Addis Ababa, May 2018

		Culture Result			
		Positive	Negative	Predictive Values	Total
Abbott Result	Positive	29 (74.4%)	10 (25.6%)	PPV= 74.4% (29/39) [95% CI: (60.7, 88.1)]	39 (100.0%)
	Negative	6 (6.8%)	82 (93.2%)	NPV= 93.2% (82/88) [95% CI: (87.9, 98.5)]	88 (100.0%)
	DSe/DSp	DSe = 82.9% (29/35) [95% CI: (70.4, 95.3)]	DSp= 89.1% (82/92) [95% CI: (82.8, 95.3)]		
	Total	35 (27.6%)	92 (72.4%)		

Table 6: Performance characteristics of Abbott real time PCR for MTB RIF/INH DST, Addis Ababa, May 2018

		Lab Result (culture)			
		RIF		INH	
		Resistance	Sensitive	Resistance	Sensitive
Abbott Result	Resistance	12 (100%)	0 (0%)	12 (92.3%)	1 (7.7%)
	Sensitive	0 (0%)	8 (100%)	0 (0%)	7 (100%)
	Below LOD	1 (100%)	0 (0%)	1 (100%)	0 (0%)
	Dse/DSp	DSe = 92.3% (12/13) [95% CI: (77.8, 100)]	DSp= 100% (8/8) [95% CI: (78.8,100)]	DSe = 92.3% (12/13) [95% CI: (77.8, 100)]	DSp= 87.5% (7/8) [95% CI: (64.6, 100)]
	Total	13 (61.9%)	8 (38.1%)	13 (61.9%)	8 (38.1%)

Below LOD: Target probe signals not detected from rpoB. KatG, and inhA upper promoter regions

6.4. Verification of IR

Eleven new cases that do not started TB treatment collected from EPHI, Addis Raye and Addis Ketema HCs and AFB smear done and all were AFB positive by two readers. Sample split done and the first group treated with NALC (control) and the other with IR and inoculated. The finding demonstrated that all (11/11) treated with NALC shown growth bone of (0/11) treated with IR has shown growth (see the table below).

Table 7: Verification of inactivation reagents for Abbott real time PCR MTB detection and DST, May 2018

SID	AFB	Growth (Yes/No)	
		Rx NALC	Rx IR
AR001	3+	*Yes*	*No*
AR003	3+	*Yes*	*No*
AK001	3+	*Yes*	*No*
E32582	3+	*Yes*	*No*
E42732	2+	*Yes*	*No*
AK002	2+	*Yes*	*No*
E22774	1+	*Yes*	*No*
AR002	1+	*Yes*	*No*
E32761	Scanty	*Yes*	*No*
AR003	Scanty	*Yes*	*No*
E12695	Scanty	*Yes*	*No*

6.5. Multivariate Analysis of Abbott real time PCR Performance

Before and after controlling for confounding variables in logistic regressing model, Abbott real time method performance significantly and positively associated with first line drug [Adjusted OR (95%CI) = 2.84 (1.01, 81.57)] and negatively with month of treatment [Adjusted OR (95%CI) = 0.01 (0.00. 0.41)] for 1st to 6th month, 0.005 (0.00, 0.20) for 7th to 12th months and 0.001 (0.00, 0.90) for the clients on treatment for more than a year. However, age, HIV status and TB classification didn't show statistical significant association with Abbott performance see the table below.

Table 8: Bi-variate and multi-variate logistic regression of Abbott real time PCR with explanatory variables for the diagnosis of smear negative MTB, Addis Ababa, May 2018

Variables	Abbott Result		Crude	Adjusted
	No/Negative Number (%)	Yes/ Positive Number (%)	OR (95% CI)	OR (95% CI)
Age in years				
<= 24	24 (70.6)	10 (29.4)	1.00	1.00
25-34	38 (79.2)	10 (20.8)	0.41 (0.13, 1.34)	0.29 (0.02, 4.35)
35-44	14 (58.3)	10 (41.7)	0.26 (0.08,0.83)*	0.10 (0.01, 5.06)
45+	9 (50)	9 (50)	0.70 (0.21, 2.44)	
HIV Status				
Negative	44 (68.8)	20 (31.2)	1.00	1.00
Positive	13 (56.5)	10 (43.5)	1.69 (0.64, 4.51)	0.34 (0.01, 8.20)
Unknown	28 (75.7)	9 (24.3)	0.71 (0.28, 1.77)	0.53 (0.01, 8.14)
TB classification				
New Case	33 (62.3)	20 (37.7)	1.00	1.00
Relapse	7 (58.3)	5 (41.7)	1.18 (0.33, 4.22)	7.67 (0.08, 69.60)
Failure	26 (74.3)	9 (25.7)	0.57 (0.22, 1.46)	2.52 (0.12, 53.60)
Previous Treatment				
New	33 (80.5)	8 (19.5)	1.00	1.00
First line	26 (63.6)	15 (36.6)	2.38 (0.88, 6.47)	2.84 (1.01, 81.57)*
Second line	12 (80.0)	3 (20.3)	1.03 (0.23, 4.54)	0.09 (0.01, 3.99)
Follow-up month				
0^{th} month	4 (22.2)	14 (77.8)	1.00	1.00
1^{st} to 6^{th}	28 (90.3)	3 (9.7)	0.31 (0.01, 0.16)*	0.01 (0.00. 0.41)*
7^{th} to 12^{th}	25 (96.2)	1 (3.8)	0.11 (0.01, 0.11)*	0.005 (0.00, 0.20)*
> 12^{th} month	26 (96.3)	1 (3.7)	0.11 (0.01, 0.18)*	0.001 (0.00, 0.90)*

*1:00- Reference * statistical significant association*

7. DISCUSSION

Almost all (98%) of the specimen used in this study was direct sputum samples or NACL-decontaminated sediments after treated by incubating with the inactivation reagent at the ratio of 3:1 to the specimen. In comparison of culture method, the overall testing process to release batch of 46 to 96 test results by using this method displayed greatest reduction to an average of 6 to 8 hours (9), as compared to culture method that takes 4 weeks (3) to 70 days for concentration method (22).

