

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES



**EVALUATION OF GENEXPERT MTB/RIF ASSAY FOR DIAGNOSIS OF
PULMONARY TUBERCULOSIS AND DETECTION OF RIFAMPICIN RESISTANCE
IN SPUTUM SPECIMENS AT KHARAMARA HOSPITAL, JIGJIGA, EASTERN
ETHIOPIA**

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Evaluation of GeneXpert MTB/RIF Assay for Diagnosis of Pulmonary Tuberculosis and Detection of Rifampicin Resistance in Sputum Specimens at Kharamara Hospital, Jigjiga, Eastern Ethiopia

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DECLARATION

I the undersigned, declare that this is my original work and has never been presented for the degree in this or any other university and all the source materials used for this thesis have duly acknowledged.

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List of abbreviations

AFB	Acid fast bacilli
ART	Antiretroviral therapy
BCG	Bacillus Calmette Guérin
CFU	Colony Forming Units
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
DST	Drug susceptibility testing
ELISA	Enzyme linked immunosorbent assay
EPTB	Extra-pulmonary TB
FM	Fluorescent microscopy
HIV	Human immunodeficiency virus
INH	Isoniazid
LJ	Lowenstein-Jensen
LM	Light microscopy
MDR-TB	Multi-drug resistant-TB
MGIT	Mycobacteria Growth Indicator Tube
MTB	Mycobacterium tuberculosis

MTBC	Mycobacterium tuberculosis complexes
NPV	Negative predictive value
NTM	Non-tuberculous mycobacterium
PCR	Polymerase chain reaction
PPV	Positive predictive value
RIF	Rifampicin
SR	Sample treatment reagent
TB	Tuberculosis
WHO	World Health Organization

Abstract

Background: TB detection rate remains critical stumbling block for TB control. Though conventional methods for TB detection exist; GeneXpert MTB/RIF assay represents a paradigm shift in the diagnosis of TB and MDR-TB by simultaneous detection of *M.tuberculosis* and rifampicin resistance bacilli. However information regarding the performance characteristics of Xpert MTB/RIF assay is scarce in Ethiopia.

Objective: To evaluate the performance of Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis and detection of Rifampicin resistance in sputum specimens at Karamara hospital, Jigjiga.

Materials and methods: A total of 227 paired expectorated sputum samples from patients with signs and symptoms of suggestive of tuberculosis at Karamara hospital during February 2014 to May 2014 were recruited by consecutive sampling method. Sputum sample was tested directly by Ziehl-Neelsen staining and Xpert MTB/RIF assay without NALC-NaOH decontamination and sputum samples were cultured for isolation and drug sensitivity testing of TB bacilli. Diagnostic performance of Xpert MTB/RIF assay and AFB smear microscopy was calculated using sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios against culture as the gold standard. Results with contaminated cultures (for both LJ and MGIT) and failed Xpert MTB/RIF were excluded from analysis.

Results: Overall 25.5% (58/227) samples were positive for MTBC by MGIT and/or LJ media of which 36.2 % (21/58) and 65.5% (35/58) were positive by AFB smear microscopy and Xpert MTB/RIF respectively. The sensitivity, specificity, positive and negative predictive value of Xpert MTB/RIF assay were 65.5% (95% CI: 53.3-77.7%), 96.3% (95% CI: 93.4-99.2%), 86.4% (95% CI: 76.2-96.5%), and 88.6% (95% CI: 83.9-93.3%) respectively. Eighteen (31.0%, 18/58) more cases, which were smear microscopy negative, were detected by Xpert MTB/RIF assay

Conclusion: Xpert MTB/RIF assay demonstrated high sensitivity in detecting MTBC in sputum specimens compared with conventional AFB smear microscopy. However, it demonstrated suboptimal sensitivity in smear negative patients compared to culture test.

Keywords: Xpert MTB/RIF assay, NPV, PPV, Sensitivity, Specificity

1. Introduction

Tuberculosis (TB) is a common and potentially serious infectious disease caused by various strains of mycobacterium, usually *Mycobacterium tuberculosis*. TB most commonly affects the lungs (pulmonary TB), but may affect any organ outside of the lungs (extrapulmonary TB), such as the brain or bones. Signs and symptoms of pulmonary TB include cough for at least two weeks, fever, chills, night sweats, weight loss, haemoptysis (coughing up blood), and fatigue [1]. Early diagnosis of active TB and detection of multi-drug resistant (MDR) strains are essential to interrupt transmission. Detecting more cases, detecting them early and rapidly identifying drug resistance are essential for improving health and avoiding transmission in the community [2].

The conventional methods for TB detection used in the laboratory require microscopy, culture, and drug susceptibility testing (DST), which is a laborious as well as time consuming process [3]. The past decade has seen unprecedented growth in the TB diagnostic pipeline and accelerated efforts to establish the necessary laboratory infrastructure. Although the World Health Organization (WHO) recommends, the latest generation liquid culture diagnostics and molecular line probe assays for rapid detection of MDR-TB; it was not yet solved the diagnostic dilemma in most resource limited settings, largely due to the need for expensive laboratory infrastructure, extensive bio-safety precautions and specialized staff [4].

In 2010 a new rapid test that may overcome many of the current operational difficulties was recommended for use by WHO. The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is an automated, real time nucleic acid amplification technology run on the multi disease platform GeneXpert (Cepheid). The Xpert MTB/RIF assay represents a paradigm shift in the diagnosis of TB and MDR-TB by simultaneously detecting *M.tuberculosis* and rifampicin resistance conferring mutations in a closed system suitable for use outside conventional laboratory settings in less than 2 h, directly from sputum samples. The test is currently recommended as first line diagnostic test in endemic countries when rapid results are crucial for TB diagnosis in HIV infected patients or for appropriate management of MDR-TB cases [5].

1.2 Statement of the problem

TB remains a major health problem among millions of people each year. It is the leading cause of morbidity and mortality worldwide. According to WHO, there were almost 9 million new cases in 2011 and 1.4 million TB deaths of which 430000 deaths from TB among HIV positive people. The absolute number of TB cases occurring each year is currently greater than at any time in history. Over 90% of TB cases develop among people living in low and middle income countries [1, 6].

Ethiopia is one of the 22 high TB burden countries. The national population based TB prevalence survey conducted in 2010/11 revealed that the prevalence of smear positive TB among adults and all age group was found to be 108 and 63 per 100,000 populations, respectively. The prevalence of bacteriological confirmed TB was found to be 156/100,000 populations and the prevalence of all forms of TB in Ethiopia is estimated to be 240/100,000 populations. There were an estimated 15,000 deaths (18 per 100,000 populations) due to TB, excluding HIV related deaths [6, 7].

The case detection rate worldwide in 2011 was 69% and the lack of rapid and accurate diagnostics remains a critical stumbling block, which is undermining progress towards the 2015 millennium development goals for TB control. Despite microscopy being the diagnostic test most widely used worldwide, only 56% of TB cases that were notified in 2011 were sputum smear positive [8]. Increasing drug resistance also poses a grave threat to TB control. An estimated 440,000 cases of MDR-TB occur worldwide each year and yet only 59,549 cases (approximately 7%) were diagnosed and notified to the WHO in 2011. Such low rates of case ascertainment again reflect the critical deficiency in diagnostic laboratory capacity [9].

The main reasons for these gaps are inadequate diagnostic capacity and an over reliance on chest radiography and sputum smear microscopy as diagnostic tools. The conventional detection techniques based on microscopic examination of acid fast stained specimens are still highly used for diagnostic purpose especially in TB endemic countries. Standard light microscopy (LM) and fluorescent microscopy (FM) are common methods. However, they fail to provide the required sensitivity and specificity and are unable to differentiate between *M. tuberculosis* and non-tuberculous mycobacteria (NTM). Patients with HIV associated TB, those with sputum smear

negative and extra-pulmonary disease, and MDR-TB patients are particularly affected by the failure of microscopy as a primary diagnostic tool [3, 10].

Histological examination has also limitations as it cannot differentiate between TB and non-TB infections caused by other related diseases (e.g. NTM) infections except for the demonstration of stained tubercle bacilli [11]. The conventional culture and DST is complex, expensive, slow and technically demanding. It takes 4-8 weeks to get those results and also requires trained technicians. The long delay required to obtain results has devastating consequences for patients who go undiagnosed or are diagnosed too late [10]. The Mantoux test is widely employed worldwide but the false positive results are observed due to the previous *Bacillus Calmette Guérin* (BCG) immunization or exposure to atypical mycobacteria and false negative results also occur in aged individuals or immune-suppressed individuals. The reports on diagnostic tests such as enzyme linked immunosorbent assay (ELISA) and slide agglutination are available; however, the specificities and sensitivities of these tests are variable besides a number of manual steps are required to perform the tests [12].

The development of molecular technique, such as real time PCR assays, has improved the speeds, sensitivities, and specificities due to its short turnaround time and automation of the procedure. Xpert MTB/RIF assay is a new rapid molecular test that overcomes many of the current operational difficulties [5]. Detecting more cases, detecting them early and rapidly identifying drug resistance are essential for improving health and avoiding transmission in the community. This requires universal access and early detection using contemporary tools and innovative strategies [2].

