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The magnitude of Pulmonary Tuberculosis and Comparison of Lipoarabinomanan Rapid Test Assays with Conventional Tuberculosis Diagnosis at Saint Peter Specialized Hospital Addis Ababa, Ethiopia

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MAGNITUDE OF PULMONARY TUBERCULOSIS AND COMPARISON OF LIPOARABINOMANAN RAPID TEST ASSAYS WITH CONVENTIONAL TUBERCULOSIS DIAGNOSIS AT SAINT PETER HOSPITAL ADDIS ABABA, ETHIOPIA,2021

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Contents

List of abbreviation

AA	Addis Ababa
AAU	Addis Ababa University
AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
CDC	Center for Disease Control
HIV	Human Immuno Deficiency Virus
ICU	Intensive Care Unit
LAM	Lipoarabinomanan
LJ	Lowenstein–Jensen
MTB	Mycobacterium Tuberculosis
NAAT	Nucleic Acid Amplification Technique
POC	Point Of Care Test
PTB	Pulmonary Tuberculosis
TB	Tuberculosis
TST	Tuberculin Skin Test
WHO	World Health Organization

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Abstract

Background: - Tuberculosis (TB) is a communicable disease that is caused by Mycobacterium tuberculosis. It is widely distributed globally with high mortality and morbidity. Africa is one of the continents which is mostly affected. Poor and shortage of diagnostic facilities were proven to be the major limitations in preventing the disease. Lowenstein–Jensen (LJ) culture and AFB smear microscopy are the gold standard diagnostic testing strategies for TB even though they both have a variety of limitations, especially in resource-limited countries. Additional rapid test that can help the diagnosis of TB in antigen and the antibody-based assay is Lipoarabinomanan (LAM) which can detect the antigen from urine and its antibody from serum in patients with active TB.

Objective: To assess the magnitude of Pulmonary Tuberculosis and Comparison of Lipoarabinomanan Rapid Test Assays with culture-based tests at Saint Peter Specialized Hospital, Addis Ababa, Ethiopia

Methods: A cross-sectional study was conducted on 314 study participants from March 2021 to June 2021. After obtaining informed consent, data were collected using a standardized questionnaire. Urine, sputum, and serum samples were collected from pulmonary tuberculosis suspected patients that showed sign and symptoms of TB. Then Lowenstein–Jensen (LJ) culture, gene expert, and LAM rapid kit testing were done following standard procedures. The data were entered and analyzed using Epi Data version 3.1 and SPSS version 23. We used descriptive statics including, KAPPA analysis.

Results: a total of 314 participants were enrolled in this study and 176 (56.1%) were male. The magnitude of Pulmonary Tuberculosis based on Lowenstein–Jensen culture methods was 20.7 %, among culture pulmonary positive cases blood Lipoarabinomanan, urine Lipoarabinomanan, GeneXpert detected 50, 42, 45 cases respectively. The sensitivity and specificity of blood Lipoarabinomanan was 76.9 and 94%; urine Lipoarabinomanan was 64.6 and 91.5%; and GeneXpert was 69.2 and 92.3% respectively.

Conclusion: Accordingly to the present finding, the Lipoarabinomanan test has good sensitivity and specificity for pulmonary tuberculosis diagnosis versus GeneXpert methods. Further large scale study is required to understand the use of LAM based assay for the diagnosis of PTB.

Keywords: - tuberculosis, Lipoarabinomanan, comparison.

1. Introduction

1.1 Background

Tuberculosis is one of the widely distributed communicable bacterial diseases that is caused by Mycobacterium tuberculosis (1,2). Organisms categorized under this genus Mycobacteria can be true pathogens, saprophytes, or opportunistic pathogens (1). Active tuberculosis is diagnosed by detecting Mycobacterium tuberculosis complex bacilli in specimens either from the respiratory tract (pulmonary TB) or in specimens from other bodily sites (extrapulmonary TB) (3,4).

Even though molecular diagnostic methods like Gene Xpert have been developed these days, acid-fast bacilli (AFB) smear microscopy and culture on Lowenstein-Jensen medium (LJ) are still found to be the "gold standards" for the diagnosis of active TB and, especially in resource-limited countries like Ethiopia, these diagnostic methods available for confirming TB in patients with a clinical presumption of active TB (4,5). AFB smear microscopy is a widely available, fast, and inexpensive method and thus is a very important method to identify highly contagious active TB patients.

Both AFB staining and LJ culture can be used to follow the effectiveness of the treatment and can help to decide whether the patient is infectious or not (4). TB culture has become a more and more valuable testing method in the last decades, particularly because of the need for drug resistance testing (3–5). AFB smear microscopy plays a significant role in the early detection of mycobacterial infections especially when it comes to resource-limited countries this is because most mycobacteria species including MTB grow slowly and culture results become available only after several weeks of incubation (4,6). Additionally, AFB smear microscopy is often the only widely available and easily accessible diagnostic method in developing countries like Ethiopia. AFB staining is based on the high lipid content (mycolic acid) of the bacterial cell wall which makes them resistant to decolorization by acid-alcohol after the primary staining (Carbolfuchsin) (4,7).

The detection of MTB infections was still practically unchanged for several years and almost certainly would have not progressed at all without the unexpected resurgence of tuberculosis that characterized the last twenty years of the century (8).