The frequency of invalid results due to inhibition, instrument error and contamination was 5/132 (3.8%) which is higher than the study done during method development which was around 1% (9). This might be due to instrument sample processing software not optimized well since the instrument showed frequent pattern of failure at specific positions. The invalid results were not included and the analysis restricted only to valid results. In comparison to previous studies, the current study included greatest 27.7% (35/127) MTB positives than the South Africa 20.7% (35/169) (33) and 10.8% in USA studies (9).

In this study the diagnostic sensitivity of Abbott real time for the diagnosis of smear negative was 82.9% [95% CI: (70.4, 95.3)]. The finding of this study is almost align with the result of the study done during method development (81%) by using similar stored specimens collected from Russia, South Africa, Uganda, USA, and Vietnam (9). It is the highest compared to the performance of GeneXpert 25.7% and 60.0% reported South Africa (33) and WHO (8) respectively. This might be due to GeneXpert target gene is *rpoB* (6) whereas Abbott uses IS610 and PAB gene sequences. It showed comparable sensitivity with Genotype MTB method (80.5-97%) but this method might take about 2 days (25,26).

It is higher than similar study done in South Africa 74.3% (26/35), of which 27 (77.1%) were HIV positives, but in the current study only 18.5% of the study participants were HIV positives. The finding also confirmed that the overall performance was reduced for individuals who had TB/HIV coinfection (33). The recent finding also slightly higher than the study done in Germany (76.2%). This might be due to specimen source differences used in the studies. In former study tracheal aspirate, bronchial aspirates, and extra pulmonary specimens including gastric aspirates, tissue biopsy, puncture samples, pleural fluid, urine, CSF, from operative sites and wounds in addition

to sputum and BAL (34). Since it is obligate aerobic organism, MTB mainly found in lung lobes (2) that can be predominantly detected from the upper respiratory specimens mainly sputum other than the above mentioned body fluids.

Diagnostic specificity of Abbott real time for the diagnosis of smear negative MTB exhibited 89.1% [95% CI: (82.8, 95.5)]. The specificity of the method within the range of other genotype MTB studies done in different countries (23, 24). It is lower performances as compared with previous similar method evaluations 97% (9), 95.8% (34), 93.1% (33) done in USA, Germany and South Africa respectively. Unlike the above similar studies, this study smear and culture negative samples from clients on treatment included and the assay as of other molecular techniques do not distinguish between live and dead bacilli; MTB DNA may persist following bacterial cell death and a positive result from the Abbott does not guarantee the existence of viable MTB.

From 22 first line drug tested samples 2 (9.1%) 1failed and the other 1 LOD (Lower Detection Limit); unable to decide the resistance status of the drug. LOD result of the Abbott method was resistance by the standard method. The failed result excluded from the study and analysis restricted to the results of resistance, sensitive and LOD. The overall concordance rate was 20/21 (95.2%) that confirms the accuracy of Abbott RT MTB INH/RIF resistance testing. The diagnostic sensitivity (identifying true resistance) of Abbott real time for both RIF and INH drug resistance testing was 92.3% [95% CI: (77.8, 100)]. The specificity (identifying the true sensitive) of the method was 87.5% and 100% for INH and RIF respectively. The findings on the current study supports the previous studies conducted in different countries with different specimen types that ranges from 94% to 99.5% (33, 34).

The current study displayed the positive predictive value of 74.4% [95% CI: (60.7, 88.1)] lower than similar study (91.2%) and the negative predictive value of this study was 93.2% [95% CI: (87.9, 98.5)] higher than the same study (75.6%) conducted on both smear positive and negatives (33). This is due to this study restricted to smear negatives and being smear negative increase the negative predictive values and, vice versa for positive predictive values.

To see the effect of independent variables on the dependent variable of Abbott real time, bivarate logistic regression analysis was carried out. Age, HIV status and TB classification had no statistical association with method performance. First line treatment significantly and positively associated

with the performance characteristics. The method also significantly and negatively associated with follow-up period in months before and after controlling confounding variables. The multivariate regression model revealed the performance associated with follow-up months by taking 0^{th} month as a reference with AOR 0.01 (95% CI:0.00, 0.09). As month on treatment increases, the probability of bacteria dying will also increases that lead to culture negative but the fragments of DNA may falsely positive the Abbott and any molecular technique.

8. STRENGTH AND LIMITATIONS

8.1. Strength

- The laboratory sample selection and testing done with technical support by experts and experienced staffs from both TB culture and molecular national reference laboratories.
- The impact of duration of anti-tuberculosis drug exposure had validated on the reliability and accuracy of the Abbott real time method.
- The performance of Abbott MTB evaluated among TB/HIV confected individuals and the effect of previous treatment also evaluated.
- The effectiveness of the reduction of infection risk procedures has been verified by using raw sputum samples from individuals who are newly diagnosed.
- As baseline information, the findings of this study will have great contribution for further studies.

8.2. Limitation

- Performance of Abbott real time to test other type of specimens including blood, CSF, stool, body fluids and tissue has not been determined that may change the performance of the assay.
- Clinical and other explanatory variables collected from secondary source, laboratory registers, that prone the data incompleteness and other explanatory variables including duration of cough not compared with performance characteristics
- The drug resistance section not adequately studied due to resources shortage

9. CONCLUSION AND RECOMMENDATION

9.1. Conclusions

- The current evaluation showed the invalidity rate of Abbott real time for the diagnosis of smear negative MTB higher than the finding of the company during method evaluation

- The diagnostic sensitivity of the method for identification of MTB and drug resistance-sensitivity and specificity almost aligns with the previous similar studies

- Abbott real time exhibited lower specificity for the diagnosis smear negatives specimens collected from clients on treatment as compared to culture (the gold standard method)

- The present study clearly demonstrated that there is undeniable association between the performance characteristics of Abbott MTB molecular with duration of treatment for the clients who are on follow-up.

