Performance evaluation of Abbott real-time PCR in the diagnosis of Mycobacterium tuberculosis in Addis Ababa, Ethiopia: A cross-sectional descriptive study

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Abstract

Background

In 2018, an estimated 10 million people developed tuberculosis, of whom more than 1.45 million died. The microscopy method used in most tuberculosis high burden and resource-limited countries is less accurate for diagnosing the disease. Thus, evaluation of the available diagnostic modalities in the country is crucial, and this study aimed to evaluate the performance of Abbott real-time PCR as a diagnostic technique for tuberculosis in Ethiopia.

Methods

A cross-sectional survey was conducted using sputum specimens collected from 150 presumptive tuberculosis patients from both public and private health facilities in Addis Ababa, Ethiopia, from May to June 2019. The laboratory investigation was conducted at the National Reference Laboratories of the Ethiopian Public Health Institute (EPHI).

Results

This finding indicated that 84.7% (127/150) and 61.3% (92/150) were smear and culture-negative, respectively. The overall diagnostic sensitivity of the Abbott real-time polymerase chain reaction (PCR) technique for the diagnosis of tuberculosis was 89.7% (52/58), that for smear-negative was 80.6% (29/36), and that for specificity was 92.4% (85/92). Drug resistance testing demonstrated diagnostic specificities of 87.5% and 100% for isoniazid and rifampicin, respectively, and a sensitivity of 92.3% for both.

Conclusions

This study demonstrated an outstanding performance of the Abbott real-time PCR technique for diagnosing tuberculosis using sputum specimens using culture as a reference standard. Thus, we recommend that Ethiopia's ministry and tuberculosis program implementers consider the Abbott real-time PCR technique for diagnosing tuberculosis and drug resistance testing, which is likely to be included in the national guidelines.

Background

Mycobacterium tuberculosis (MTB) remains one of the world's deadliest communicable diseases. In 2018, an estimated 10 million people developed *tuberculosis* (TB) globally; of these, more than 1.45 million died from the disease, 251,000 (17.3%) of whom were HIV-positive. Ethiopia has been classified among the 30 high TB, high TB/ human immunodeficiency virus (HIV), and high multidrug-

resistant (MDR) TB (MDR-TB) burden countries (1). The causative agent, acid-fast bacilli (AFB), is obligate aerobic, so *tuberculosis* mainly affects the lung lobes where complete aeration is available (2).

Diagnostic methods of the disease continue to evolve. As of 2016, more than 50 diagnostic methods were developed for the diagnosis of MTB (3, 4). Although less accurate, the smear microscopy method using acid-fast stains and a light microscope is the most commonly used method in most high-burdened and resource-poor countries (5). The sensitivity of this method ranges from 32–94% (6). The sensitivity of fluorescence microscopy (FM) using auramine stain is 52–97% compared to light microscopy (5, 7). There were no significant differences in specificity between the Ziehl-Neelsen (ZN) and FM techniques (98.2% and 98.4%, respectively) (8).

Mycobacterium Growth Indicator Test (MGIT) 960 is a culture method that minimizes the time for MTB detection and drug resistance testing (9). However, this method is not accessible for high-burden countries such as Ethiopia since it requires sophisticated infrastructure and high technical skills. The sensitivity of GeneXpert for the diagnosis of pulmonary TB ranges from 51–81%, with an average of 62%, while the specificity ranges from 97–99%. In pulmonary specimens, sensitivities were 100% and 68.6% for smear-positive and smear-negative specimens, respectively (10). The aggregated sensitivity and specificity of GeneXpert to detect rifampicin resistance (RIF) were 93% and 98%, respectively (11). However, the cost per test is a challenge for the rollout of this method, especially in low-resource settings (12).