1.3. Significance of the study

The TB Xpert Project will provide approximately 1.4 million Xpert MTB/RIF test cartridges and over 220 GeneXpert instruments for the rapid detection of TB and rifampicin resistance in 21 recipient countries in 2013 – 2015 [13]. Ethiopia is among one of the 21 recipient countries to implement the TBXpert project. So far twenty three GeneXpert instrument are imported and distributed to Regional Health Bureau [14]. However, no performance related data from highly suspect of TB patient at point of treatment settings in Ethiopia are available to inform recommendations to use the assay for testing sputum samples when investigating suspected pulmonary TB. Even if WHO has recommended this instrument for use, it has to pass the validation study in the Ethiopian context.

Therefore the present study is aimed to give information on the performance of Xpert MTB/RIF assay on patients with suspected of pulmonary TB on row sputum samples and its agreement with the current TB diagnostic test in the country. This study will also have implication for policy makers in scaling up implementation of Xpert MTB/RIF assay in the national algorithms. Governmental and non-governmental organizations working in the area of TB control may utilize the information generated to accelerate and improve detection and treatment rates for TB and MDR-TB. The data will be indispensable for local government and non-governmental organizations, researchers and students.

2. Literature review

Different studies were carried out to evaluate the effectiveness of Xpert MTB/RIF assay for detection of MTBC and RIF resistance in specimens. Studies on performance of Xpert MTB/RIF assay are diverse. Mostly the performance of Xpert MTB/RIF assay has been evaluated against existing reference standards, microscopy and culture, for TB testing and phenotypic DST for rifampicin resistance testing.

2.1. Performance of Xpert MTB/RIF assay

In USA, 79 MTB isolates and 89 NTM isolates were studied. The Xpert MTB/RIF assay correctly identified all 79 MTB isolates and correctly excluded all 89 NTM isolates. Rifampicin resistance was correctly identified in all 37 resistant isolates and in none of the 42 susceptible isolates. Dynamic range was assessed by adding 102 to 107 Colony forming units (CFU) of MTB into MTB negative sputum samples. The assay showed a log-linear relationship between cycle threshold and input CFU over the entire concentration range. Resistance detection in the presence of different mixtures of rifampicin resistant and susceptible DNA was assessed. Resistance detection was dependent on the particular mutation and required between 65% and 100% mutant DNA to be present in the sample for 95% certainty of resistance detection [15].

Another study in USA, the bio-aerosols generated by the Xpert MTB/RIF assay was compared to AFB microscope slide smear preparation. The Xpert MTB/RIF assay sample treatment reagent (SR) was also studied for its sterilizing capacity, stability, and effect on assay sensitivity after prolonged treatment. Neither the sample preparation steps for the Xpert MTB/RIF assay nor its automated processing produced any culturable bio-aerosols. In testing of SR sterilizing capacity, clinical sputum samples from strongly smear positive TB patients treated with SR at a 2:1 ratio eliminated MTB growth in all but 1/39 or 3/45 samples cultured on solid or liquid medium, respectively. These few un-sterilized samples had a mean 13.1 day delay in the time to culture positive. SR treatment at a 3:1 ratio eliminated growth in all samples. These results suggest that bench-top use of the Xpert MTB/RIF assay limits infection risk to the user [16].

In South Africa, a TB prevalence survey conducted in gold mining companies. 6,893 participants provided a sputum specimen. 187/6893 (2.7%) were positive for MTB in culture, 144/6893

(2.1%) were positive for MTB by Xpert MTB/RIF, and 91/6893 (1.3%) were positive for AFB by microscopy. Sensitivity, specificity, PPV and NPV for detection of MTB by Xpert MTB/RIF were 62.6%, 99.6%, 81.3%, and 98.9; agreement between Xpert MTB/RIF and culture was 98.5%. Sensitivity of microscopy was 17.6%. When individuals with a history of TB treatment were excluded from the analysis, Xpert MTB/RIF specificity was 99.8% and PPV was 90.6% for detection of MTB [17].

In New Zealand, Xpert MTB/RIF assay and detection of false-positive rifampicin resistance in *M.tuberculosis* were evaluated. A total of 169 specimens were analyzed. The overall sensitivity and specificity of the Xpert MTB/RIF assay for the detection of MTB were 100% (141/141) and 100% (28/28) respectively. The Xpert MTB/RIF assay detected rifampicin resistance in 13/169 (7.7%) specimens. However, using standard phenotypic methods, rifampicin resistance was detected in only 7/13 (54%) isolates. In 2 of the remaining 6 isolates, amplification and sequencing of the *rpoB* gene revealed mutations associated with increased but low level rifampicin resistance. In this study, the Authors found the MTB/RIF assay highly sensitive and specific for the detection of MTB, when used for both smear-positive pulmonary and extra-pulmonary specimens as well as for isolates in liquid culture media. However, the Authors found that the assay was less reliable for the detection of rifampicin resistance, producing false-positive results in 4/13 (31%) specimens [18].

In South Africa a study was conducted in primary health care. Consecutive adults with suspected TB attending a primary health care clinic were prospectively enrolled and evaluated for TB, including assessment for sputum smear, chest X-ray, clinical evaluation, and HIV testing. A single sputum sample underwent routine decontamination, AFB smear microscopy, liquid culture, and phenotypic DST. Residual sample was batched for molecular testing. Compared to liquid culture, sensitivity of all the test methodologies, determined with a limited and potentially underpowered sample size ($n = 177$), were 59% for smear microscopy, 76% for MTBDRplus, 76% for LCTB, and 86% for Xpert MTB/RIF with specificities all >97%. Among HIV positive individuals, sensitivity of the Xpert MTB/RIF assay was 84%, while the other molecular tests had sensitivity reduced by 6%. TB detection among smear negative, culture positive samples was 28% (5/18) for MTBDRplus, 22% (4/18) for LCTB, and 61% (11/18) for Xpert MTB/RIF. A

few rifampicin resistant cases were detected using phenotypic DST. Xpert MTB/RIF detected four of these five cases and two additional phenotypically susceptible cases [19].

The accuracy of the Xpert MTB/RIF assay for diagnosing TB and drug resistance was assessed in comparison with other tests, including fluorescence smear microscopy and MGIT and DST. Of 515 patients enrolled, 468 patients produced at least one sputum sample, yielding complete sets of results from 839 samples. MTB was cultured from 81 patients. The overall sensitivity of the Xpert MTB/RIF assay for culture TB was 73.3% (specificity, 99.2%) compared to 28.0% (specificity, 100%) using smear microscopy. All smear positive culture positive disease was detected by Xpert MTB/RIF from a single sample (sensitivity, 100%), whereas sensitivity for smear negative culture positive TB was 43.4% from one sputum sample and 62.3% from two samples. Xpert MTB/RIF correctly identified rifampicin resistance in all four cases of MDR-TB but incorrectly identified resistance in three other patients whose disease was confirmed to be drug sensitive by gene sequencing (specificity, 94.1%; PPV, 57%) [20].

A multi-central study was conducted in Peru, Azerbaijan, South Africa, and India to assess the performance of Xpert MTB/RIF 1730 patients with suspected pulmonary tuberculosis. Among culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%). The test was specific in 604 of 609 patients without TB (99.2%). Among patients with smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity by 12.6 % and a third by 5.1 percentage points, to a total of 90.2%. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant bacteria and 504 of 514 (98.1%) with rifampin-sensitive bacteria. Sequencing resolved all but two cases in favor of the MTB/RIF assay [21].

In similar study, 6648 individuals were prospectively enrolled in South Africa, Peru, India, Azerbaijan, Philippines and Uganda, comparing Xpert MTB/RIF with microscopy in decentralized microscopy centers, and with culture and phenotypic DST in centralized laboratories. Xpert MTB/RIF detected 90.3% (933 out of 1033) of the culture-confirmed TB cases, compared with 67.1% (699 out of 1041) using microscopy. In sputum smear-negative,

culture-positive TB patients Xpert MTB/RIF test sensitivity was 76.9% (296 out of 385) and specificity was 99.0% (2846 out of 2876). Sensitivity for rifampicin resistance was 94.4% (236 out of 250) and specificity was 98.3% (796 out of 810) [22].

2.2. Comparative study with other molecular assays

In central hospital laboratory, South Africa Xpert MTB/RIF was compared with Genotype MTBDRplus (version 2) on smear positive and negative patient specimens. 282 consecutive specimens were tested by the two new molecular assays and routine diagnostics. Both assays showed similar diagnostic performance characteristics. Sensitivity of the Genotype MTBDRplus (v2.0) and Xpert MTB/RIF assay for the detection of culture-negative MTB was 73.1% and 71.2% respectively; specificity for both assays was 100%. Both assays diagnosed MTB in 57 – 58% of smear negative cases suggesting that the performances depend on bacillary load. Detection of MTB in culture negative specimens confirmed that molecular-based assays should not be used for treatment monitoring. Sensitivity and specificity for rifampicin for resistance detection was 100% in both assays. However, Genotype MTBDRplus (v2.0) assay provided additional information on isoniazid (INH) susceptibility [23].

In Spain, 340 samples were processed using Xpert MTB/RIF and Cobas TaqMan MTB. Sensitivity and specificity of the Xpert assay were 95% and 100%, respectively, compared to 78% and 98% for the Cobas assay. Both molecular techniques represent an important contribution to the detection of MTB, since they can provide results in a matter of hours, whereas the reference culture method takes days. Real-time PCR techniques afford greater sensitivity and specificity and a much reduced response time, as well as enabling visualization of amplification curves. This study point out that one limitation of molecular techniques is that, they cannot distinguish between viable and nonviable microorganisms. For that reason, although these assays are semi-quantitative, they can't be used for monitoring patient progress or treatment efficacy [24].