Most diagnostic tests for tuberculosis rely on sputum microscopy in Ethiopia. This is because low resource settings like Ethiopia have limited TB culture facilities all over the country. Thus,

evaluation of available rapid test kits working in serum and urine samples will be very crucial to enhance the uptake and implementation of service in the health facilities.

1.2 Statement of the problem

Mycobacterium Tuberculosis is a global problem that causes morbidity and mortality in humans (2). According to the World Health Organization (WHO) report, between the year 2000 and 2020 approximately 1 billion people will become recently infected with this bacilli, 200 million of which will Progress to active disease(active TB) with an estimated 35 million mortalities (9). Old Statistics by the year 2002 showed that the incidence of MTB in the Americas alone was 43 cases per 100,000 in population, while in Africa the rate was 350 per 100,000 (10). This can be an evidence to prove that the magnitude of the problem is very large. The problem is even much worse in the case of sub-Sahara Africa where a large number of these patients are co-infected with (Human immune-deficiency Virus) HIV(11).

A disease like TB has a deep social and economic impact (9),(12). Low-income societies with a large number of families, people living in dense urban communities with poor housing conditions, have a high chance of becoming infected with this bacillus and they can develop active TB and die from it (13,14). The risk of transmission of MTB among several populations is also Higher, people that live in congregated institutions (fumigated living conditions), such as prisons with many prisoners that live in low housing conditions or fumigated environments, social shelters, youth correctional facilities, nursing homes for elderly people, day nurseries and schools; the same is valid in case of elderly people, peoples with diabetics and other immune-compromised diseases like Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/ADIS) (11,13,15)

Regardless of worldwide MTB eradication efforts, it is still found to be global public health anxiety these days, particularly in the case of low and middle-income countries. Active TB can easily be curable if it is early diagnosed and followed by effective treatment. However, multi-drug-resistant tuberculosis (MDR-TB) is the biggest challenge. Therefore, cost-effective, rapid, and accurate point-of-care tools for TB diagnosis are very important (16).

The outcomes of tuberculosis on society are huge. Globally, one out of three persons (1/3) is infected with MTB. Almost two billion people in total are affected these days (17) (18). It also accounts for 2.5 % of the global epidemiology of disease and is the well-known cause of mortality in the case of women, killing more women gather than all causes of maternal mortality combined (19). This could be even larger in the case of low resource-limited countries like Ethiopia.

Nowadays TB ranks the seventh cause of mortality rate globally(9). If rigorous efforts are not made, it is likely to keep up that place in2020 or even more, despite a considerable predictable decrease in disease burden from other communicable diseases (20,21). This is because it hinders socioeconomic development: about 75 % of people with TB are within the economically productive age crowd of 15-54 years(22). Ninety-five percent (95%) of all cases and 99 % of mortalities happen in countries with the low resource(23) (24).

In most countries, more cases of TB are reported among men than women (27, 28). In the case of Ethiopia, women are mostly engaged with unfavorable housing conditions, this can leads them to be exposed to MTB easily. This difference is partly because women have less access to diagnostic facilities in some settings especially in Ethiopia where rapid TB diagnostic kits are not introduced, but the broader pattern also reflects real epidemiological differences between men and women, both in exposure to infection and in susceptibility to disease (25)(27-28). In regions where the transmission of *M. tuberculosis* has been stable or increasing for many years, the incidence rate is highest among young adults, and most cases are caused by recent infection or reinfection. As transmission falls, the caseload shifts to the older age groups, and a higher proportion of cases come from the reactivation of latent infection(25) (27-28).

Sputum specimens (2.5-10 ml) were processed by the standard N-Acetyl-L-Cysteine-Sodium Hydroxide (NALC-NaOH) method [9] and concentrated at 4000 × g for 15 minutes. The sediment, irrespective of the original sample volume, was reconstituted to 2.5 ml with phosphate buffer pH 6.8, to make the inoculums for the smears and cultures. Two Lowenstein-Jensen slants, one containing 0.75% glycerol and the other containing 0.6% pyruvate, were inoculated with the sediment and incubated at 37°C. The final result will be approved after 8 weeks incubation period.

In our country has limited TB culture facilities. Most diagnostic tests for tuberculosis rely on sputum microscopy in Ethiopia. From the point of our experience, there was a problem to obtain appropriate sputum samples particularly among Immuno-suppressed individuals that induce false-negative results in smear microscopy, Culture diagnostic tests pass a lot of processes and it also takes time, this process may increase the transmission of MTB and it may prolong starting date of treatment. We must be in mind, other diagnostic tests by considering time, cost, and availability only a few studies have been conducted in LAM test by urine and blood but not have enough evidence in our country

1.3 Significance of the study

Since the LAM test is an easy test to detect pulmonary tuberculosis, it motivates the concerned body to implement LAM diagnostic tests and it will minimize the transmission of MTB and also facilitate effective treatment. It will also contribute to generate information that might help fully to understand the current situation of the MTB diagnostic method. This study would also bring to light various diagnostic methods that detect MTB and it can be used as baseline data for other researches. Besides, it can be a source of information for policy and decision-makers in this area.