Abbott real-time PCR can release a batch of 93 test results in one run, which takes approximately 6–8 hours (13). For the diagnosis of MTB, the assay uses fluorescently labeled primers targeting protein antigen B (PAB) and insertion sequence 6110 (IS6110). IS6110 is a multicopy target to maximize the sensitivity and minimize false negativity due to target gene mutation or deletion (14). The Abbott MTB RIF and Isoniazid (INH) Resistance assay is performed separately or in reflex mode using the remaining eluents from Abbott real-time MTB (15).

The overall invalidity rate of Abbott real-time PCR is 1% when compared to the culture method, which is the gold standard, and the overall sensitivity is 93%. The assay sensitivity increased to 99% for smear positivity and 81% for smear negativity. In contrast, the test has 97% specificity for culture-positive MTB (15). Abbott real-time exhibited INH sensitivity and specificity of 88.3% and 94.3%, respectively. At the same time, RIF demonstrated a sensitivity of 95.8% and a specificity of 100% (16).

In view of the preceding, there is a need to introduce and implement rapid batch testing molecular methods in high-burden countries such as Ethiopia to improve case detection and management at an early stage of the disease. Therefore, this study evaluated the performance of Abbott real-time PCR to produce evidence for policymakers and stakeholders for informed and objective decision-making on the use of the technology for MTB diagnosis.

Methods

Study design and setting

We conducted a cross-sectional study using specimens collected from presumptive MTB patients from both public and private health facilities in Addis Ababa, Ethiopia, from May to June 2019. Addis Ababa, the capital city of Ethiopia, has a population of more than 3 million people. It is home to the African Union, the Economic Commission for Africa, and other international organizations. We conducted the study at the EPHI National Reference Laboratory, which is based in Addis Ababa and is the national reference laboratory. We used specimens for MTB culture, drug resistance, and GeneXpert MTB and RIF testing services.

Sample size and testing procedure

A total of 150 patients who provided specimens and were tested for culture-based detection and drug resistance testing were included in the study. Leftover sputum specimens stored at -25°C to -15°C up to 28 days before the study and adequate for the required tests (Abbott real-time PCR for detection and drug resistance testing) were included. The specimens were tested directly or after the sedimentation procedure by Abbot PCR MTB assay at the National Reference Laboratory.

A sample inactivation step was performed to reduce the infection risk associated with clinical specimens that may contain MTB (17). Specimen material directly in contact with inactivation reagent (IR) for at least 1 hour was liquified and considered to have a reduced infection risk (18). Five hundred (500) (ml) of IR was prepared by mixing 20 mL of 10 molar sodium hydroxide (NaOH), 300 mL of isopropanol, 0.9 mL of Tween-20, and 179.1 mL of purified water (18). The proportion of IR to sample volume was 3:1 with a minimum of 0.3 mL sample volume. The mixture was then mixed for 30 seconds and incubated for 24 hours for inactivation. The process was handled in a biological safety cabinet class II (18, 19).

Eleven new TB cases with different microscopic grades (four cases with 3 + grade, two with grade 2+, two with grade 1+, and three scanty) were tested using AFB smear to determine the effectiveness of the IR, of which all tested AFB positive by two readers (20). A sample split was performed, and the first group was treated with N-acetyl-l-cysteine (NALC) as a control and the other with IR and inoculation. The findings demonstrated that all (11/11) treated with NALC showed growth, while patients treated with IR showed no growth (0/11).

Preparation of target deoxyribose nucleic acid (DNA) was performed using an Abbott m2000sp automated sample preparation protocol. Then, 180 microliters (µL) of internal control (IC) was added to the bottle of analysis DNA buffer, and 25 µL of distilled water was added to 200 µL of 95% ethanol in lysis buffer. Negative and positive controls and specimens were loaded onto the Abbott 2000sp sample rack and then processed (21).

After sample preparation, purified DNA and master mix were added to a 96-well PCR plate. After addition, each plate was sealed and transferred to an Abbott m2000rt for amplification. The targets of PCR were IC and two different MTB targets, IS6110 (IS6110) and protein antigen b (PAB) (22). The presence of MTB

amplification products was detected during the annealing and extension step by measuring the real-time fluorescence signal of the MTB IS6110 and PAB probes. The presence of IC amplification products is detected by measuring the real-time fluorescence signal of the IC probe (22).