Van Z *et al* compared the turnaround-time, detection threshold, dynamic range, reproducibility, relative discriminative ability, of 4 mycobacterial load determination techniques: MGIT-960, [3H]-uracil incorporation assays, luciferase-reporter construct bioluminescence, and Xpert MTB/RIF using serial dilutions of *M.bovis* and MTB H37RV. Mycobacterial CFU using 7H10-

Middlebrook solid media served as the reference standard. All 4 assays correlated well with the reference standard, however, bioluminescence and uracil assays had a detection threshold $\geq 1 \times 10^3$ organisms. By contrast, MGIT-960 liquid culture, although only providing results in days had the lowest detection threshold (< 10 organisms), the greatest discriminative ability (1 vs. 10 organisms), and the best reproducibility. Xpert MTB/RIF correlated well with mycobacterial load, had a rapid turn-around-time (< 2 hours) but had a detection limit of ~ 100 organisms [25].

A comparative study was conducted to compare the sensitivities of the Xpert MTB/RIF test and an in-house IS6110 based real-time PCR using TaqMan probes (IS6110-TaqMan assay) for the detection of MTBC DNA were compared by use of 117 clinical specimens. Both methods were highly specific and exhibited excellent sensitivity (100%) with smear-positive specimens. The sensitivity of the Xpert MTB/RIF test with smear-negative specimens was more reduced than that of the IS6110-TaqMan assay (48 *versus* 69%). Finally, the sensitivities of the Xpert MTB/RIF test and the IS6110-TaqMan assay were 79% and 84%, respectively. The Xpert MTB/RIF test correctly detected the rifampin resistance in smear-positive specimens but not in the one smear-negative specimen. The Xpert MTB/RIF test is sensitive as the IS6110-TaqMan assay with respiratory specimens but less sensitive with paucibacillary specimens, such as smear-negative non-respiratory specimens [26].

2.3. Xpert MTB/RIF in diagnosis of TB among HIV patients

Maximum benefit from the Xpert MTB/RIF assay is obtained by targeted testing of individuals considered at risk of drug-resistant TB and smear-negative TB, such as those co-infected with HIV. Risk groups for drug-resistant TB include all re-treatment categories (failure, relapse and default cases), as well as those with HIV infection [1].

Published studies have shown significant increases in TB case detection when Xpert MTB/RIF is used as an add-on or replacement test for microscopy, especially in settings with high HIV prevalence [5]. In Santiago, Chile, a cross sectional study was conducted in five hospitals to assess the accuracy of rapid testing for pulmonary TB detection in routine care in HIV infected patients. 166 HIV positive subjects with suspected pulmonary TB were included in the study. The prevalence of TB was 8.1%. Diagnostic sensitivity increased from 66.7% for AFB smear to 91.7% for Xpert MTB/RIF assay, with comparable specificity at 98.6% and 99.3%. Xpert

MTB/RIF assay allowed early detection of rifampicin resistance in 16.6% of cases, with rapid adjustment to MDR treatment. Xpert MTB/RIF provided earlier TB diagnosis in 25% more cases than AFB smear alone [27].

In Zambia the performances of Xpert MTB/RIF assay for diagnosis of TB in HIV infected patients were compared with conventional technique. Culture confirmed TB was found in 22.8% patients. The specificity of the Xpert MTB/RIF was 95.0% and sensitivity 86.1%. In sputum smear-negative, culture positive the assay was 74.7% sensitive identifying an additional 71 TB cases compared to smear microscopy. The sensitivity and specificity of the Xpert MTB/RIF assay to detect culture confirmed rifampicin resistant TB were 81.3 and 97.5% respectively [28].

In Peru, detection of TB by Xpert MTB/RIF was assessed in HIV patient. 131 patients, who had median CD4 cell count 154.5 cells/mm³ and 45 had TB, were included. For TB detection among HIV patients, sensitivity of Xpert MTB/RIF was 97.8% (44/45); specificity was 97.7% (84/86); PPV was 95.7% (44/46); NPV was 98.8%. Xpert MTB/RIF detected 13/14 smear negative TB cases, outperforming smear microscopy 97.8% vs. 68.9%. For rifampicin resistance detection, sensitivity of Xpert MTB/RIF was 100%; specificity was 91.0%; PPV was 66.7%; NPV was 100%. In HIV patients with a high clinical suspicion of TB, Xpert MTB/RIF performed well for TB diagnosis and outperformed smear microscopy [29].

Another study in Tanzania assessed the accuracy of the Xpert MTB/RIF assay including HIV positive study participant. Sputum samples from 292 consecutively enrolled adults were included in the study. The diagnostic performance of Xpert MTB/RIF assay was compared to standard sputum smear microscopy and culture. Confirmed MTB in a positive culture was used as a reference standard for TB diagnosis. Xpert MTB/RIF assay achieved 88.4% sensitivity among patients with a positive culture and 99% specificity in patients who had no TB. HIV status did not affect test performance in 172 HIV-infected patients (58.9% of all participants). Seven additional cases (9.1% of 77) were detected by Xpert MTB/RIF assay among the group of patients with clinical TB who was culture negative. Within 45 sputum samples which grew NTM the assay's specificity was 97.8% [30].

Balcha TT *et al* were conducted a study to evaluate diagnostic yield of Xpert MTB/RIF in intensified TB case finding in HIV positive adults in Ethiopian Health Centers. A total of 812

study participant recruited in 5 Health Center. TB was diagnosed and bacteriological confirmed in 16.9% participants. Among bacteriological confirmed cases, 70.1% were Xpert positive. Xpert MTB/RIF increased the TB detection rate by 47.4% compared with smear microscopy. The overall sensitivity of Xpert MTB/RIF was 66.4%. While Xpert MTB/RIF was 46.7% sensitive among patients with CD4 cell counts >200 cells/mm³ and it was 82.9% in those with CD4 cell counts <100 cells/mm³. Compared with Xpert positive TB patients, Xpert negative cases had less advanced HIV and TB disease characteristics [31].

3. Objective of the study

3.1. General objective

To evaluate the performance of Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis and detection of Rifampicin resistance in sputum specimens at Karamara Hospital, Jigjiga, Ethiopia

3.2. Specific objectives

- To determine sensitivity, specificity, positive and negative predictive values of Xpert MTB/RIF assay.
- To calculate % agreement between Xpert MTB/RIF assay and the gold standard TB culture.
- To determine performance of Xpert assay in detection of Rifampicin resistance.

4. Materials & Methods

4.1. Study Design

A cross-sectional study was conducted on patients with suspected of pulmonary TB to evaluate the performance of Xpert MTB/RIF assay for detection of MTBC at Karamara hospital, Jigjiga, Ethiopia.

4.2. Study Site

The study was conducted at Karamara hospital, Jigjiga town. Jigjiga is the capital of Somali region, which is located 631 km away from Addis Ababa, capital city of Ethiopia. The town is located in eastern part of Ethiopia and 60km west of the border with Somalia. Based on figures from the Central Statistical Agency in 2005, Jijiga has an estimated total population of 98,076 of whom 50,355 are men and 47,721 are women [32]. Karamara hospital is one of the largest hospitals in Somali region. It lies on about 6400 square meter surface area. Karamara hospital provides health care services for outpatient, in-patient and comprehensive services including TB diagnosis services for ten kebeles with more than 200, 000 peoples on catchments area of 4800 Km². It is also used as a referral hospital for private clinics and governmental health centers of neighboring zones and woredas [33].

4.3. Study Period

The study was conducted from January 2014 to May 2014 GC.

4.4.1. Source Population

All patients who were visiting Karamara hospital during the study period were a source population.

4.4.2. Study Population

Patients who were suspected of having pulmonary tuberculosis and provide sputum sample were the study population.

4.5. Sampling Procedure and Sample Size

4.5.1. Sampling Procedure

Non-probability, consecutive sampling technique was used. Two morning expectorated sputum samples were collected on the same day from enrolled patients. The minimum acceptable volume of sputum was 2ml in each collection tube. One sample was tested directly by Ziehl-Neelsen staining microscopic examination and the Xpert assay and the other sputum was used for TB culture. Specimens for TB culture were stored at -20°C after collection. The sputa were placed on an ice pack in a cool box and transported to ICL TB laboratory within Five days of collection and analyzed.

4.5.2. Sample Size Determination

The sample size required for this study was calculated using Buderer's formula for estimating sample sizes for evaluating sensitivity and specificity of diagnostics test [34]. To calculate the sample size, 95% sensitivity and specificity with absolute precision of less than 5% at 95% confidence interval (CI) is used. Prevalence of pulmonary tuberculosis of 30% is also used [7]. With this given data, 243 study participants were supposed to be enrolled in the study. However, only 227 patients who fulfill the inclusion criterion were available during the study paired. Sample size at the required absolute precision level for sensitivity and specificity was calculated by:

$$\text{Sample size (n) based on sensitivity} = \frac{Z_{1-\alpha/2}^2 \times S_N \times (1 - S_N)}{L^2 \times \text{Prevalence}}$$

$$\text{Sample size (n) based on specificity} = \frac{Z_{1-\alpha/2}^2 \times S_P \times (1 - S_P)}{L^2 \times (1 - \text{Prevalence})}$$

Where n = required sample size,

S_N = anticipated sensitivity,

S_P = anticipated specificity,

α = size of the critical region ($1 - \alpha$ is the confidence level),

$z_{1-\alpha/2}$ = standard normal deviate corresponding to the specified size of the critical region (α), and

L = absolute precision desired on either side (half-width of the confidence interval) of sensitivity or specificity.