9.2. Recommendations

- The current study showed the higher invalidity rate with patter of failure further studies need to be continued before to use the machine for the clinical purpose.

- Abbott real time PCR identifies more than 80% of the culture positive smear negative cases that can be used for the clinical diagnosis of smear negative MTB cases.

- With the current method and procedure, the results can be released within same day that will contribute for early identification, treatment and control of MTB among smear negative suspected clients.

- The finding also revealed the specificity of Abbott real time the lowest as of other molecular methods for the diagnosis of smear negative MTB cases who are on MTB treatment. Therefore, we strongly recommend not to use the method for all follow-up MTB cases except zero month.

- To sum up, using this as a preliminary study, further investigation should be continued to explain exhaustively the effect of explanatory variables on Abbott MTB performance

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11. LIST OF ANNEXES

11.1. Laboratory Test SOPs

Specimen Management

Unprocessed Specimen Collection and Storage

Sputum samples, or NALC-treated sediments of sputum and samples may be used with the Abbott RealTi*m*e MTB assay. Follow standard procedures for collecting and storing specimens from TB suspects. Specimens are to be stored per the following conditions:

- Raw sputum samples - store at a maximum of 35°C for up to 3 days and then at 2 to 8°C for an additional 7 days.
- NALC-treated sediments of sputum samples - store at 2 to 8°C for up to 7 days.
- Raw sputum and NALC-treated sediments of sputum and BAL samples may be stored at –25 to –15°C for up to 28 days.

Specimen Storage

- IR-treated samples - store at ambient temperature conditions for a minimum of 1 hour and no more than 24 hours.
- *m*2000*sp* or manually extracted sample eluates may be stored sealed at 15 to 30°C for 4 hours or at –25 to –15°C for up to 90 days.

Instrument Procedure

The Abbott RealTi*m*e MTB assay file must be installed on the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments from the Abbott RealTi*m*e MTB ROW System Combined Application CD-ROM prior to performing the assay

- The optional amplification reagent extended use feature with the automated procedure requires Abbott *m*2000*sp* software version 6.0 or higher. The procedures differ in how the amplification reagents are used and stored.

Reduction of MTb Infection Risk

Prior to DNA extraction, clinical specimens must be incubated with inactivation reagent (IR) to reduce the infection risk. Only specimen material directly in contact with IR for at least 1 hour is considered to have a reduced infection risk. Specimen droplets on the sides or lid of the container may not be effectively inactivated if not in continuous contact with the IR. Therefore, this inactivation procedure may not completely eliminate the infection risk. Caution should be observed when handling the IR-treated specimens.

Preparation of Inactivation Reagent (IR)

Materials Required:

- Polypropylene or glass container
- 10M NaOH
- Isopropanol
- Tween-20
- Purified water

Preparation of IR

1. Add 179.1 mL of water to an empty polypropylene or glass container (do not use a polystyrene 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 1 month

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.
4. Add IR at a ratio of 1:3 (eg, 1 mL specimen + 3 mL IR)
5. Invert the container to ensure contact between the IR and the specimen.
6. Vortex the mixture for 20 to 30 seconds.
7. Incubate the mixture at ambient temperature for at least 1 hour and no more than 24 hours.
8. Vortex the mixture one final time for 20 to 30 seconds at 20 to 30 minutes into the incubation period.

Automated Assay Procedure

Materials Provided

- Abbott RealTime MTB Amplification Reagent Kit (List No. 08N15-090): **new** and/or **partial** amplification packs and internal control.
- RealTime MTB Control Kit (List No. 08N15-080)
- Abbott RealTime MTB ROW System Combined Application CD-ROM (List No. 08N15-

001 or higher)

- Abbott RealTime MTB RIF/INH Resistance Control Kit (List No. 08N28-080)
- Abbott RealTime MTB ROW System Combined Application CD-ROM (List No. 08N15-002 or higher)
- Abbott m2000sp Instrument (m2000sp software version 6.0 or higher is required); Abbott RealTime MTB ROW System Combined Application CD-ROM (List No. 08N15-001); and Abbott m2000sp Operations Manual, (List No. 09K20-06 or higher)
- For use with the Optional Reflex feature: Abbott m2000sp Instrument (m2000sp software version 8.0 or higher is required); Abbott RealTime MTB ROW System Combined Application CD-ROM (List No. 08N15-002 or higher); and Abbott m2000sp Operations Manual, (List No. 09K20-008 or higher)
- Abbott mSample Preparation SystemDNA (List No. 06K12-24)
- Uracil-N-glycosylase (UNG) Protocol (List No. 08N15-067)
- 5 mL Reaction Vessels 12 x 75 mm tubes (List No. 4J71-20)
- 200 mL Reagent Vessels (List No. 4J71-60)
- Transport Tubes (Master Mix Vials) (List No. 4J71-80, 4J71-90)
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70) Abbott 96-Deep-Well Plate (List No. 04J71-30)
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Abbott Optical Adhesive Cover (List No. 04J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32-01)
- 200 µL and 1000 µL Disposable Tips for Abbott m2000sp
  (List No. 4J71-17 and 4J71-10)
- Waste bags (List No. 3N17-01)
- Biohazard bags (List No. 4J71-45)
- USP grade 190 to 200 proof ethanol (95 to 100% Ethanol).
- Do not use ethanol that contains denaturants.
- Sample racks
- Vortex mixer
- Centrifuge capable of 2000g for Abbott 96-Deep-Well Plate and Abbott 96-Well Optical Reaction Plate (Required for optional reflex feature or manual sample preparation)
- Calibrated precision pipettes capable of delivering 20 µL to 1000 µL (Calibrated

precision pipettes capable of delivering < 10 µL may be required if using UNG).

- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes (aerosol barrier pipette tips capable of delivering < 10 µL may be required if using UNG).
- 500 mL Polypropylene or glass container
- 10 M NaOH (Sigma, Cat No. 72068 or equivalent)
- Isopropanol (OmniSolv, Cat No. PX1834-1 or equivalent)
- Purified water for IR buffer (Distilled, or higher grade)
- Tween-20 (Sigma-Aldrich, Cat No. P7949 or equivalent)
- Molecular Biology Grade Water (DNase/RNAse Free)
- 1.7 mL molecular biology grade microcentrifuge tubes)

Other materials

- Sealable plastic bags

Sample Preparation Area

All specimen preparation must take place in the dedicated Sample Preparation Area. Refer to the handling Precautions section of this insert before preparing samples.

1. A maximum of 96 samples can be performed per run
2. Before use, vortex IR treated samples for 3 to 5 seconds. Using a pipette, transfer IR treated samples to the reaction vessels
3. 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.
    - If not using the optional amplification reagent extended use feature:
    - Thaw new amplification reagents at 2 to 8°C or 15 to 30°C. Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours, prior to use.
    - If using the optional amplification reagent extended use feature:

Select new and/or partial amplification reagent packs to be used in the run. Refer to Abbott m2000sp Operations Manual (List No. 9K20-06 or higher), Operating Instructions, for instructions pertaining to amplification reagent pack inventory management. Amplification reagent packs must have the same lot number.

4. Vortex each control 3 times for 2 to 3 seconds each time before use. Ensure that bubbles or foam are not created. If found, remove them with a new sterile pipette tip for each tube. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial.

5. Gently invert the Abbott mSample Preparation System DNA bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each bottle
6. Vortex the IC vial 3 times for 2 to 3 seconds each time before use. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip.
7. Using a calibrated precision pipette, add 180 µL of IC to 1 bottle of mLysisDNA buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming. Each bottle of mLysisDNA buffer supports up to 48 sample preparations. Add 180 µL of IC to a second bottle of mLysisDNA buffer for 49 to 96 samples. If using the optional amplification reagent extended use feature, partial vials of IC can be recapped and stored at 2 to 8°C for a second use.
8. Add 25 mL of USP grade 190 to 200 proof ethanol (95 to 100% ethanol) to the mLysisDNA buffer + IC reagent bottle. Do not use ethanol that contains denaturants. Gently invert the container to ensure homogeneous solution. For 49 to 96 samples, add 25 mL of ethanol to a second bottle of mLysisDNA buffer + IC. Gently invert to ensure a homogeneous solution
9. Add 70 ml USP grade 190 to proof ethanol to wash 2 bottle supports up to 48 reactions. Gently invert to ensure a homogenous solution
10. Place the negative and positive control and the patient specimens into the Abbott m2000sp sample rack.
11. Place the 5 mL Reaction Vessels into the Abbott m2000sp 1 subsystem carrier.
12. Load the carrier racks containing the Abbott mSample Preparation SystemDNA reagents and the Abbott 96-Deep-Well Plate on the Abbott m2000sp worktable as described in the Abbott m2000sp Operations Manual, Operating Instructions.
13. From the Run Sample Extraction screen, select and initiate the sample extraction protocol as described in the Abbott m2000sp Operations Manual, Operating Instruction.
    NOTE: Change gloves before handling the amplification reagents.
14. Load the amplification reagent pack and master mix vial (if needed) on the Abbott m2000sp worktable after sample preparation is completed.
    - Each amplification reagent pack supports up to 24 reactions.
    - Thaw 1 set of reagents for 1 to 24 samples, 2 sets for 25 to 48 samples, 3 sets for 49 to 72 samples and 4 sets for 73 to 96 samples.

- Ensure the amplification reagents are thoroughly thawed before use.
- Ensure that the contents are at the bottom of the vials by tapping the vials in an upright position on the bench.
- Remove the amplification reagent vial caps.
- If using the optional amplification reagent extended use feature, a combination of new and partial reagent packs may be used.
- Partial amplification reagent packs can only be used on the same Abbott m2000sp instrument used for the reagent pack's initial preparation. Using an amplification reagent pack for a second time on a different instrument was result in an error, which may delay the run.
- Partial and new amplification reagent packs may be used together.
- Ensure that the contents of new amplification reagent packs are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench.
- Do not tap partial amplification reagent packs being used a second time. Tapping may result in loss of master mix volume in the cap.
- Remove caps. If a new amplification reagent pack was stored for a subsequent use, the vials was need to be recapped for storage using either the saved original caps or new fresh caps.
- Partial amplification packs are loaded to the left of new amplification packs on the Abbott m2000sp worktable.
- Ensure that the amplification reagent packs are firmly seated on the instrument.

15. Select appropriate deep well plate from the run master mix. Addition screen that matches the corresponding sample preparation extraction. Initiate the Abbott m2000sp Master Mix Addition protocol. Follow the instructions as described in the Abbott m2000sp Operations Manual, Operating Instructions section.

NOTE: The assembly of the amplification master mix and sample eluates into the Abbott 96-Well Optical Reaction Plate (step 15) must be initiated within 1 hour after completion of Sample Preparation.

NOTE: The Abbott m2000rt protocol (step 20) must be started within 90 minutes of the initiation of the master mix Addition protocol.

NOTE: If the run is aborted for any reason subsequent to step 15, the amplification reagents are to be discarded and a new 96-well PCR plate must be used if the Abbott m2000sp master

mix Addition Protocol (step 15) was repeated.

16. Switch on and initialize the Abbott m2000rt in the Amplification Area.

NOTE: The Abbott m2000rt requires 15 minutes to warm up.