The MTB gene encoding the beta subunit of RNA polymerase (rpoB), oligonucleotides, is used as a hybridization probe that is highly resistant to RIF(6). KatG is a catalase-peroxidase that changes INH to an active form in MTB, and its mutations are major causes of INH resistance by MTB (23, 24). Resistance to RIF was detected by using eight rpoB wild-type probes, and resistance to INH was detected by using wild-type and mutant (S315T) katG probes and wild-type and mutant (-15T) inhA promoter probes (mutations of INH-resistant MTB). Amplification and detection reagents, including activating MTB RIF and INH resistance amplification reagent A, reagent B, reagent C, and DNA polymerase, were gently vortexed and loaded for sample processing and initialized for real-time PCR. Reagents A and B contain a primer pair for identifying the 81 base pair extended RIF Resistance Determining Region (RRDR) of rpoB using eight labeled dye probes (6). Reagent C labeled probes to detect katG wild type sequence, katG S315T1 drug resistance mutation (usually associated with high-level INH resistance), inhA wild type sequence, and inhA C-15T mutation primarily associated with low-level INH resistance (25).

Data Collection and Analysis

We extracted the sociodemographic and clinical characteristics of the patients from the MTB laboratory logbook available at the national MTB laboratory. We transferred them to the worksheet developed for this purpose. Crude data from worksheets were entered into Statistical Package for Social Sciences (SPSS) version 23. After data cleaning, we calculated frequencies, proportions, and summary statistics to describe the study population's sociodemographic and clinical characteristics. The Abbott Real-Time PCR performance was calculated using culture as a gold standard. A two-by-two table was used to determine the performance characteristics of Abbott real-time PCR against the standard gold technique. Culture results were inserted as the gold standard (at the top row), and Abbott real-time MTB results were inserted in the first column (method under evaluation). Finally, performance characteristics such as sensitivity, specificity, and predictive values were calculated with a 95% confidence interval (CI).

We conducted bivariate analyses to calculate the odds ratio (OR) with 95% CI to assess the association between Abbott performance characteristics and clinical and specimen-related factors. For all statistical significance tests, the cutoff point was 0.05, and $p < 0.05$ was considered a statistically significant association with Abbott MTB performance.

Results

Sociodemographic and clinical characteristics

One hundred fifty (150) presumptive MTB patients were enrolled in the study. The majority were males (60.4%) aged 25 to 34 years (38.8%), mainly from the Addis Ababa city administration (66.7%). The mean age of the patients was 31.7 ± 12.3 standard deviation (SD) years. The study participants were

predominantly new MTB cases (55.1%). Approximately 18.4% of the study participants were HIV positive, and 15% were previously treated with second-line anti-MTB treatment. The laboratory findings also verified that 84.7% (127/150) and 61% (92/150) were smear and culture negative, respectively (Annex 1)

Diagnostic performance characteristics

Abbott real-time PCR's overall sensitivity and specificity were 89.7% (95% CI: 81.82–97.49%) and 92.4% (95% CI: 86.97–97.81%), respectively, for the detection of MTB in pulmonary specimens. However, for smear-negative pulmonary TB, the sensitivity of Abbott real-time PCR was 80.56% (95% CI: 67.63–93.48%). Of the total tested, 52/58 of the positives and 85/92 of the negatives were correctly identified by the method under evaluation (Abbott real-time PCR). The overall accuracy was 91.3% (137/150) (95% CI: 86.83–95.84%) for pulmonary specimens (Table 1 and Table 2).