2. Literature review

Several studies have been conducted on Tuberculosis. The majority of the studies showed that TB is a communicable disease with the highest morbidity and mortality across the globe. Besides, there is a limitation in the diagnosis of TB in resource-limited countries. Where It's difficult to put in place the gold standard for tuberculosis diagnosis. However, various rapid tests based on different concepts have been developed to identify antibodies or antigens in a specific sample, but evaluating these rapid tests remains problematic. In various investigations, the quick tests have showed differing degrees of sensitivity and specificity. Generally, Antibody-based immunodiagnostic assays can give indirect evidence of current and historical infections in organisms of relevance, such as MTB. However, obtaining approval for such quick diagnostic test kits for tuberculosis remains a challenge. Immunodiagnostic methods, such as the tuberculin skin test (TST), have not been widely utilized to diagnose chronic tuberculosis infections due to their low sensitivity and specificity (30).

A study from Malaysia on active TB patients showed that the registered new PTB cases according to the National Tuberculosis Information System (TBIS) , the prevalence of smear-positive PTB was 68.6% and Most of the cases were male (71%) (26).Another study from India also indicated that the prevalence of symptoms suggestive of PTB disease was to be 35% and it was common among males than females. The symptoms were 17% cough more than two weeks, 14% chest pain. The prevalence of smear-positive PTB was 4 (216/100,000 population), smear-negative and culture-positive 6 (324/100,000) and 6 (324/100,000) were both smear and culture-positive PTB (27). A cross-sectional study conducted in Nigeria revealed that out of 527 sputum specimens LJ cultured 411 (78%) were positive, 89 (17%) negative, 22 (4%) contaminated and 5 (1%) had non-tuberculosis mycobacteria (NTM) (28).

In the Southern Ethiopian study the main symptoms Of the 436 individuals who suspected patients were cough 433 (99.3%), sputum 426 (97.7%), tiredness 416 (95.4%), chest pain 406 (93.1%), difficulty in breathing 402 (92.2%), night sweats 383 (87.8%), fever 374 (85.8%) and loss of appetite 357 (81.9%) (29). Another study institutional-based cross-sectional study was conducted three health facilities in Addis Ababa, Zewuditu Memorial Hospital, Federal Police Hospital, and Teklehayimanot Health Center in 2014GC from the out-patient clinics on HIV

seropositive individuals of 117 eligible participants All the 117 subjects had a cough, had night sweet 111(94.9%) and fever 99 (84.6%). All patients suspicion of pulmonary TB sputum samples were sent to the TB laboratory. Overall, 23(63.9%) were PTB positive by sputum culture-confirmed(30).

In the southwest Ethiopian study, A total of 257 of the households of TB suspects cross-sectional survey was conducted from February to March 2009 in the Gilgel Gibe 17 new cases of TB were identified by culture growth positive(31).Some studies have evaluated gene Xpert and sputum culture, in one research done in US 992 enrolled from 21 sites. 638(64%) of US and 354(36%) of non-US participants were inpatients at the time of evaluation. The result of Xpert 187(18.85%) MTB positive and sputum culture 175(17.6%) MTB positive, Xpert MTB negative 783(78.9%) and sputum culture MTB negative 735(74.1%), Xpert false positive 10(1%)and false-negative 40(4%)(32).

Immuno-diagnostic examinations have not been broadly used for active TB infections diagnosis because of their low sensitivity and specificity (33). Antigens like Lipoarabinomanan (LAM), cord factor, A60, 38 kDa, and 16 kDa have been used for antibody detection kits previously in studies. The outcome for such Immuno-diagnostic tests varied markedly with sensitivity ranging from 15.7% to 89.2% and specificity from 50% to 100 % (33). TB-LAM has been most frequently used as the target antigen, and its monoclonal and polyclonal antibodies have been used to detect the antigen in sputum samples. According to this study, the problem with using TB-LAM as target antigen is the specificity because of the presence of antigen in other species of Mycobacteria. Other antigens such as whole cells, culture filtrate protein (CFP), PPD, 65 kDa, and 14 kDa have been used in this study. However, The sensitivity varied from 50% to 90% and specificity from 80% to 100 % (33).

LAM, is a lipopolysaccharide that forms the main part of the cell wall of TB bacilli, is released from metabolically active or degrading organisms, and is filtered by the kidneys and later excreted in the urine. A laboratory-based urine LAM ELISA test had varying levels of sensitivity, but it is highly specific, especially in detecting active TB (34). A study from South Africa also showed that Determining ATB-LAM is the first HIV-associated tuberculosis (TB)

point-of-care test (POC), and it quickly detects TB in people who are at high risk of short-term death. (35).

A cross-sectional study from Ethiopia conducted in March 2012, showed that the sensitivity of the Determine TB LAM test increased in patients with HIV co-infection. Thus, the Determine TB LAM test is a promising point-of-care assay for the diagnosis of smear-negative TB patients and HIV-infected individuals, especially those with advanced immune suppression. Moreover, the combination of smear microscopy and Determine TB LAM tests could improve the diagnosis of TB in peripheral health settings (36). TB culture has high sensitivity compared to other methods, but it is time-consuming and is not suitable at POC levels in resource-constrained settings. So that, new diagnostic tests that are inexpensive, fast, sensitive, and capable of working with direct clinical samples such as sputum, (serum) blood, or urine are necessary for detecting and monitoring TB diseases (16).