17. Place the Abbott 96-Well Optical Reaction Plate into the Abbott Splash-Free Support Base after the Abbott m2000sp instrument has completed addition of samples and master mix.
18. Place the Abbott 96-Well Optical Reaction Plate in the Abbott m2000rt instrument. Import the Abbott m2000sp test order via CD (or directly to a mapped Abbott m2000rt via a network connection) per the Import Order instructions in the Abbott m2000rt Operations Manual, Operating Instructions section.
19. Place the Abbott 96-Well Optical Reaction Plate in the Abbott m2000rt and initiate the Abbott RealTime protocol as described in the Abbott m2000rt Operations Manual.

If the Abbott m2000rt instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott m2000rt Operations Manual along with the gloves used to handle the plate.

20. After the Abbott m2000rt instrument has completed the amplification and detection protocol, remove the Abbot 96-Well Optical Reaction Plate and dispose according to the instructions in the handling Precautions section of this insert.

Reference: Abbott m2000sp Operations Manual

Purpose	This procedure provides instructions for smear preparation
Abbreviations	AFB:Acid-Fast Bacilli N/A: Not applicable
Definition	N/A
Utility	N/A
Principle	When preparing a smear from a sputum specimen, the laboratory personnel must decide which part of the specimen to use. Making a good smear is not as easy as it may seem and requires practice and patience. A good smear is neither too thick nor too thin, and is evenly spread, which may require prolonged smearing using circular movements.

Materials

Supplies
Waste receptacles (including splash proof receptacle for liquids) Discard bucket with biohazard bag insert, containing appropriate disinfectant Paper towel soaked in appropriate disinfectant Microscope slides, frosted at one end, new and clean Pencil for labelling slides Sterile, transfer pipettes with graduations marking volume (individually wrapped) Sterile plastic loop or disposable applicator stick Slide drying rack Forceps Little plastic bag for the waste disposal 7 0% ethanol 50ml conical tubes Distilled water Wash bottle
Equipment
Biological safety cabinet Vortex mixer

Sample

Sample type	Amount required	Transport and Storage	Stability
Processed sediment	1 drop	Not applicable	Not applicable
LJ culture	1 colony		
MGIT culture	1 drop		

Limitations: Spontaneous sputum: Sputum from suspects should be rejected if they are liquid and clear as water, with particles or streaks of mucous material. However, sputum from follow-

	up patients should be accepted and examined even if they look like saliva, since these patients often cannot produce mucous specimens.
Environmental and safety controls	During the smear preparation care should be taken to avoid spillage and if there any spillage, the area should be cleaned. For staining procedures, especially for decolourization with acids, protective glasses should be worn.

Quality Control

Control	Level	Stability	Frequency	Preparation (y/n)
Unstained positive(1+)	positive	6 month	At least for every newly prepared staining solution	As need arise
Unstained negative	Negative			

Control preparation: Refer reference Book for Panel slide preparation

Note: Positive and negative control slide should have expected result. If it hasn't this could be problem associated with staining solution preparation, staining procedure or reagent used for staining solution preparation. The sample of that run should be retested and patient result is not released.

Procedure

General for smear preparation

Step	Action
	Put a Paper towel in Biological safety cabinet
	Place the west containers in Biological safety cabinet
	Open the containers and do the smearing in Biological safety cabinet
	Label the slides properly using the laboratory register serial number
	Place each slide on the corresponding container
	When dry, hold the slides in forceps and fix them by passing three times

Preparation of Smears from direct sputum

Step Action

Label the frosted end of the slide in pencil with the laboratory accession number
Working in a biological safety cabinet, Select a small portion of purulent or muco-purulent transfer it to the slide
Use the ragged ends for dissecting sputum and one of them for smearing
Make a smear by repeated loop formation
Spread the material carefully over the area equal to about 2-3 cm
Make the smear as even as possible by continuing this process for a sufficient time.
Remove excess material with the second part of the stick and discard in the bag.
Let the smears dry at the air at room temperature in Biological safety cabinet

Preparation of Smears from Processed Sputum

Step	Action
1	Label the frosted end of the slide in pencil with the laboratory accession number
2	Working in a biological safety cabinet, vortex the decontaminated sediment
3	The concentrated material has to be transferred to the slide with a sterilized loop or Use a transfer pipette to place ~100 µl (2 drops) of well-mixed re-suspended pellet from the digested-decontaminated specimen onto the slide
4	Spreading over an area approximately 1 x 2 cm.
5	Air-dry the smear.

Preparation of Smears from Positive Cultures

Step	Action
	Label frosted end of slide in pencil with laboratory accession number.
	If examining a positive MGIT culture: dispense 1drop of egg albumin on a glass slide with a transfer pipette.
	Vortex tube well, unscrew MGIT tube cap and sample an aliquot of broth using a disposable pipette. Place ~100 µl (2 drops) of broth onto the slide, spreading it to cover an area approximately 1 x 2 cm
	Dispose of transfer pipette into the biohazard discard bucket.
	I f examining colonies on solid medium, dispense ~100 µl of distilled water or sterilized NaCl (0,9%) on a glass slide with a transfer pipette. Using a sterile loop or disposable applicator stick, transfer 2 to 3 colonies to the water and gently mix to make a smooth, thin suspension
	Dispose of applicator stick into the biohazard discard bucket.
	Air-dry smear.