4.6. Inclusion and Exclusion Criteria

Inclusion criteria:

Patients with age of ≥ 18 (male or female), willing to participate and comply with the instructions of the study, and who fulfill at least one of the listed criteria were included in the study:

- Patients who present with signs and symptoms suggestive of tuberculosis and/or who have suggestive of Tb chest x-ray
- Had a prior history of tuberculosis or MDR TB suspect
- Patients who were produce a sputum sample

Exclusion criteria:

- Patients who were receiving tuberculosis treatment

4.7. Study Variables

4.7.1. Dependent Variable

Test status: Sensitivity, specificity, positive and negative predictive values

4.7.2. Independent Variables

- AFB smear result
- AFB smear grading
- Time to test positivity in liquid culture

4.8. Measurement and Data Collection

Data collection was conducted after having signed informed consent of the study participants. Demographic data were collected from TB registration book.

4.9. Laboratory Methods

Ziehl-Neelsen direct AFB smear, Xpert MTB/RIF assay, solid (LJ) and liquid (MGIT 960) medium for TB culture, were included. Xpert assay and AFB smear was performed at Karamara hospital from fresh sputum sample and TB culture was performed at ICL.

4.9.1. Xpert MTB/RIF assay

The Xpert MTB/RIF assay consists of a single use multichambered plastic cartridge preloaded with the liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction, and heminested real-time PCR. Sputum samples or decontaminated sputum pellets are treated with NaOH and isopropanol-containing sample reagent (SR). The SR is added at a 2:1 ratio to the sputum sample or sputum pellet and incubated for 15 min at room temperature. The treated sample is transferred into the cartridge, the cartridge is loaded into the GeneXpert instrument, and an automatic process completes the remaining assay steps. The assay cartridge also contains lyophilized *Bacillus globigii* spores which serve as an internal sample processing and PCR control. The spores are automatically resuspended and processed during the sample processing step, and the resulting *B. globigii* DNA is amplified during the PCR step. The standard user interface indicates the presence or absence of *M. tuberculosis*, the presence or absence of RIF resistance, and a semi quantitative estimate of *M. tuberculosis* concentration (high, medium, low, and very low). Assays that are negative for *M. tuberculosis* and also negative for the *B. globigii* internal control are reported as invalid [35].

The PCR assay amplifies a 192 bp segment of the *M. tuberculosis rpoB* gene in a heminested real-time PCR. The internal control heminested *B. globigii* assay is multiplexed with the *M. tuberculosis* assay. *M. tuberculosis* is detected using five overlapping molecular beacon probes (probes A to E) that are complementary to the entire 81-bp RIF resistance determining core region of the wild type *rpoB* gene. Mutations in the *rpoB* gene target inhibit hybridization of one or more of the *rpoB* specific molecular beacons, reducing or eliminating the signal from the corresponding probes [35].

4.9.2. Mycobacterial Culture

The sputa were collected in a sterile container (falcon tube) which placed on an ice pack in a cool box, sent to ICL and processed within the same day of sample arrival. Digestion-Decontamination of sputum by N-acetyl L-cysteine (NALC) Sodium Hydroxide method was performed. LJ and MGIT 960 culture media were inoculated and incubated for 6 weeks on MGIT and 8 weeks on LJ culture [36].

A culture was considered positive if MTB growth was confirmed on either LJ or MGIT media. A culture was considered negative if no growth was confirmed on both LJ and MGIT media or if one culture result was negative and the other is contaminated. A culture was considered contaminated if both LJ and MGIT demonstrated contamination. The positive culture was confirmed to contain *M.tuberculosis* complex by using Capilia Tb antigen test. Drug susceptibility testing was performed using MGIT SIRE.

4.9.2.1 Principle of MGIT 960

The MGIT consists of liquid broth medium that is known to yield better recovery and faster growth of mycobacteria. The MGIT contains 7.0 ml of modified Middlebrook 7H9 broth base. This medium is terminally sterilized by autoclaving. An enrichment, MGIT OADC (Oleic acid, Albumin, Dextrose and Catalase) or MGIT 960 Growth Supplement, is added to make the medium complete. This Growth Supplement is essential for growth of many mycobacteria, especially those belonging to *M.tuberculosis* complex. Addition of the MGIT PANTA is necessary to suppress contamination.

In addition to Middlebrook 7H9 liquid media, the MGIT tube contains an oxygen-quenched fluorochrome, tris 4, 7-diphenyl-1, 10-phenonthroline ruthenium chloride pentahydrate, embedded in silicone at the bottom of the tube. During bacterial growth within the tube, the free oxygen is utilized and is replaced with carbon dioxide with depletion of free oxygen; the fluorochrome is no longer inhibited, resulting in fluorescence within the MGIT tube when visualized under UV light. The intensity of fluorescence is directly proportional to the extent of oxygen depletion [37].

4.10. Quality Assurance

Morning sputum sample and data collection was conducted by trained laboratory personnel. The sputa were collected in a sterile container (falcon tube) which placed on an ice pack in a cool box, during sample transportation to the laboratory. All laboratory tests were performed by well trained laboratory personnel. Standard operational procedures of the host laboratory were used to ensure the reliability and validity of test result. To avoid subjective interpretation of test results the laboratory personnel processing the sputum samples for TB culture and Xpert MTB/RIF was blinded to the results of the other test.

4.11. Statistical Analysis

The results of Xpert assay was compared with smear microscope and culture for the presence of AFB and MTBC respectively. The data collected from TB registration book were entered to excel spreadsheet and transported to and analyzed by SPSS version 20.0. Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios with their 95% CI for Xpert MTB/RIF assay and AFB smear was calculated using sputum culture as the gold standard. Chi-square test was used to check the association between test status and independent variables. P values less than 0.05 was considered as significant.

4.12. Ethical Considerations

Ethical clearance (Protocol number DRERC062/13/MLS) was collected from research and ethics review committee of the department of medical laboratory sciences, school of allied health sciences, college of health sciences; Addis Ababa University. Additional ethical clearance paper was also collected from Jigjiga health bureau. Then the participants after reading the consent form, and when agree upon, and sign, involved on the study. Concerning the confidentiality of the result, since all clients who were participated in the study had unique code number confidentiality was kept well throughout the study. The results of laboratory findings were provided to the study participants and those in need of medical attention were communicated to respective physicians and laboratory personal.

5. Results

5.1. Demographic and diagnostic characteristics

In the current study 227 paired sputum specimens, ordered for routine AFB smear microscopy in TB clinic of Karamara hospital, Jigjiga, Ethiopia were included. Among the sputum specimens, 63.0% (143/227) and 37.0% (84/227) were obtained from male and female study participants respectively resulting male to female ratio of 1.7 to 1. The age of the study participants ranged from 18 to 82 years with median age of 35 years (Table 1).

A pair of spontaneously expectorated morning sputa specimen were concurrently collected from each patient on the same day. One arbitrarily selected sample was submitted for MGIT 960, LJ and phenotypic DST. The second fresh sputum sample was analyzed for Xpert MTB/RIF and direct AFB smear testing. Smear grading was performed according to the WHO/International Union Against Tuberculosis and Lung Disease method [38]. A confirmed positive culture of MTBC was used as a reference standard. All 227 study participants had cultures (both LJ and MGIT), AFB smear and Xpert MTB/RIF tests. Since culture was used as a reference for the study, results with contaminated cultures (for both LJ and MGIT) were excluded. Results with failed Xpert MTB/RIF results were also excluded.

Table 5.1: Diagnostic characteristics of study population by sex at Karamara hospital, Jigjiga, Ethiopia (n=227)

Microbiological classification						
Sex	Number (n)	Percent (%)	Smear (+)	Smear (-)	Contaminated Samples	
			Culture (+)	Culture (+)		
			Male	143	63.0%	14(6.2%)
Female	84	37.0%	7(3.1%)	14(6.6%)	62(27.3%)	1(0.4%)
Total	227	100.00%	21(9.3%)	37(16.3%)	166(73.1%)	3(1.3%)

Overall, 224 sputa had an interpretable result for MGIT and/or LJ. Three sputa with a MGIT and LJ final result of “contaminated” were excluded from this analysis. Out of 224 specimens, 58 (25.9%) were positive for MTBC by MGIT and/or LJ media. When the specimen stratified by

AFB smear microscopy status; 9.3 % (21/227) had smear-positive, culture-positive TB, whereas 16.3% (37/227) had smear-negative, culture-positive TB (Table 5.1). The median time for *M.tuberculosis* liquid culture positivity was 15 days (range 4–27), 10 days for smear positive and 19 days for smear-negative cases ($P<0.05$). Compared with culture, the sensitivity, specificity, PPV, and NPV for AFB smear microscopy examination was 36.2% (95% CI: 23.8%-48.6%), 99.4% (95% CI: 98.2-100%), 95.5% (95% CI: 86.8-100%), and 80.4% (95% CI: 74.5-85.5%) respectively.