Table 1
Performance comparison of Abbott real-time PCR against culture results (2X2 table) for the detection of MTB in pulmonary specimens, June 2019

	Culture +	Culture -	Total
Abbott RT—PCR +	52 (overall)	7	59
	29 (smear negative)	7	36
Abbott RT—PCR -	6	85	91
Total	58	92	150

Table 2
Performance of Abbott real-time PCR for the detection of MTB in pulmonary specimens, June 2019

Statistic	Value	95% CI
Sensitivity- Overall	89.70% (52/58)	81.82–97.49%
Sensitivity- Smear Negative	80.56% (29/36)	67.63–93.48%
Specificity	92.40% (85/92)	86.97–97.81%
Positive Predictive Value	88.14% (52/59)	79.88–96.39%
Negative Predictive Value	93.40% (85/91)	88.31–98.51%
Accuracy (TP + TN/total)	91.33% (137/150)	86.83–95.84%
TP: true positive, TN: true negative		

Drug Sensitivity Testing

Twenty-two positive samples were tested for RIF and INH drug resistance. The study data revealed 92.3% (95% CI: 63.9% – 99.8%) sensitivity in detecting resistance to both RIF and INH. Specificity values of

87.5% (95% CI: 47.3% – 99.7%) and 100% were recorded for INH and RIF, respectively (Table 3 and Table 4).

Table 3
Performance comparison of Abbott real-time PCR against culture results (2X2 table) for MTB RIF and INH drug resistance, Addis Ababa, June 2019

		Culture- Resistance Detected	Culture- Resistance Not Detected
Abbott	RIF-Resistance Detected	12 (100%)	0 (0%)
	RIF-Resistance Not Detected	1 (100%)	8 (0%)
	INH-Resistance Detected	12 (92.3%)	1 (7.7%)
	INH-Resistance Not Detected	1 (7.7%)	7 (87.5%)

Note: Not Detected/Below LOD: Target probe signals not detected from rpoB. KatG and inhA

Table 4
Performance of Abbott real-time PCR for the detection of RIF and INH resistance in pulmonary specimens, June 2019

Statistics	RIF resistance % (95% CI)	INH resistance % (95% CI)
Sensitivity	92.3% (64% – 99.8%)	92.3% (63.9% – 99.8%)
Specificity	100% (63% – 100%).	87.5% (47.3% – 99.7%)
Positive Predictive Value	100% (-)	92.3% (65.6% – 98.7%)
Negative Predictive Value	88.9% (54.9% – 98.1%)	87.5% (51.1% – 97.9%)
Accuracy	95.24% (76.2% – 99.9%)	90.5% (69.6% – 98.8%)

Bivariate Analysis of Abbott MTB Performance

Abbott's real-time PCR method did not show a statistically significant association with sex, age group, HIV status, TB classification, or previous TB treatment status.

Discussion

This study sought to assess the effectiveness of the Abbott real-time PCR technique in diagnosing MTB and drug resistance to the disease. The findings showed that the sensitivity of the Abbott real-time PCR technique for the diagnosis of smear-negative MTB aligned with the result of the study performed during method development (81%) for smear negatives and the overall sensitivity of 93% by using similar specimens collected from Russia, South Africa, Uganda, the United States of America (USA), and Vietnam

(13, 26). Compared to the performance of GeneXpert, it was the highest for the diagnosis of smear-negative MTB, and the overall performance was almost comparable (91.3%) (13, 27). This could be because the GeneXpert target gene is rpoB, whereas Abbott uses IS610 and PAB gene sequences (28, 29). The findings also showed comparable sensitivity with the Genotype MTB method (80.5–97%), which is more time-consuming than Abbott's real-time PCR (26, 29).

The sensitivity of the current finding is higher than that of a similar study performed in South Africa, 74.3% (26/35), of which 27 (77.1%) were HIV positive. However, in the current study, only 18.4% of the study participants were HIV positive. The former finding also confirmed that the overall performance was reduced for individuals with TB/HIV coinfection (30).