Result Interpretation	The thickness of the smear should be such that a newspaper can barely be read through the dried smear held about 10 cm above it
Expected Values	NA
Limitations	The minimum fields to be scanned before reporting a negative result is 100 to 150 fields
Related Documents	Document Number Document Name EPHI NRL/TBL/SOP 5.5-006: Procedure for Ziehl-Neelsen Staining Solution Preparation EPHI NRL/TBL/SOP 5.5-008: Procedure for Ziehl-Neelsen staining technique
References	1.Collins C, Grange J, Yates M. Organization and practice in tuberculosis bacteriology. London: Butterworths; 1985. 2.Kent PT, Kubica GP. Public Health Mycobacteriology: a guide for the level III laboratory. Atlanta, Ga: U.S. Department of Health and Human Services, Centers for Disease Control,; 1985. 3.Lumb R, Bastian I. Laboratory diagnosis of tuberculosis by sputum microscopy. Adelaide: Institute of Medical and Veterinary Science; 2005. 4.Smithwick RW. Laboratory manual for acid-fast microscopy. 2nd ed. Atlanta: CDC; 1979. 5.World Health Organization. Laboratory services in tuberculosis control. Part II: Microscopy. Geneva; 1998.

Purpose	This procedure provides instructions for Ziehl-Neelsen staining technique.
Abbreviations	AFB: acid-fast bacilli LPO: low power objective N/A: Not Applicable
Definition	N/A
Utility	N/A
Principle	The property of acid-fastness is based on the presence of mycolic acids in the cell wall of mycobacterium. Primary stain (fuchsin) bindsto cell-wall mycolic acids. Intense decolourization (strong acid or acid/alcohol) does not release the primary stain from the cell wall and the mycobacterium retain the red colour of fuchsin – hence acid-fastness. Counterstaining (with methylene blue) provides a contrasting background. While mycobacteria are AFB, very few other bacteria possess the property of acid-fastness, and then only weakly (e.g. *Nocardia*). AFB found in extra pulmonary specimens, particularly gastric washings, stool or urine, should never be automatically assumed to represent TB bacilli
Materials	

Reagents
1%Carbolfuchsin staining solution.
3% Acid- alcohol.
0.1% Methylene blue counterstaining solution.

Reagents preparation: ReferEPHI NRL/TBL/SOP5.5-006.
Reagents stability and storage: Refer EPHI NRL/TBL/SOP5.5-006.

Supplies
Burning Spirit diamond pencil or lead pencil(if frosted end slides are used). filter paper funnel Immersion oil, synthetic, with refractive index of 1.5180+/-0.0004. Do not use cedar wood oil. lens paper or soft tissue paper little plastic bag for the waste disposal disposable loops staining reagents oil absorbing paper forceps slide drying rack slide staining rack slide box new, clean slides timer 250 ml staining bottles with spout beaker for rinsing water

Disinfectant solution.

Equipment
LED Microscope BSC Sink and water supply.

Sample

Sample type	Amount required	Transport and Storage	Stability
Processed sediment	1 drop	Not applicable	Not applicable
LJ culture	1 colony		
MGIT culture	1 drop		

Limitations: Not applicable.
Sample retention: Samples are discarded after the smear result issued if no further test is required.

Environmental and safety controls

For staining procedures, especially for decolourization with acids, protective glasses should be Worn.

Quality Control

Control	Level	Stability	Frequency	Preparation (y/n)
Unstained positive(1+)	positive	6 month	At least for every newly prepared staining solution	As need arise
Unstained negative	Negative			

Control preparation:
Note: Positive and negative control slide should have expected result. If it hasn't this could be problem associated with staining solution preparation, staining procedure or reagent used for staining solution preparation. In this case root cause analysis shouldbe done. The sample of that batch should be retested and patient result is not released.

Procedure

Step	Action
	place the slides with smear upwards on the staining rack over a sink about finger-width apart and heat fix.
	Add carbol-fuchsin staining solutions over the smears
	Prepare the torch by dipping its cotton wool end in burning spirit and light it
	Heat all slides keeping the torch a little below them until steam arises
	do not let staining solution dry on the slides
	Leave the heated stain on the slide a minimum of 5 minute.
	Tilt each slide using forceps to drain off the staining solution
	Rinse the slides well with clean water from a beaker.
	Pour decolourising solution over the smears covering them completely
	Allow to act for 3 minutes
	Tilt each slide with forceps to drain off the acid
	Gently rinse each slide again with clean water. do not splash adjacent slides.
	If needed, repeat until all macroscopically visible stain has been washed away.
	Flood smear with methylene blue solution for 1 minute.
	Gently rinse each slide with water. Do not splash adjacent slides
	Tilt each slide with forceps to drain off excess water
	Clean back of slide with moist paper
	Using forceps place it on draining rack. Always keep smears out of direct sunlight.
	Examine slides after it has dried

Examination

	Check smear is facing upward
	Apply one drop of oil immersion. the drop must fall freely onto the smear and Never allow the oil applicator to touch the slide
	Use 10X Objective to focus the first smear. Scan the smear, looking for purulent or cord material
	Carefully rotate to 100X objective
	Carefully adjust the fine focus until cells are sharp. Never allow lens to touch the glass slide.
	Examine at least 100 high power fields (one length) before recording a negative result
	Store examined slide.
	Wipe the microscope lens gently with tissue paper to remove immersion oil after each positive slide and at the end of examining a batch of slide

Appearance of AFB
Viewed with oil immersion , AFB are red , slender rods, some time with one or more granules. the bacilli may occur singly, V-shaped forms, cords, or as clumps. fragments of bacilli often seen during treatment.

Result Interpretation	Recording Observation	Reporting
	No AFB found in at least 100 fields	Negative
	1-9 AFB in100 fields	Record exact number of bacilli
	10 - 99 AFB per 100 fields	1+
	1 to 10 AFB per field (check 50 fields)	2+
	More than 10 AFB per field (check 20 fields)	3+
Limitation	N/A	
Related Documents	Document Number Document Name EPHI NRL/TBL/F 5.5-031: Ziehl-Neelsen/Fluorescence Microscopy Exanimation Worksheet EPHI NRL/TBL/SOP 5.5-007: Procedure for smear preparation EPHI NRL/TBL/SOP 5.5-006: Procedure for Ziehl-Neelsen Staining Solution Preparation	
References	Laboratory Diagnosis of Tuberculosis By sputum Microscopy, The Handbook. GLI Smithwick RW. Laboratory manual for acid-fast microscopy. 2nd ed. Atlanta: CDC; 1979. World Health Organization. Laboratory services in tuberculosis control. Part II: Microscopy. Geneva; 1998.	