Lipoarabinomanan in urine has been evaluated recently, for the detection of tuberculosis in HIV-positive patients. And showed that urinary LAM ELISA (Alere, USA) has an overall sensitivity of 59–67%, increasing to as high as 85% in patients with their CD4<50 cells/ml, and an overall specificity of 80–94%. Additionally, LAM positivity has been associated with, more critical illness, higher mycobacterial burden, and death. As an alternative to the ELISA-kit, LAM can now be detected by a simple, low-cost (<US$3.5) point-of-care lateral flow assay that can deliver results in 25 min from just 60 µl of urine. Initial evaluation also showed the equivalent performance of the urine LAM strip test compared to the LAM ELISA assay in a different context (37).

3. Objective of the study

3.1 General objective

To assess the magnitude of Pulmonary Tuberculosis and Comparison of Lipoarabinomanan Rapid Test Assays with culture-based tests at Saint Peter Specialized Hospital, Addis Ababa, Ethiopia, from March 2021 to June 2021.

3.2 Specific objectives

- To assess the magnitude of pulmonary Tuberculosis among Tuberculosis suspected patients.
- To compare the yield of detection of PTB using LAM Antigen and Antibody tests against culture-based tests.
- To determine the sensitivity and specificity of TB LAM based assays
- To determine KAPPA value

4. Material and Methods

4.1 Study Area

The study was conducted at St .peter's Specialized Hospital, which is located in the Gullele sub-city. St .peter's Specialized Hospital was established in 1953 EC. According to the 2011 statistic Gullele sub-city has a total population of 267,624 from which 129,396 are males and 138,228 are females. The hospital serves as a Specialized Hospital with more than 300 beds and gives different inpatient and outpatient services including counseling, HIV testing, and ART.

4.2 Study design

A cross-sectional study design was conducted at St. Peter specialized hospital.

4.3 Study period

The study was conducted from March 10, 2021, to June 30, 2021

4.4 Population

4.4.1 Source population

All patients who visited St. Peter Specialized Hospital TB clinic during the study period were the source population.

4.4.2 Study population

All patients who are suspected TB that show signs and symptoms and who come to St. Peter Specialized Hospital during the data collection period, that fulfill the inclusion criteria.

4.5 Inclusion and exclusion criteria

4.5.1 Inclusion criteria

Patients suspected of pulmonary Tuberculosis and who show signs and symptoms of PTB and volunteers to participate in the study.

4.5.2 Exclusion criteria

Patients who started anti-tuberculosis drug, critically ill patients and pediatrics suspected of PTB.

4.6 Study variables

4.6.1 Dependent variables

The magnitude of pulmonary Tuberculosis

Performance of rapid test kit versus Lowenstein–Jensen (LJ) culture.

4.6.2 Independent variables

Sociodemographic and clinical data such as weight loss, fever, cough, and chest pain were extracted by structured questionnaires through interview**s.**

4.7 Measurement and data collection

4.7.1 Sample size and Sampling procedure

Non-probable convenient sampling method was used. The sample size required was calculated using the formula for the single population proportion formula. The prevalence (p)=28.7% was taken from a study conducted in Ethiopia Addis Ababa selected health centers by LJ culture TB confirmed bacteriology(36).

$$n = \frac{Z_{1-\alpha/2})^2 \times P(1-P}{d^2} = \frac{(1.96)^2((0.287)(0.713))}{(0.05)^2} = 314$$

Where: n = required sample size

$Z_{1-\alpha/2}$ = standard normal value at 95% CI which is 1.96

P = estimated prevalence 28.7%

1-P= degree of accuracy

d = possible margin of error 5%

Therefore, the final sample was 314

Enrollment of the study participants was consecutive until the required sample size is obtained.

4.8 Sampling method and procedure

The study area (St. Peter Hospital) was selected purposively. The participants for the current study were selected by using non-probable convenient sampling method. The study enrolled

adults ambulatory hospitalized and outpatients with at least one sign or symptom of TB. By using standardized questionnaires self-reported and laboratory measurements. The questioners including socio-demographic (sex, age, residence, and occupation) and clinical data (cough, weight loss, night sweating, chest pain, contact PTB patient…) and a sample collected using sterile urine, sputum cups, and serum collection tubes.

4.8.1 Laboratory Methods

4.8.1.1 Specimen collection, Handling & Transportation

All samples were collected aseptically by using a flacon tube, SST tube, and sterile screwed urine cup for sputum, whole blood, and urine respectively from patients attending St. Peter Hospital for TB screening. Specimens were labeled with all the necessary information. Samples were transported to the laboratory immediately after collection and processed accordingly following the laboratory protocol (36,38).

4.8.1.2 Sample processing

For the sputum sample, we use 4-6 ml mixed with an equal amount of L-Cystine (NALC/sodium hydroxide (NaOH) disinfectant solution. Following vortex for 10 minutes adding phosphate buffer saline up to 40 ml of falcon tube. Centrifuge for 15 min at 3000rpm and sediment was ready for culture (39). Whole blood sample centrifuge and collect serum before examine using LAM Ab and urine specimen directly using for LAM Ag test. Sputum sample processing for Gene X-pert through pouring of the SR buffer into sputum sample with 2:1 proportion volume ratio. Close the lid and vortex for 15 seconds and, allow the mixed specimen for 15 minutes at room temperature (39).

4.8.1.3 TB mycobacterial cultures

By adding 3-4 drops of phosphate buffer saline on Sputum sediment from sample processing for ruminants contaminants and inoculating into LJ (companies) media with special precautions. For positive and negative result interpretation the Lj culture wait for 4-8 weeks for positive results typical non pigmented, rough, dry colonies are seen (32).