5.2 Performance of Xpert MTB/RIF assay

A total of 227 sputum samples were run in Xpert MTB/RIF assay. Out of 227, 98.2 % (223/227) samples gave an interpretable Xpert MTB/RIF assay result. Four samples (1.8%) with an Xpert final result of “Invalid” were excluded from the analysis, even if 1 sample was culture positive and 3 were cultures negative. From the valid Xpert MTB/RIF results; 19.4% (44/227) were positive and 78.8% (179/227) were negative. Twenty isolates (8.8%) of MTBC were not detected by Xpert MTB/RIF assay. Six false positive (2.6%) Xpert MTB/RIF results were recorded in the study (Table 5.2).

Table 5.2: Xpert MTB/RIF assay and culture results

Xpert MTB/RIF	Culture result			
	MTB	Negative	Contaminated	Total
Positive	38(16.7%)	6(2.6%)	0	44(19.4%)
Negative	20(8.8%)	156(68.7%)	3(1.3%)	179(78.9)
Invalid	1(0.4%)	3(1.3%)	0	4(1.8%)
Total	59(25.9%)	165(72.6%)	3(1.3%)	227(100%)

Eighteen (7.9%) AFB smear negative samples were Xpert MTB/RIF and culture positive. When the specimens stratified by AFB smear status, Xpert MTB/RIF assay has a sensitivity of 95.2% (95% CI: 86.1-100%) for smear positive-culture positive specimens and sensitivity of 48.6%

(95% CI: 32.5-64.8%), specificity of 96.9 % (95%CI: 94.2 – 99.6%), PPV of 78.3 % (95% CI: 61.4-95.1) and NPV of 89.1% (95% CI: 84.5 – 93.8%) for smear negative-culture positive specimens. The sensitivity were higher for smear-positive than for smear-negative specimens (p=0.007). Overall, the sensitivity of Xpert MTB/RIF was 65.5% (95% CI: 53.3-77.7%), specificity was 96.3% (95% CI: 93.4-99.2%), PPV was 86.4% (95% CI: 76.2-96.5%), and NPV was 88.6% (95% CI: 83.9-93.3%) compared to the culture respectively (Table 5.3). Overall agreement between Xpert MTB/RIF and culture was 88.8% (95%CI: 83.9 – 92.4%). Test agreement was similar by smear status, 90.9% for smear positive and 87.8% for smear negative.

Table 5.3: Sensitivity, Specificity, and Predictive values of AFB smear and Xpert MTB/RIF assay using culture as a gold standard

Method and test type	Sensitivity %(95% CI)	Specificity %(95% CI)	PPV %(95% CI)	NPV %(95% CI)
AFB Smear	36.2 (23.8- 48.6)	99.4 (98.2-100)	95.5 (86.8-100)	81.3 (75.9-86.7)
GeneXpert MTB/RIF assay				
Smear Negative	48.6(32.5-64.8)	96.9(94.2-99.6)	78.3(61.4-95.1)	89.1(84.5-93.8)
Smear Positive	95.2(86 -100)	N/A*	95.2(86-100)	N/A
Total (SPTB &SNTB)	65.5(53.3-77.7)	96.3(93.4-99.2)	86.4(76.2-96.5)	88.6(83.9-93.3)

* N/A – not applicable, SPTB – Smear positive TB, SNTB - Smear negative TB

Xpert MTB/RIF gives a semi-quantitative result (as very low, low, medium, and High) when the test detect MTBC. Eight samples (18.2%) had a high semi-quantitative result (average cycle threshold (CT) value of 14.3), 10 samples (22.7%) had a medium result (average CT value of 19.1), 12 samples (27.3%) had a low result (average CT value of 27.0), and 14 samples (31.8%) showed a very low semi-quantitative result (average CT value of 33.1). The average Ct value was significantly lower in smear-positive compared with smear-negative cases (19.9 vs. 28.8; P<0.05). An inverse association between Ct of Xpert MTB/RIF assay and AFB smear grading was found. Time to growth in MGIT medium and of Xpert MTB/RIF result was also compared.

The median time required to grow in liquid medium for Xpert positive samples with MTBC isolates was statistically lower than that for Xpert negative samples (9 versus 17 days; $P<0.05$).

5.3. Xpert MTB/RIF result

A total of 38 (86.4%) valid RIF result were obtained from positive Xpert MTB/RIF assay. Among 38 specimens with MTB detected by Xpert, rifampicin resistance was detected in 9.0% (4/38) of specimens. Indeterminate RIF results were recorded in 13.6% (6/44) specimens, among these rifampicin indeterminate result, 4 results occurred in smear negative-culture positive samples and 2 results occurred in smear negative-culture negative samples with very late Ct (33-37). Among 4 rifampicin resistance result, 3 strains were identified from smear-negative culture-positive samples and 1 strain from smear-positive culture-positive sample.

Conventional DST was only available for 10 MTBC isolates. Out of 10 isolates, 8 were susceptible to all primary drugs, and 2 patients had strains resistant to INH and RIF. Discrepancy between Xpert RIF and DST results were detected in two cases. The Xpert MTB/RIF assay's semi-quantitative result in these specimens was very low. Since DST results were not available for all valid RIF result, we were unable to calculate the sensitivity and specificity of Xpert MTB/RIF assay for detection of RIF resistant in sputum specimen.

6. Discussions

Xpert MTB/RIF assay is a new rapid molecular test that overcomes many of the current operational difficulties in TB diagnosis [5]. WHO endorsed Xpert MTB/RIF assay for use in TB prevalent resource limited countries [4, 5]. Many studies have assessed the performance of Xpert MTB/RIF assay for detection of MTBC and RIF resistance in sputum specimens. However, there are limited data about performance outcomes of Xpert MTB/RIF at point of treatment in health care facility in resource poor settings where smear microscopy is a formidable diagnostic challenge.

In the present study, high prevalence (26.4%) of culture confirmed TB was found among the study participants. The conventional diagnostic tool, smear microscopy detected only 9.3% (21/227) of cases. Xpert MTB/RIF detected 16.7% (38/227) of cases. Xpert MTB/RIF diagnosed more patients than did smear microscopy.

Overall sensitivity of Xpert MTB/RIF was 65.5% (95% CI: 53.3 – 77.7%). The sensitivity observed in this study was comparable to Dorman SE, *et al*, who reported 62.6% in South Africa [17]. Balcha TT *et al* also reports 67.6% sensitivity in Adama, Ethiopia, which was consistent with the finding of this study [31]. However reports in literature indicated that the sensitivity of Xpert MTB/RIF is high as 96.7% in Lima, Peru and 100 % in New-Zealand [18, 29]. Direct comparisons of overall sensitivity may be challenging owing to different methodologies and sample type applied for the study.

When the specimen stratified by smear microscopy result, Xpert MTB/RIF demonstrated 95.2% (95% CI: 86.1 – 100%) sensitivity for smear-positive, culture-positive and 48.6 % (95% CI: 32.5 -64.8%) for smear-negative, culture-positive specimen. The results for smear-positive samples do not differ from other study conducted in different place which have shown a sensitivity ranging from 95.6 to 100 [18, 20,21]. However, the sensitivity found for smear-negative, culture-positive was lower than reported by others ranging from 61%to 76.9% [18, 19, 21, 22]. Relatively modest sensitivity (48.6%) found in this study is consistent with that reported by previously published in South Africa by Lawn SD *et al* (43.%) [20]. The sensitivity in smear-negative, culture-positive samples was also varied substantially in different studies, ranging from

43.4% to 91.7% [18-22]. Using culture as a gold standard, 34.4%(20/58) false negative Xpert results were detected in smear negative specimens. These false negative results in smear negative specimen substantially decreased the sensitivity of Xpert MTB/RIF assay in this study. The possible explanation for this discrepancy could be differences in study design and the presence of PCR inhibitors or insufficient nucleic acid material in the specimens. Despite its low sensitivity in smear negative specimen, Xpert MTB/RIF had almost 31% increases in TB case detection compared with smear microscopy alone. Xpert MTB/RIF detected 18 more cases than smear microscopy did.

Specificity of Xpert MTB/RIF assay found in this study was 96.3% (95% CI: 93.4 – 99.2%). The result of specificity is comparable with other study conducted in different place which have shown a specificity ranging from 94.1 to 100 [18-31]. However, 6 (2.6%) patients had positive Xpert MTB/RIF results but no bacterial growth in MGIT or LJ media. All specimen tested positive in the ‘very low and low’ range of Xpert MTB/RIF detection with average Ct of 28.3. Possible reasons might be also non viable organism or excretion of residual fragments of DNA from persistent non-viable organisms. This has been suggested to occur in patient receiving anti-TB treatment [21,24].

Different studies reported that the semi-quantitative MTB complex load estimates from the Xpert MTB/RIF assay have good correlation with the smear status of the patients. Similarly this study found good correlation between smear gradeing and Xpert MTB/RIF assay load as demonstrated by other studies [20, 21, 31]. Consistent with previous report with higher Xpert MTB/RIF assay loads were associated with decreased MGIT culture TTP in our study [31]. Low semi-quantitative Xpert MTB/RIF assay loads were significantly more common in smear-negative than smear positive specimen.

To minimize a relative high cost of Xpert MTB/RIF assay, it is important to control the chance of getting failed test report. Invalid and error reports have important cost implication. A total of 4 invalid Xpert MTB/RIF test results were obtained in this study. These results counted as test failure. We found acceptably low occurrence of test failure rate (1.7%) in the current study. The

frequency of invalid results was lower than previously reported studies (4.5% to 6.2%) [28, 29,31].