Purpose	This procedure provides instructions for processing of samples using NALC-NaOH method, inoculation to MGIT and LJ tube and reading of the growth.
Abbreviations	LJ: Lowenstein Jensen BSC: Biological Safety Cabinet NALC: N-acetyl L-cysteine NaOH: Sodium Hydroxide PANTA: Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim, Azlocillin MGIT -Mycobacterium Growth Indicator Tube
Definition	N/A
Utility	N/A
Principle	Specimens for tubercle bacilli isolation do usually contain associated flora which has to be eliminated before inoculation of the specimen onto culture media. Culture examination detects fewer bacilli than microscopy and increases the number of tuberculosis cases found, in the range of 20-50%, subject to the local incidence. Culture methods provide definitive diagnosis by establishing the viability and identity of the organisms and enable the detection of drug resistance.
Materials	

Reagents
NaOH-Trisodium citrate
NALC-NaOH solution
Phosphate buffer 0.067M, pH 6.8
NALC
PANTA solution
Sterile distilled water

Reagents preparation: See EPHI NRL/TBL/SOP5.4-003 forNaOH-Trisodium citrate and Phosphate buffer 0.067M, pH 6.8
Reconstitution of PANTA solution
Dissolve PANTA in 15 ml MGIT Growth Supplement.
Label the date and initial of reconstitution
NALC-NaOH solution, freshly prepared for daily use only
Add 0.5 g NALC to 100ml of the mixture of sodium hydroxide-Trisodium citrate just before use
Reagents stability and storage:
Reconstitution of PANTA: store at 2-8^{o}C and stable for 5 days.
NALC-NaOH solution is stable only for one day. Sore 2 to 25^{o}C

Supplies:
Slides
Falcon tubes (50 ml)

Rack for tubes
Pipette tips of 1.0 ml, sterile, single use,
Repeater pipette
Multi tip for repeater pipette
Pipette boy
10ml serological pipette
Sterile plastic pipettes (with graduation)
Pipetting aids
Sterile forceps
Disinfectants
waste containers
Buckets, stainless steel or polypropylene
Timer
General lab glass ware
Decontamination reagents

Equipment:
Vortex mixer
Refrigerated centrifuge with safety shield
BSC
Incubator
MGIT machine

Sample

Sample type	Amount required	Transport and Storage	Stability
EPHI NRL/TBL/SOP5.4-006	EPHI NRL/TBL/SOP5.4-006	EPHI NRL/TBL/SOP5.4-006	EPHI NRL/TBL/SOP5.4-006

Limitations: If sample collected at the referral center, it should be refrigerated and sent to the laboratory within 4 days in order to minimize contamination
Sample retention: The sediment was discarded after the result issued

Environmental and safety controls

Material that have any contact with infectious material should not leave the laboratory unlessit has been decontaminated or autoclaved.
Procedures that can cause the generation of aerosols must be minimized and carried out in a class II bio-safety cabinet.
Minimize the aerosol production by opening the caps of specimen containers slowly, avoiding vigorous shaking of the specimen and avoiding the expulsion of the last drop from the pipette.

Quality Control

Control	Level	Stability	Frequency	Preparation (y/n)
Sterile distilled water	Start	Store at 18 to 25 ºC for 3 months	For each test run	No
Sterile distilled water	End	Store at 18 to 25 ºC for 3 months	For each test run	No

Note: Start and End control shouldn't show any growth. If it has growth it could be cross-contamination or any contamination from reagent or material. In this case cause analysis was done and patient result is not issued. The sample of that run should be retested.

Procedure

Pre-processing preparation:
Label the sample and request form with culture number
Check all the material required for sample processing is in place
Arrange sample, and required material in the biological safety cabinet

Step	Action

1	Use the TB processing checklist to collect supplies, reagents, and specimens Label the specimen, slide and LJ media (with lab code, date inoculated) Disinfect BSC and perform daily maintenance Clean slides with 70% alcohol Set up BSC for specimen processing with absorbent liner, disinfectant, and waste con
2	Sort specimens into batches using sterile water blanks as the first and last negative pr controls Batch size is based on the centrifuge load including negative processing controls

Preparation for direct inoculation

1.1 Sample collected in swabs

Step	Action
	Use sterile forceps to transfer the swab to a sterile centrifuge tube
	Add 2 ml of sterile distilled water and mix-well
	Remove the swab from the tube with forceps
	Ready for direct inoculation, the remaining suspension is handle as for sputum

Body fluid

Step	Action
	When volume is 5 ml or less, ready for direct inoculation, the remaining suspensi handled as for sputum
	When volume is more than 5 ml centrifuge at 3,000 g for 15 min
	If it is mucopurulent body fluid, add 50 to 100mg of NALC powder for 50ml of t then mix-well and centrifuge at 3,000 g for 15 min
	Pour off the supernatant, re-suspend the sediment in 3 ml of sterile distilled wate
	Ready for direct inoculation, the remaining suspension is handled as for sputum

Preparation for process using NALC-NaOH

Sputum

Step	Action
	Check the volume of the sputum (at least 2 ml, not more than 5 ml) (if the volum sample is above 5ml, transfer 5ml of the purulent part to another falcon tube and this allocate for the subsequent steps)
	Add equal volumes of NALC-NaOH solution. Use aliquots of NALC-NAOH(1 v NALC-NAOH per one specimen)
	Tighten cap of container and vortex slowly
	Shake intermittently to aid homogenization and decontamination
	Invert each bottle to ensure that NALC-NaOH solution contacts all the sides and portion of caps.
	Keep at 20°C – 25°C for 15 min for decontamination
	Fill the tube with phosphate buffer up to 45 ml mark on the tube. . Use aliquots o phosphate buffer(1 vial of of PBS per one specimen)
	Mix-well or vortex
	Centrifuge at 3,000 ×g for 15 minutes

	Carefully pour off the supernatant into a discard container containing 5% sodium hypochlorite or other germicide. Make sure the final concentration of bleach is 1% after pouring off the supernatant.
	Prepare smear on slide labelled with culture number for microscope
	Re suspend the deposit with 2ml PBS.