4.8.1.4 Blood Lipoarabinomanan testing

LIO detect rapid tests for serums were done simultaneously to compare the yield of detection of pulmonary tuberculosis. This qualitative rapid test kit detects the cell wall antibody from

MTB in human serum specimens. The test was done by pipetting 2 drops of serum into the sample portion of the kit and followed by adding an equal amount of LIO detect buffer. Finally, the result was interpreted after 20 minutes of incubation at room temperature result interpreted for Negative one pink/purple line appears in the control area "C", there is no other line appear. For Positive two pink/purple bands appear one in control area ”C” and the other one in the test area ”T”. for Invalid no line appears or in the control area or line appears only test area (40).

4.8.1.5 Urine Lipoarabinomanan testing

LIO detect rapid urine tests were done simultaneously to compare the yield of detection of pulmonary tuberculosis. This qualitative rapid test kit detects the cell wall antibody from MTB in human urine specimens. The test was done by pipetting 2 drops of urine into the sample portion of the kit and followed by adding 3 drops of LIO detect buffer. Finally, the result was interpreted after 20 minutes of incubation at room temperature result interpreted for Negative one pink/purple line appears in the control area "C", there is no other line appear. For Positive two pink/purple bands appear one in control area ”C” and the other one in the test area ”T”. for Invalid no line appears or in the control area or line appears only test area (40).

4.8.1.6 Gene Xpert MTB/RIF testing

By using a stile pipette slowly dispense >2ml of the specimen into the open port of the X-Pert cartilage. Finally were taking the X-pert cartilage into the Gene X-pert instrument. The result interpretation of this experiment took accordingly the manufacturer instruction as follow, MTB target DNA is present the patient is TB positive, and MTB target DNA is was not detected considers as negative (32).

4.9 Data Quality Assurance

4.9.1 Pre analytical

Data quality was ensured through the use of standardized operation protocols and materials. The questionnaire is prepared based on the previous literature. The training was given for sample collectors to ensure the quality of data generated.

4.9.2 Analytical

Positive and negative control samples were run simultaneously during the testing. Additionally, continuous supervision was made by advisors and the principal investigator during data processing. All the laboratory aspects were performed with an experienced laboratory technologist in the facility of Akililu Lemma institute and St. Peter Hospital.

4.9.3 Post analytical

Results were reviewed and approved by the principal investigator.

4.10 Data management and analysis

After completion of the laboratory data collection, each result was entered, coded, and Data cleaning was using Epi Data version 3.1 and SPSS version 23. Descriptive, frequency and crosstab KAPPA analyses were done. KAPPA value shows -1(disagreement) and 1 (agreement). Finally, Results were displayed using Tables.