The diagnostic specificity of the Abbott real-time PCR technique for the diagnosis of MTB was 92.4%. The specificity of the method is within the range of other genotype MTB studies performed in different countries (28). In comparison to the culture method, the overall testing process to release a batch of 48 to 96 test results by using this method displayed the most significant reduction to an average of 6 to 8 hours compared to the culture method, which took four weeks to 70 days for the concentration method and up to 27 days for the MIGIT technique. The frequency of invalid results due to inhibition and instrument error was 2/152 (1.3%), similar to the study performed during method development, which was approximately 1% due to instrument sample processing optimization.

Of 22 specimens tested for first-line drugs (INH and RIF), two (9.1%) failed, and the other one had a lower detection limit, meaning that the drug's resistance status could not be determined. The overall concordance rate was 20/21 (95.2%), confirming Abbott's accuracy in real-time PCR MTB INH and RIF resistance testing. Abbott's real-time PCR diagnostic sensitivity for RIF and INH drug resistance testing was 92.3% (95% CI: 77.8, 100). The specificity of the method was 87.5% and 100% for INH and RIF, respectively. The current study's findings support previous studies with different specimen types ranging from 94–99.5% (24).

The findings of this study should be interpreted within the context of some limitations. Abbott's real-time PCR technique's performance in testing other types of specimens, including blood, CSF, stool, body fluids, and tissue, has not been determined, which may change the assay's performance. Clinical and other explanatory variables were collected from an incomplete secondary source, so other explanatory variables, such as cough duration, were not compared with Abbott real-time PCR performance characteristics. The drug resistance section is not adequately studied due to resources and sample shortages.

Conclusion

This study demonstrated a very good performance of the Abbott real-time PCR technique for the diagnosis of MTB using sputum specimens. The diagnostic sensitivity and specificity of the Abbott real-time PCR method for both identification of MTB and drug resistance almost align with the previous similar studies done during method development and how the technique is suitable for the clinical

diagnosis of MTB cases and drug resistance patterns of INH and RIF in the study setting. With this method and procedure, the results can be released within the same day, contributing to early identification, treatment, and control of MTB among suspected clients.

At the policy level, it is essential to consider the Abbott real-time PCR technique for diagnosing MTB and RIF and INH treatment monitoring in the Ethiopian MTB guidelines. Further research to explore the effect of explanatory variables such as age, HIV status, and MTB classification on the performance of the Abbott real-time PCR technique for the diagnosis of MTB is needed.

Abbreviations

AFB	Acid Fast Bacilli
CSF	Cerebrospinal fluid
DNA	Deoxyribose Nucleic Acid
EPHI	Ethiopian Public Health Institute
FM	Fluorescence Microscopy
HIV	Human Immunodeficiency Virus
IC	Internal Control
INH	Isoniazid
IR	Inactivation Reagent
IS6110	Insertion sequence 6110
MGIT	Mycobacterium Growth Indicator Tube
MTB	Mycobacterium tuberculosis
NaOH	Sodium Hydroxide
NALC	N-Acetyl-l-cysteine
PCR	Polymerase Chain Reaction
PAB	Protein Antigen B
RIF	Rifampicin
TB	Tuberculosis

ZN Ziehl-Neelsen

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics committee of Addis Ababa University School of Medical Laboratory Technology. The need for informed consent was waived by the Ethiopian Public Health Institute Institutional Review Board (EPHI IRB) since no human subjects were used for the study. Confidentiality of the results was assured by keeping the documents secured and the study subjects anonymous.

The authors alone are responsible for the views expressed in this editorial, which do not necessarily represent the views, decisions, and policies of the institutions with which they are affiliated.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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I declare that there is no financial conflict. However, the author requested a publication fee waiver, as the manuscript originated from a low-income country (Ethiopia).

Authors' contributions

MH, KZ, and AK conceived and coordinated the study. MH, KZ, AK, BR, GD, GTB, GG, SA, AY, and ET participated in the laboratory work and write-up of the manuscript. MH, AK, KKB, and OOO: data analyses and manuscript writing. All the authors have read and provided significant inputs into all manuscript drafts, agreed to be accountable for all aspects of the work, and approved the final draft of the manuscript for publication.

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