2.2

Step	Action
	Use a tissue grinder/mortar or stomacher
	Handle as for sputum

Gastric Lavages (If specimen is watery)

Step	Action
	Centrifuge at 3,000 g for 20 min.
	Pour off the supernatant, resuspend the sediment in 5 ml of sterile distilled water
	Add equal volumes of NALC-NaOH solution
	Tighten cap of container and vortex slowly
	Shake intermittently to aid homogenization and decontamination
	Invert each bottle to ensure that NALC-NaOH solution contacts all the sides and portion of caps.
	Keep at 20°C – 25°C for 15 min for decontamination
	Fill the tube within with phosphate buffer up to the 50 ml mark on the tube
	Mix-well or vortex
	Centrifuge at 3,000 g for 15 minutes
	Carefully pour off the supernatant into a discard container containing 5% sodium hypochlorite or other germicide
	Prepare smear on slide labeled with culture number for microscope
	Resuspend the deposit with 2ml PBS

Gastric Lavages (If specimen is mucoid)

Step	Action
	Add 50 to 100mg of NALC powder to 50ml of gastric lavage
	Replace and tighten the cap
	Mix by swirling on a test tube mixer
	Continue the steps 1-13 of the above procedure

Inoculation

3.1 MGIT Media

Step	Action
	Mark each MGIT tube with laboratory number.
	Using repeater pipette Add 0.8 ml of the PANTA solution to each MGIT tube jus inoculation.
	with disposable Pasteur pipette, Add 0.5 ml of a well-mixed processed and/or dir specimen to the appropriately labelled MGIT tube

	Tightly recap the tube and mix by inverting the tube several times.
	Wipe tubes and caps with a mycobactericidal disinfectant.
	Leave inoculated MGIT tubes at room temperature for 30 min.

LJ Media

Step	Action
	Mark each LJ tube with laboratory number
	Decant excess water from the media
	Inoculate 2 to 3 drops of sediment and/or direct preparation in to two LJ media
	Wipe tubes and caps with a mycobactericidal disinfectant.

Incubation and follow up of the culture tube

MGIT tube

Step	Action
	Open the desired MGIT 960 drawer and press the “tube enters” key
	The barcode scanner was light up
	Scan the inoculated MGIT tube and load into the slot identified by the MGIT 960
	Check MGIT 960 daily for indicator lights flagging positive and negative culture
	Incubate MGIT tubes until the instrument flags them as positive (red flag) or neg (green flag)
	Positive and negative tubes was issued by pull the respective drawer and press “p negative button as needed”, the machine displayed by the indicator light changing at the exact location of the tube in the instrument drawer
	Remove the tube and scan
	Continue MGIT culture work up (refer SOP TB-TP-SP-03)
	Register the result in the work sheet if it is final result register in the final registra (Annex Work sheet for culture reading)

LJ tube

Step	Action
	Loose the cap slightly and put the LJ tube in slant position facing upward in the inc
	Make sure the fluid cover the surface
	Keep in a slant position for one week
	Check LJ two times a week for any contamination and fast grower Mycobacterium

		Read LJ tubes weekly until a positive growth obtained (Refer Annex characteristic of reference strain)
		If the LJ tube have growth and enough (>50 matured colony) for subsequent work , quantify the colony (Annex quantification scheme) and issue
		If the LJ tube have growth and not enough for subsequent work write "P" on the work sheet and wait 1 to 3 week till enough growth obtained
		Positive tube was kept in the incubator till (maximum of 1 week) the subsequent work is done
		If the LJ tube have no growth in 8th week issued as negative
		Negative tube was safely discarded from the laboratory
		Register the result in the work sheet weekly if it is final result register in the final registration book (Annex work sheet for culture reading)

Result Interpretation

For MGIT refer to EPHI NRL/TBL/SOP 5.5-004

For LJ: Growth on culture media shall be recorded in accordance with the following schema:

Description	Result/Grade	
Confluent growth	3+	
More than 100 colonies	2+	
20-200 colonies	1+	
< 20 colonies	Exact count	
No growth	Negative	

Expected Values

N/A

Limitations

N/A

Related Documents

References

Global Laboratory Initiative; Mycobacteriology Laboratory Manual. Page number 19-22, 1st Edition 2014

Laboratory Services in Tuberculosis Control. Part III: Culture. WHO/TB/98/258 pp 79-84 and pp 48-52.

Barrera L , B. Lopez, N. Simboli, O. Latini and M. D. Sequeira Quality Control of the Culturing of Mycobacteria, Geneva, Reviewed, adapted and translated from the Spanish original by A. Laszlo, for World Health Organization.

11.1. Data Capturing Worksheet

SN	Data collection Date	Age	Sex	Region	HIV status R/NR/UK	Lab Plate #	Abbott Results	TB classification N/R/F/L/T/O	Previous Rx 1.N 2,FL 3.SL	Diagnosis/ Follow-up month	Specimen collection date	Processed date	AFB smear result	Culture result P/N/C	DST method used	Method used 1. Genotypic 2.Phenotipic

10.3. 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: **Million Hailu (BSc, MPH)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Adane Bitew (PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Gebreab Teklebirhan (BSc, MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.