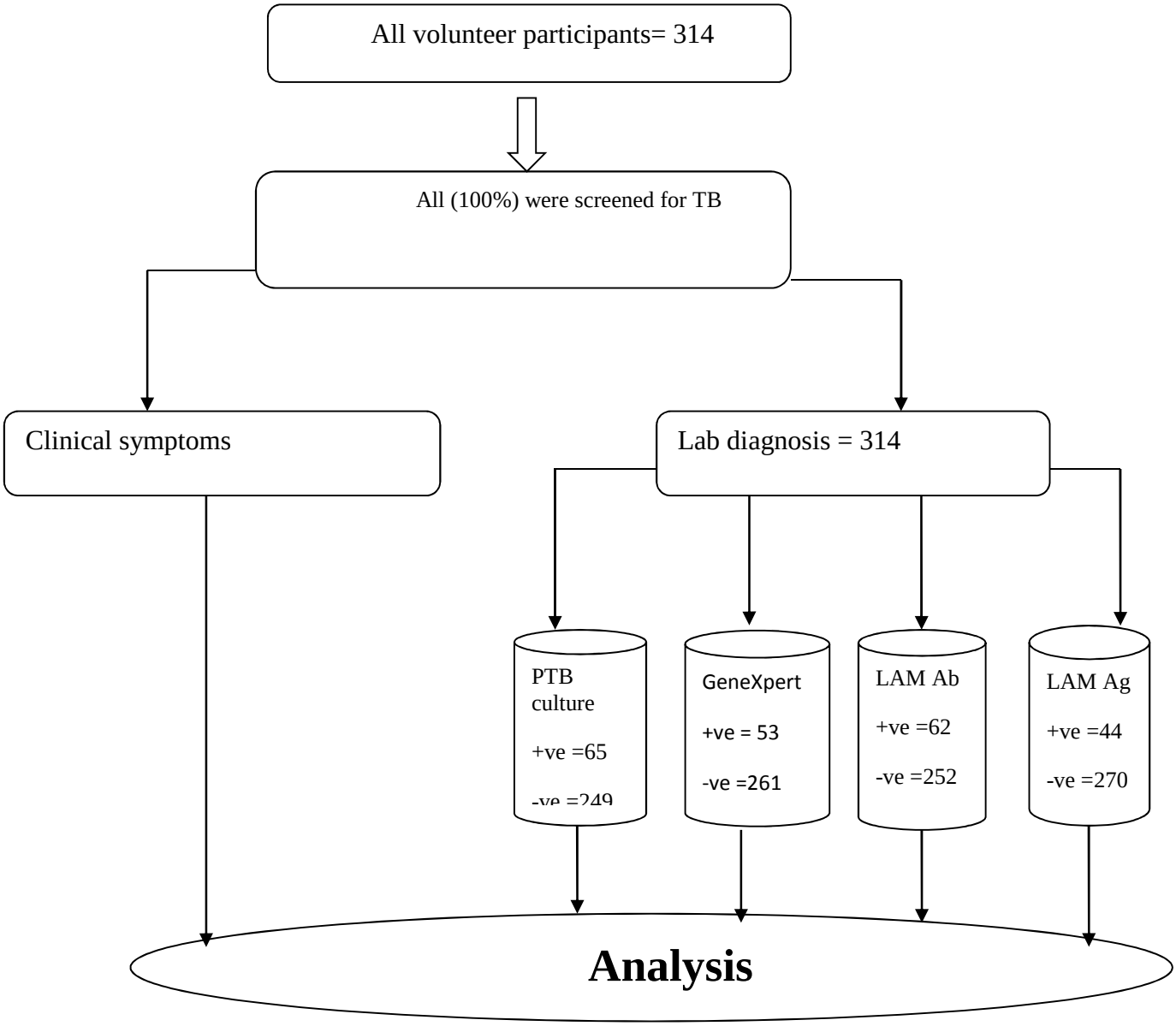

Figure1: diagram of a diagnostic evaluation of the LAM and Xpert assay in comparison to mycobacterial culture

4.11 Operational Definitions

Sensitivity: Refers ability to a TB LAM test's designate participants with a disease as positive (true positive)

Specificity: The specificity of a TB LAM test is its ability to designate participants who do not have a disease as negative(true negative)

Positive predicting value: The probability that a Participants with a positive (abnormal)TB LAM test result have the disease.

Negative predicting value: The probability that a participant with a negative (normal) test result is truly free of disease.

4.12 Ethical consideration

This study was conducted after it is ethically reviewed and approved by the Department of Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Sciences, College Of Health Sciences, and Addis Ababa University. A permission letter was also obtained from St. Peter hospital. Data collection was conducted after verbal and written consent was obtained from participants. Written informed consent was taken from each selected participant after an explanation of the survey purpose, a description of the benefits, and an offer to answer all inquiries were made to the respondents. The participants were given the right to refuse to take part in the study as well as to withdraw at any time during the study period. Identification and any information of the participants that do harm was not revealed, coded, and secured by password.

4.13 Dissemination of the results

The result of this study was presented for partial fulfillment of masters of Science in diagnostic and public health microbiology track Addis Ababa University Department of Medical Laboratory Sciences, to peer-reviewed journals for publication. The findings of this study were also disseminated to St. Peter Hospital

5. Results

5.1 Sociodemographic characteristics of participants

Out of the total 314 participants, 176 (56.1%) were males and the rest were females and the male to female ratio was found to be 1.2:1. The mean age of respondents was 47.3 ± 16 years with a minimum age of 16 years and maximum age of 83 years. The majority, 296 (94.3%), of the participants knew about the disease and few participants did not know TB .Almost two-thirds of the participants attend school (Table 1).

Table 1: Socio-demographic characteristics of study participant at St. Peter specialized hospital, Ethiopia, 2021.

Variable		Frequency (n)	Percent (%)
Sex	Male	176	56.1
	Female	138	43.9
Educational status	Illiterate	31	9.9
	Read & write only	85	27.1
	Primary	46	14.6
	Secondary	84	26.8
	Higher	68	21.7
Occupation	Housewife	76	24.2
	Farmer	18	5.7
	Government	66	21
	Private employee	8	2.5
	Self employed	56	17
	Student	28	8.9
	Jobless	62	19.7

5.2 Clinical profile of participants

Among the 314 participants who were suspected and tested for PTB, only 65(20.7%) had PTB. Symptoms of cough 310(98.7%), and night sweating 231(73.6%). Among participants of study 304(96.8%) had no contact with TB patients (Table 2).

Table 2: Clinical profile of participants suspected of PTB, AA, Ethiopia, 2021

Variable		**Frequency (n)**	**Percent (%)**
Cough	Yes	310	98.7
	No	4	1.3
Night sweeting	Yes	231	73.6
	No	83	26.4
Fever	Yes	42	13.4
	No	272	86.6
Chills	Yes	4	1.3
	No	310	98.7
Chest pain	Yes	158	50.3
	No	156	49.7
Weight loss	Yes	158	50.3
	No	156	49.7

5.3 Yield of tuberculosis using different Laboratory test

Among 314 suspected patients, only 16.9% of them had PTB using the GeneXpert method, 20.7% with the LJ culture method. TB LAM Ab (using blood) was positive in 19.7% of suspected PTB patients and only 14 % of suspected cases were positive for PTB based on urine TB LAM Ag assay (Table 3).