Detection of simultaneous MTBC and RIF resistance are a key advantage of Xpert MTB/RIF over smear microscopy. Rapid detection of RIF resistance is considered crucial for the control of MDR-TB [2]. Overall 86.3% valid RIF results were obtained from the total positive Xpert MRB/RIF assay. RIF resistance was detected in 4 (8.8%) specimens and was indeterminate in 6 (13.6%) specimens. The two RIF resistant result were confirmed on conventional DST. However the other two results were discrepant with conventional DST. There are reports of false positive RIF resistance in different literature [18-20]. Although we have got discrepant RIF results, we are unable to drown conclusions on the sensitivity and specificity of Xpert MTB/RIF assay for detection of RIF resistance due to availability of limited number of conventional DST result.

The conventional culture (both LJ and MGIT) can take several weeks to months to yield detectable growth and DST result. It requires specialized laboratory facility including sophisticated bio-safety and highly trained laboratory personnel. TB culture is only available at national or reference laboratory level. It is also difficult to get access to these laboratory for the district level of the health system. Xpert MTB/RIF assay will represent an important contribution by providing MTB and RIF resistance results in a matter of hours. As observed in this study, it only took one day training to run the Xpert MTB/RIF assay. The turnaround time to get Xpert result was one day. However, short expiry date of cartilage, constant supply of consumables and requirement of annual instrument calibration were observed challenge during the study paired.

7. Strengths and limitation of the study

The study provides information about real-life performance of Xpert MTB/RIF assay; since information regarding Xpert detection rate in Ethiopian context is sparse. Moreover it informs on how Xpert MTB/RIF tests perform at the point of treatment, where the assay can have greatest impact on patient care in remote health care facility. However, this study is not without limitations. The following were the limitation of the study.

- Unable to run phenotypic DST for all Xpert MTB/RIF positive specimen. Large number of MTB positive specimens, in comparison with conventional DST method will be useful to assess the ability of Xpert MTB/RIF assay in the diagnosis of MDR TB.
- Due to the nature of the study design (cross-sectional), we are unable to clarify culture negative Xpert MTB/RIF positive samples, whether it is false positive or not, using follow up culture and clinical data.
- Operational and logistic difficulties to increase the sample size and to link Xpert MTB/RIF result with clinical outcomes of the study participant.

8. Conclusion and Recommendations

Our findings demonstrate that the Xpert MTB/RIF assay is a useful tool for the detection of MTBC with high sensitivity in sputum specimens compared with conventional AFB smear microscope. It outperformed smear microscopy in significant proportion of patients who are smear-negative. However, it demonstrated suboptimal sensitivity in smear negative patients compared to conventional culture test. Negative Xpert MTB/RIF result might be insufficient to rule out active TB.

Requirement of minimal training to run the test and simultaneously detection of MTBC and RIF resistance would make Xpert MTB/RIF assay to be a good candidate to roll-out in national TB control programs. However, cost, constant supply of consumables, short expiry dates of cartilage, and requirement of annual instrument calibration will be a major challenge for the roll-out of Xpert MTB/RIF assay in Ethiopia. Due to some limitation Xpert MTB/RIF cannot replace conventional TB diagnostic tools. All the above points should be considered to rule-out Xpert MTB/RIF in national TB control programs to use for detection of TB in particular group or for all TB suspect patient. So, further studies are required to clarify operational difficulty, challenges and limitations in rule-out Xpert MTB/RIF in current TB control/treatment algorithms in the country.

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10. Annexes

10.1. Annexe I: Informed consent form (English version)

Dear Sir/Madam;

My name is Dereje Assefa. I am currently a student of Addis Ababa University, department of medical laboratory sciences and undertaking a master's degree (MSc) in field of Diagnostic and Public Health Microbiology. I am going to carry out a scientific study on evaluation of the performance of Xpert MTB/RIF assay which is a new TB diagnostic instrument.

We are inviting all adults who attend Karamara Hospital to participate in the research on the evaluation of this machine. If you are volunteer, you will be asked to give two morning sputum samples and to give permission to access of your medical record. Your socio-demographic data and medical history will be taken from your record. We would like to indicate that your name will not be written in the form and we assure you all the information you give will be kept strictly confidential.

Signature of person taking the consent: _____

Date: _____

Day/month/year

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily and give permission for my sputum sample to be used in the current research project: Evaluation of Xpert MTB/RIF Assay for Diagnosis of Pulmonary Tuberculosis and Detection of Rifampicin Resistance in Sputum Specimens

Print Name of Participant: _____ Signature of Participant: _____

Date: _____

Day/month/year

10.2. Annexe II: Informed consent form (Somali version)

SOOGUDBIN WAR SUGGAN

MUDANE/MARWO

Magaceygu waa Dereje assefa. Waxan ahay ardey dhigta jamacada addis ababa university, qeybta caafimaadka ee medical laboratory sciences iyo diyaarinta aqoon heer sare ee master's degree (MSs) gaar ahaan diagnostic iyo public health microbiology. Taas oo waliba wax walba ay kugu diyar'yihin qalaab casri ah sida **Xpert MTB/RIF** taas oo Alladda casriga ah ee lagu baadho cuddurka duumada ama **TBda**. Waxan xaflad martiqaad ugu sameyneyna dhamman dhalinyarada in ay ka soo qayb qaataan karamara hospital si ay uga bogtaan qalabka casriga ah ee an idin hayno. Hadii aad tahay diyaar waxaa laga rabba 2 malmood in aad subbax walba laga qaaddo qandhuf amma xaaqo laguna baadho wakhti yar gudoohood....

Waxaad ahaaneysa qof bulshada u ku nool yahay kadheer dhibaataada ay ledahay cudurka duumada ama **TBda** lehna aqoonsi shahaado, waxaan rabnaa in aan ku shacino in ann lagu qayixi doonin (amma lagu sooo bandhiigi doonin) magacaga liistada ama diwaanka booga. waxaa si cad kugu so gudbineynaa amaano iyo karti buuxda.

Sexex qadaha: _____

Date: _____

Malinta/bisha/sanadka

Waa helay akhriinta warka, ama waa la ii akhriyay waxaan ahaa diyaar si aan u waydiyo su'aal wana la iiga jawaabay su'asheydi si buuxda oo leh qaneecaad.... Waxaan ahay heegan waxa kaloo diyaar u ahay ugu deqida candhuufteyda si logu baadho, badhitaanka casriga ahee **GeneXpert MTB/RIF** for diagnosis of pulmonary tuberculosis and detection of rifampicin resistance in sputum specimens

Magaca qaybgalaha: _____ **sexexa qaybgalaha:** _____

Date: _____

Malinta/bisha/sanadka

10.3 Annexe III: Informed consent form (Amaric version)

የፈቃደኝነት መጠየቂያ ቅጽ

ስሜ ደረጃ አሰፋ ይባላል በአዲስ አበባ ዩኒቨርሲቲ ጤና ሣይንስ ኮሌጅ የድህረ ምርቃ ተማሪ ነኝ፡፡ የቲቢ ምርመራን በተሻለ ጥራት ለመስራት የሚያስችል አዲስ የምርመራ ማሽን (ጂን ኤክስፐርት) ለመጠቀም አሁን ያሉትን የምርመራ ዘዴዎች ከአዲሱ መሳርያ አንጻር በመገምገም ጥናት እያደረጉ እገኛለሁ፡፡ የጥናቱ አላማ ማሸኑ በአለም የጤና ድርጅት የተሰጠው የጥራት ደረጃ ከእኛ ሀገር አንጻር በስራ ላይ ቢወል ጠቀሜታውን ለማሳየትና የመፍትሄ ሀሳብ ለመስጠት ነው፡፡ የእርስዎ ትብብርና ፍቃደኝነት ለጥናቱ ጠቀሜታ ስላለው መረጃዎችን በመስጠት እንዲሁም ምርመራ በሚደረግ ትብብርዎን እየጠየኩ ከዚህ በመቀጠል ጥያቄዎች የማቅርብልዎ ሲሆን ትብብሮ በፈቃደኝነት ላይ የተመሰረተ እንዲሁም መመለስ የማይፈልጉት ጥያቄም ካጋጠመዎ አለመመለስ ይችላሉ፡፡ እንዲሁም በመረጃ መሠብሰቢያው ላይ ስምዎ የሚጠቀስና መረጃውም በሚሰጥበት የሚያዝ መሆኑን እገልጻለሁ፡፡

እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ በጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ፡፡

ፊርማ: _____

መረጃውን የሰበሰበው ግለሰብ ስም: _____

ፊርማ: _____

10.4 Annexe IV: Data collection sheet

Code No.: _____

Sample ID: _____

Date: _____

Section I: Demographic Characteristics

1. Sex: M ☐ F ☐
2. Age: _____ years

Section I I: Patient's medical history

1. Symptoms of TB disease:

- ☐ Cough (especially if lasting for 3 weeks or longer) with or without sputum production
- ☐ Fatigue
- ☐ Pain in the chest when breathing or Malaise
- ☐ Fever
- ☐ Coughing up sputum or blood
- ☐ Night sweats
- ☐ Unexplained weight loss

2. Classification of Chest radiographs views:

- ☐ Probable TB (findings consistent with TB)
- ☐ Possible TB (atypical findings),
- ☐ TB unlikely (findings not consistent with TB)
- ☐ Normal chest radiograph

3. Reason for TB test request:

- ☐ Had a prior history of tuberculosis
- ☐ Follow up (patients who are receiving tuberculosis treatment)
- ☐ HIV co-infection

- ☐ Patients present with signs and symptoms suggestive of tuberculosis
- ☐ Suggestive of Tb chest x-ray

10.4.1 Culture result collection sheet (Laboratory data)

[illegible]

10.4.2. Xpert MTB/RIF result collection sheet (Laboratory data)

[illegible]

10.5 Annexe V: Standard Operating Procedures (SOPs)

10.5.1 Principles and Procedures of Xpert MTB/RIF assay

Test principle:

The Xpert MTB/RIF assay consists of a single use multichambered plastic cartridge preloaded with the liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction, and heminested real-time PCR. The PCR assay amplifies a 192 bp segment of the *M. tuberculosis rpoB* gene in a heminested real-time PCR. The internal control heminested *B. globigii* assay is multiplexed with the *M. tuberculosis* assay. *M. tuberculosis* is detected using five overlapping molecular beacon probes (probes A to E) that are complementary to the entire 81-bp RIF resistance determining core region of the wild type *rpoB* gene. Mutations in the *rpoB* gene target inhibit hybridization of one or more of the *rpoB* specific molecular beacons, reducing or eliminating the signal from the corresponding probes.