Table 3: Summary of the yield of PTB using TB Culture (Lj), TB LAM Ab, TB LAM Ag, and GeneXpert at St.Peter hospital, AA, Ethiopia, 2021

Variable		Frequency (n)	Percent (%)
TB Culture (Lj)	Growth	65	20.7
	No-growth	249	79.3
TB LAM Ab	Positive	62	19.7
	Negative	252	80.5
TB LAM Ag	Positive	44	14
	Negative	270	86
GeneXpert	Detected	53	16.9
	Not-detected	261	83.1

5.4 validity of TB LAM based assay and GeneXpert for the diagnosis of PTB

All the 314 participants were tested for PTB LAM assay with blood and urine samples in addition to the conventional, culture-based tests. The sensitivity of LAM Ab test was 76.9 % higher than LAM Ag-based test with a sensitivity of 64.6 %. Specificity was 98.3 % for LAM Ab and 99.1 % for LAM Ag for blood and urine respectively, the sensitivity of GeneXpert test was 69.2% and the specificity 96.7%. Agreement between LAM Ab and culture results was 91.4 % (kappa statistics 0.73) and the agreement between LAM Ag and culture was 92 % (Kappa statistics 0.725). (Table 4). Agreement between LAM Ab and GeneXpert results was 90.8 % (kappa 0.73) and the agreement between LAM Ag and GeneXpert was 92.7 % (KAPPA 0.725) and (Table 5).

Table 4: validity of LAM Ab, Ag and GeneXpert tests for the diagnosis of PTB for who had culture was positive, AA, Ethiopia, 2021

Variable		Culture		
LAM Ab		Yes	No	Total
	Positive	50	12	62
	Negative	15	237	252
	Total	65	249	314
Sensitivity (a/a+c)	76.9%			
Specificity (d/b+d)	95.1%			
Agreements	0.73(0.048-13.0)*			

Variable		Culture		
LAM Ag		Yes	No	Total
	Positive	42	2	44
	Negative	23	247	270
	Total	65	249	314
Sensitivity (a/a+c)	64.6%			
Specificity (d/b+d)	99.1%			
Agreements	0.725(0.051-13.2)*			

Variable		Culture		
Gene-Xpert		Yes	No	Total
	Positive	45	8	53
	Negative	20	241	261
	Total	65	249	314
Sensitivity (a/a+c)	69.2%			
Specificity (d/b+d)	96.7%			
Agreements	0.709(0.051- 12.654)*			

*KAPPA value

Table 5: The validity of LAM Ab and Ag tests for the diagnosis of PTB for who GeneXpert was positive, AA, Ethiopia, 2021

Variable		Gene-Xpert		
LAM Ab		Yes	No	Total
	Positive	43	19	62
	Negative	10	242	252
	Total	53	261	314
Sensitivity (a/a+c)	82.2%			
Specificity (d/b+d)	92.7%			
Agreements	0.692(0.053-12.314)*			

Variable		Gene-Xpert		
LAM Ag		Yes	No	Total
	Positive	37	7	44
	Negative	16	254	270
	Total	53	261	314
Sensitivity (a/a+c)	69.8%			
Specificity (d/b+d)	97.3%			
Agreements	0.720 (0.055-12.836)*			

*KAPPA value

5.5 Reliability of TB LAM based assay and GeneXpert tests for the diagnosis of PTB

The ability of LAM Ab to predict those who have PTB is 80.6% (50/62) which is lower than the predictive value of LAM Ag and GeneXpert 95.5% (42/44), 84.9% (45/53).The negative predictive value of LAM Ab is 94.0(237/252) which is higher than LAM Ag and GeneXpert 91.5% (247/270), 92.3% (241/261). (table6)

Table 6: Reliability of LAM Ab and Ag tests for the diagnosis of PTB for who culture was positive, AA, Ethiopia, 2021

Variable		Culture		
LAM Ab		Yes	No	Total
	Positive	50	12	62
	Negative	15	237	252
	Total	65	249	314
PPV (a/a+b)	80.6%			
NPV (d/c+d)	94.0%			

Variable		Culture		
LAM Ag		Yes	No	Total
	Positive	42	2	44
	Negative	23	247	270
	Total	65	249	314
PPV (a/a+b)	95.5%			
NPV (d/c+d)	91.5%			
Variable		Culture		
GeneXpert		Yes	No	Total
	Positive	45	8	53
	Negative	20	241	261
	Total	65	249	314
PPV (a/a+b)	84.9%			
NPV (d/c+d)	92.3%			

6. Discussion

Diagnosis of TB mostly depends on sputum smear microscopy (SSM), which is regarded as an inexpensive and popular method for the diagnosis of TB and the evaluation of the response to treatment. Many studies have been conducted on Tuberculosis. Almost all studies agreed that TB is a communicable disease with a significant worldwide mortality and morbidity rate [14]. However, most literature argued that TB is still difficult to diagnose especially in limited-resource countries where the gold standard for TB diagnosis is difficult to be implemented. However several rapid tests have been developed based on different principles to detect either antibodies or antigens in the precise sample but the evaluation of these rapid tests remains difficult. Those rapid tests have shown varying degrees of sensitivity and specificity in different studies. This study was intended to assess the magnitude of pulmonary TB using conventional and serological-based assays and comparison of Lipoarabinomanan rapid test assay with conventional tuberculosis diagnosis among TB suspected patients.

In this study all 314 participants had signs and symptoms of MTB, among them 20% of participants confirmed they had MTB by using the gold standard method of diagnosing (culture), this result was consistent with the study done in Ethiopia, Addis Abeba selected health centers in which 28.7% of participant were confirmed MTB by using culture (36). The result of this study was lower than the results in health facilities in Addis Ababa in which 63.9% participants were smear positive (30) and a study conducted in Nigeria revealed that new PTB LJ culture confirmed 78% (28). This could be due to differences in the study period, participants in Nigeria were HIV seropositive and there is variation in taking the sample size.