Sample preparation:

The sputum samples are treated with sample reagent (SR). The sample reagent contains NaOH and isopropanol. The SR is added at a 2:1 ratio to the sputum sample and incubated for 15 min at room temperature. The treated sample is transferred into the cartridge, the cartridge is loaded into the GeneXpert instrument, and an automatic process completes the remaining assay steps.

Assay cartridge:

The assay cartridge contains:

- Lyophilized *Bacillus globigii* spores which serve as an internal sample processing and PCR control
- Processing chambers
- Valve body
- Reaction tube

Running a test:

Each GeneXpert Dx module processes one sample. insert the sample and applicable reagents into a GeneXpert cartridge, create a test, load the cartridge into an available instrument module, and then start the test. During the test, the system performs the following steps:

- Moves the sample and reagents into different chambers in the cartridge for sample preparation.

- Hydrates the reagent beads
- Performs probe checks to ensure that the sample preparation is successful (only if the assay definition requires this step).
- Moves the sample and reagent mixture into the reaction tube.
- Starts the PCR cycles and real-time detection

System Calibration:

The thermal reaction chamber thermistors are calibrated to ± 1.0 °C using National Institute of Standards and Technology (NIST)-traceable standards. During the manufacturing process, the temperature of the heating system is measured at two temperatures: 60 °C and 95 °C. Calibration coefficients that correct for small errors in the raw thermistor readings of the heaters are stored in the memory of each I-CORE module. The optical system is calibrated using standard concentrations of individual unquenched fluorescent dye-oligos. For each optical channel, the signal produced by a tube alone (the blank signal) is subtracted from the raw signal produced by the dye-oligo standard to determine the spectral characteristics. Using the individual spectral characteristics of the pure dye-oligos, signals from an unknown mixture of dye-oligos can be resolved into corrected signals for the individual dye-oligos in the mixture.

10.5.2. Standard Operating Procedures (SOPs) for TB culture

Specimen Digestion/Decontamination for Mycobacteriology Culture

Analytic Procedure

Specimen types

- Specimens that require decontamination include sputum, bronchial secretions, washings, or biopsies, skin, soft tissue, gastric lavage, stool specimens, urines and all other specimens from sites contaminated with normal microbial flora.
- When a specimen is determined to be unacceptable, a repeat specimen must be requested.
- Unacceptable specimens include unlabeled or inadequately identified specimens, dry swabs, and those received in previously used containers, containers that are non-sterile or cannot be tightly sealed.
- Specimens held unrefrigerated prior to processing for greater than one hour may lead to bacterial overgrowth at levels that prevent detection of mycobacteria
- Sputum specimens collected at different times must never be pooled as this greatly increases the contamination rates.

Specimen storage

- Specimens should be delivered to the laboratory as soon as possible to avoid overgrowth by contaminants and normal respiratory flora.
- Specimens not processed within one hour of collection must be refrigerated at 2 – 8° C.

Reagents/Media:

Reagents used for digestion/decontamination are dependent on precise adherence to the required procedures.

1. Fresh working NALC-NaOH Solution - Directions for preparing NALC-NaOH Solution or alternatively BD BBL™ MycoPrep™ Specimen Digestion/Decontamination Kit are included
2. Phosphate buffer solution (pH 6.8)
3. MIT™ Mycobacteria Growth Indicator Tube
4. BACTEC™ MGIT™ 960 Supplement Kit including:
 - a. BACTEC MGIT Growth Supplement

b. MGIT PANTA Antibiotic Mixture vial

Required Supplies:

1. 50 ml sterile conical centrifuge tubes
2. Vortex mixer
3. Centrifuge- capable of speed 3,000–3,500 x *g*, fixed angle rotor with aerosol-free safety centrifuge cups
4. Funnel and waste container filled 1/3 full with an approved disinfectant solution
5. Disposable sterile pipettes
6. Slide warmer set between 65 and 75°C
7. Glass microscope slides (a frosted end for labeling with a pencil is useful)

Quality Control:

Negative - Process a negative water or buffer control with each run of specimens.

Positive - Sputum spiked with a mycobacterial species should be processed as a positive control once per week.

Digestion, Decontamination and Concentration Procedure Steps:

Follow laboratory bio-safety practices for all procedure steps.

1. Prepare fresh working digestant/decontamination solution or BD MycoPrep™ Specimen Digestion/Decontamination Kit.
2. Prepare the BSC for use following the *Use of the Bio-Safety Hood in the Mycobacteriology Laboratory SOP*.
3. Reconstitute a lyophilized vial of MGIT PANTA Antibiotic Mixture with 15 mL of BACTEC MGIT Growth Supplement.

**Once reconstituted, the PANTA mixture must be stored at 2 – 8°C and used within 5 days.*

4. Label the MGIT tubes with the specimen number.
5. Unscrew the cap and aseptically add 0.8 mL of Growth Supplement/MGIT PANTA Antibiotic Mixture to each labeled MGIT tube.

**For best results, the addition of Growth Supplement/MGIT PANTA Antibiotic Mixture should be made just prior to specimen inoculation.*

6. If the specimen was not collected in a sterile, labeled 50 ml disposable centrifuge tube, transfer the entire specimen to a labeled 50 ml conical tube.
**No more than 10 ml of specimen may be processed per conical tube. The remaining sample should be transferred to second tube and processed. Repeat for all patient specimens.*
7. Stagger the tubes in the rack to prevent cross contamination.
**Do not process more specimens in a batch than the centrifuge will hold.*
8. Opening only one tube at a time, to the first specimen tube add an equal amount of fresh working NACL-NaOH solution, rotate and invert the tube, ensuring the mixture coats the entire interior surface. Vortex the mixture for 10-15 seconds. Repeat this step for each specimen in the batch.
**Start the timer for 15 minutes after adding the solution to the first tube in the batch.*
9. Allow the specimens to stand the entire **15** minutes. During the incubation time check each specimen by slightly tilting the tube and observing for liquefaction.
**If a specimen is very mucoid with no change during these checks, add a small amount of NALC directly to the tube, vortex and allow to stand until the end of this incubation time.*
10. After the digestant has remained in the first tube for **15** minutes, begin with the first specimen and fill the first tube to the 50 ml mark with phosphate buffer by slowly pouring the buffer down the side of the tube avoiding splashing or contamination. Tighten the cap and wipe the outside of each tube with the disinfectant soaked towel, then invert the tube several times to mix thoroughly. Repeat this step on each of the remaining specimens in the batch, mixing well after each addition.
11. Load tubes in aerosol-free safety centrifuge cups. Centrifuge tubes for 15 minutes at 3,000 - 3,500 x g. Allow aerosols to settle a few minutes before removing tubes from the centrifuge cups.
12. Opening one tube at a time, pour off the supernatant into a waste container filled 1/3 full with approved disinfectant solution. Wipe the lip of the conical tube with a disinfectant soaked towel and then recap.
**The use of a funnel is preferred. (Pour slowly so as not to disturb the pellet and be sure to not touch the funnel while pouring.*

13. Using a sterile disposable transfer pipette, re-suspend the pellet by adding 1-2 mL of phosphate buffer. Gently mix the tube contents.
14. Add 0.5 mL of the concentrated specimen suspension to the prepared BBL™ MGIT™ Mycobacteria Growth Indicator Tube. Also add a drop (0.1 - .25 mL) of specimen to a Lowenstein-Jensen agar slant or other conventional solid medium.
**Refer to the Mycobacteriology Culture SOP.*
15. With the same pipette, place a drop of the suspension onto a clean, labeled glass microscope slide. Prepare a smear over an area of 1 by 2 cm of the slide.
**Refer to the AFB smear SOP.*
16. Tightly recap the MGIT tube and mix well. Leave the inoculated tubes at room temperature for 30 minutes before loading in to the MGIT system.
**Refer to the Mycobacteriology Culture SOP.*
17. Load inoculated BBL™ MGIT™ Mycobacteria Growth Indicator Tube into the instrument following manufacturers' instructions for the duration of the recommended 42 day testing protocol.
18. For specimens in which mycobacteria with different incubation requirements are suspected, a duplicate MGIT tube can be set up and incubated at the appropriate temperature; e.g., 30 or 42°C. Inoculate and incubate at the required temperature. These tubes must be manually read (refer to the BACTEC MGIT Instrument User's Manual).

Post-analytic Procedure

Specimen Retention:

Store processed specimens in AFB refrigerator for as long as space allows (approximately two weeks).

Calculations:

Quality monitors are compiled monthly to monitor the digestion process of the lab.

Expected Values:

- Contamination rates of 8 - 10% are considered to be acceptable for solid culture media.
- Liquid culture media will have a higher contamination rate which will be monitored for acceptability.
- A contamination rate of less than 5% suggests overly harsh decontamination.
- A contamination rate of greater than 10% growth suggests inadequate decontamination, incomplete digestion, reagent and/or media contamination or environmental contamination.

Interpretation of Results:

Contamination rates higher than 10% on solid media will be investigated to determine whether equipment, reagent or personnel are causing the high rates. Data is included in the monthly quality assurance report.

Method Limitations:

- The procedure is dependant on strict adherence to recommended techniques, timing, temperature, and biochemical requirements. Any deviation from the SOP will not provide appropriate clinical care.
- The NaOH procedure is very robust and may kill up to 60% of tubercle bacilli in clinical specimens, and may give a false negative result, especially in cases of paucibacillary disease as seen in early disease, or in many HIV positive patients.
- Additional contributory factors such as heat build-up in the centrifuge step may also kill tubercle bacilli.

10.5.3. Standard Operating Procedures (SOPs) for DRUG SUSCEPTIBILITY TESTING (DST) BY MGIT 960

Purpose

Drug resistant tuberculosis has become a serious public health problem. Resistance to first line anti-TB drugs makes the disease more difficult and expensive to treat. Rapid detection of these resistant isolates is critical to effective patient management. Antimycobacterial susceptibility testing is valuable for proper treatment of patients. WHO recommends the use of liquid culture systems for rapid detection of drug susceptibility. MGIT provide DST results approximately at the same time frame.

Principle

Susceptibility testing by MGIT is a 4-13 day qualitative test. The test is based on growth of the *M. tuberculosis* strain in a drug containing tube compared to the drug free tube (Growth control). The instrument continuously monitors the tubes for increased fluorescence. Analysis of fluorescence in the drug containing tube compared to the fluorescence of the growth control tube is used by the instrument to determine susceptibility results. Results are interpreted automatically and reported as susceptible or resistant.

Available DST kit

- **SIRE** kit
- **SIRE** supplement
- **PZA Kit**
- **PZA** supplement

Storage and reconstitution

- Lyophilized drug vials and SIRE supplement are to be stored at 2-8°C
- Once reconstituted, the antibiotic solutions may be stored at -20°C or colder up to 6 months. Once thawed, drugs should be used immediately
- Reconstitute SIRE drug vial should be reconstituted with 4ml of sterile distilled water and PZA vial with 2.5ml.
- Date of preparation should be marked on the vial and the Cryovials (during storage)

Kit components

SIRE kit

- One vial each of lyophilized first line drugs namely, STR, INH, RIF & EMB.
- 8 vials of SIRE supplement consisting of 20ml of OADC enrichment
- One kit can be used to perform 40 tests

PZA kit

- Two vials of lyophilized PZA drug and 6 vials of PZA supplement

Concentration of drugs used

Drug	Concentration (µg/ml)
STR	1.0
INH	0.1
RIF	1.0
EMB	5.0
PZA	100

DST preliminary setup

- Label 5 individual MGIT 7ml tubes (Used for isolation) for each test isolate
- In each set, label one tube as growth control (GC) and the set as S, I, R& E
- Label one tube each as growth control (GC), and the rest as STR, INH, RIF & EMB.
- Use 2 tubes of 7ml PZA tubes (green labeled) one for GC and one for drug
- Aseptically add 0.8ml of MGIT SIRE / PZA Supplement to tube.
- Add 0.1ml of the respective drugs into the corresponding labeled tubes

Types of cultures: Positive MGIT tubes and LJ

Preparation of cultures suspension a.

From positive MGIT tubes General note

- It is important to prepare the inoculum using the time reference to obtain the appropriate organism concentration for DST
- The first day of an instrument positive is considered as “0” day and can be used for DST set up from the next day (Day1) till the Day 5
- DST can be set up without any dilution of cultures ranging between 0-2 days after positivity but should be diluted (1 in 5) in case of cultures ranging between 3-5 days
- Beyond 5 days from the day of positivity the culture should be sub cultured into one fresh MGIT tube (with / without PANTA if required) and DST should be carried out.

Inoculum preparation from Day1-2

- Vortex the MGIT tubes and let stand in the cabinet for allowing the coarse particles to settle (at least 20min).
- Place 3 sterile screw capped vials in the MGIT carrier rack and label as neat, 1:10 & 1:100.
- Add 4.5ml of sterile saline (0.85% - 0.85gms in 100ml of distilled water, autoclaved and used) to each of 1:10 & 1:100 vials. Carefully transfer 3-4ml of the culture from positive MGIT tube in to the neat vial using a sterile disposable transfer pipette.

- Add 0.5ml of the culture from the neat vial into 1:10 vial and mix for at least 10 times for even distribution (care must be taken to avoid aerosols while mixing)
- Similarly transfer 0.5ml from 1:10 vial to 1:100 vial and mix it thoroughly.
- Add 0.5ml of the suspension from 1:100 vial to the MGIT tube labeled as Growth control for SIRE – GC
- Add 0.5ml of suspension from 1:10 vial for PZA- GC
- Add 0.5ml of the culture from neat vial to all the labeled drug containing tubes.
- Gently tilt the tubes 3-4 times for even mixing (take care to tighten the tubes completely to avoid any sort of leakage)

Inoculum preparation from Day 3-5

- Add 1ml of the well mixed inoculum into 4ml of the vial labeled as neat (1 in 5 diluted culture will be considered as neat in this case)
- Prepare 1 in 10 and 1 in 100 dilution from neat (1 in 5) as before in saline. (Final dilution for control tubes would be 1: 50 for PZA & 1:500 for SIRE)

b. Inoculum preparation from LJ medium

- Prepare culture suspension in 4ml of Middlebrook 7H9 broth with 8-10 glass beads (preferably the whole growth)
- Vortex the suspension and adjust to 1.0 McFarland standard
- Let the suspension STAND for 20min without disturbing
- Transfer the supernatant into another screw capped vial and let stand for 15min
- Again transfer the supernatant into another vial and match with 0.5 McFarland standard
- Dilute 1ml of the suspension in 4ml of sterile saline (1 in 5 dilution). This will be considering as neat.
- Prepare 1 in 10 and 1 in 100 dilution from neat (1 in 5) as before in saline. (Final dilution for control tubes would be 1: 50 for PZA & 1:500 for SIRE)

Loading the MGIT DST set into the instrument

- Place the GC and drug tubes for a particular culture in a standard order of arrangement
- Select the appropriate DST carrier set (carrier sets are available as 2, 3, 4, 5 & 8 set) depending on the number of tubes tested for each culture
- Always place the GC tube behind the bar code of the DST carrier set as bar coding is done only for the carrier set and not for the tube
- Arrange the drug tubes adjacent to the growth control
- Use separate DST carrier set for PZA (preferably 2 carrier set)
- Note down the culture number and the drugs set up in the DST register
- Open the segment in the instrument (which is allocated for DST) and select the culture input option on the screen
- Pass the barcode of the DST carrier set through the barcode reader
- Place the carrier set in the position defined by the instrument
- Note down the position of the DST set in the register for each culture

Quality control

- Annually: Perform WHO DST EQA panel

10.6. Annexe VI: Definition of terms

acid-fast bacilli (AFB) – mycobacteria that when stained, retain color even after they have been washed in an acid solution; may be detected under a microscope in a stained smear

culture – to grow organisms on media (substances containing nutrients) so that they or the product of this process can be identified; a positive culture for *M. tuberculosis* contains tubercle bacilli, whereas a negative culture contains no detectable tubercle bacilli

drug susceptibility pattern – the list of drugs to which a strain of tubercle bacilli is susceptible and to which it is resistant

MDR-TB: is defined as tuberculosis that is resistant to at least isoniazid (INH) and rifampicin (RIF), the two most powerful first line treatment anti TB drugs

media – substances containing special nutrients and used for growing cultures of bacteria found in specimens

medical history – the part of a patient's life history that is important for diagnosing and treating TB infection or disease, including history of exposure, symptoms, previous diagnosis of TB infection or disease, and risk factors for TB disease

negative predictive value (NPV): is the probability that subjects with a negative screening test truly don't have the disease

polymerase chain reaction (PCR) – a technique used to copy small segments of DNA

positive predictive value (PPV)- is the probability that subjects with a positive screening test truly have the disease

resistant – an organism's ability to grow despite the presence of a particular drug

sensitivity- The probability of the screening test correctly identifying diseased subjects

smear – a specimen that has been smeared onto a glass slide, stained, washed in an acid solution, and then placed under the microscope for examination; used to detect acid-fast bacilli in a specimen

specificity- the probability of the screening test correctly identifying non-diseased subjects

sputum – phlegm from deep in the lungs, collected in a sterile container for processing and examination

susceptible – an organism's ability to be killed by a particular drug

symptoms of TB disease – noticeable conditions caused by TB disease. The symptoms of pulmonary TB disease include coughing, pain in the chest when breathing or coughing, and coughing up sputum or blood. The general symptoms of TB disease (pulmonary or extrapulmonary) include weight loss, fatigue, malaise, fever, and night sweats. The symptoms of extrapulmonary TB disease depend on the part of the body that is affected by the disease.