Our study findings showed that the performance of the LAM Ab and Ag test against culture was sensitive and specific (76.9%, 94%) in Blood, urine (64.60%, 91.50%) and GeneXpert (69.20%, 92.30%) respectively. This is also supported by one study from Brazil that was intended to evaluate the diagnostic performance of LAM in People Living with HIV with CD4 counts ≤200 cell/mm^3, in conjunction with smear microscopy or Xpert, compared to MTB culture. In this study, the overall LAM sensitivity was found to be 46.9% and specificity was 90.7%. The sensitivity and specificity in our study from blood samples showed a significant increase. The difference might be because of the source population and immunity (41).

A sensitivity of TB LAM was 93.5% and a specificity of 85.3% in discriminating healthy controls from culture-confirmed TB patients from Bangladesh, This may have accounted for the lower sensitivity and specificity reported as participants who may have been symptomatic with respiratory illnesses caused by pathogens other than TB could have been included in the clinical disease group (42).

In our study the performance of LAM sensitivity and the specificity was (76.9%), (94%) respectively. A multicenter study conducted in the Philippines and India reported a sensitivity of 71.2% and a specificity of 72% for a rapid POC device comparing participants suspected of having TB with controls (43).

A lower sensitivity (69.20%) and higher specificity (92.3%) were detected when the culture/Xpert®-negative participant group was compared to the culture/Xpert®-positive participants in our study. Bruins *et al*. also reported a sensitivity of 76.5% and specificity of 87.2% (42). A similar study by Kolk *et al*. found a sensitivity of 72% and specificity of 86% in a South African cohort comparing culture-confirmed TB participants to culture-negative participants. This study used GC-MS to differentiate between TB and non-TB breath samples (45).

There are several possible explanations for the limitations of sensitivity and specificity reported. Firstly, an individual's samples with poor quality contain hundreds of compounds with combinations that are usually distinctive of an individual (46). A study from Ghana conducted to assess the diagnostic accuracy of FujiLAM and AlereLAM assays in biobanked urine samples showed that the sensitivity of FujiLAM was 74.2% compared to 53.0% for AlereLAM, which showed a difference of 21.2%. Where the Specificity was 89.3% versus 95.6% for FujiLAM and AlereLAM, a difference of 6.3% (47).

When we compare the findings of this study with our study sensitivity 64.60% and specificity 91.50% respectively. It showed high sensitivity but poor specificity for AlereLAM. On the other hand, we have seen poor sensitivity and high specificity when we compare our results with FujiLAM. Such difference might be because the study participants from Ghana were people with HIV. There has been considerable work on the technology of urine LAM in the diagnosis of *Mtb* as well as its association with mortality related to TB. However, the research is mostly limited to few Western countries and mostly limited to Immuno-compromised patients.

Moreover, a study from Ethiopia showed the sensitivity and specificity of the TB LAM test were 30.8% and 97.3% respectively (36).We found higher sensitivity (64.6)% than in a study conducted by Lawn et al. (that was 43.7%) and Sahle et al (48,49). This difference might be because they used frozen samples and the effect of freezing urine samples is not well known.

7. Strengths and Limitations of the study

Strengths

- Use of multiple measurement tools for data collection
  - ✓ LJ culture
  - ✓ Gene Xpert
  - ✓ LAM assay

Limitations

- Test kits shortage leads to limited sample size

8. Conclusion

Pulmonary tuberculosis is still a high burden in our country. This finding of a new diagnostic approach is encouraging. Accordingly, the present finding indicated that LAM method has good agreement both with culture and GeneXpert diagnostic method. On the other hand concerning sensitivity and specificity of PTB diagnostic versus culture methods, blood LAM is best of all (urine LAM and GeneXpert) while GeneXpert is better than urine LAM test.

9. Recommendation

Since LAM method is relatively simple and inexpensive compared to GeneXpert, in the prevention and control activities of PTB, the application of LAM method in PTB diagnosis laboratories could be considered. To strengthening the present findings further large-scale studies in another part of the country need to be conducted. Policymakers should give attention to TB LAM because it has higher sensitivity and specificity than GeneXpert, is less expensive, and is easy to work with. Further extended studies must be done by fellow researchers.

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11. Annexes

11.1 Annex- I. Informed Consent Form

Dear Sir/Madam;

My name is Mekdes bekele. I am currently a postgraduate student of Addis Ababa University, Department of Clinical laboratory sciences in the field of diagnostic and public health microbiology specialty. I am going to conduct a research study. The objective of the study is to assess the magnitude of pulmonary TB using conventional and serological-based assays using serum and urine at St. Peter Specialized Hospital. Your cooperation and willingness the study are very helpful. If you are a volunteer you will be required to give serum, urine, and sputum samples. Your sample will be tested for TB culture and Gene-Xpert free of charge.

I would like to indicate that your name will not be written in the form and I assure you that all the information you give me will be kept strictly confidential. Your participation is voluntary and you are not obliged to answer any question that might be important during the testing. If you are not comfortable with the study, please feel free to stop it at any time you like.

Do you agree to be a part of the study?

Yes _____ No _____

Thank you for your participation in the study!

I hereby give my consent to participate in the study and give serum, urine, and sputum samples as instructed by health personnel. I understand there is no serious invasive procedure at the beginning as well as at the end of the study. I agreed to the terms and the conditions indicated above.

Participants signature: _____ Date: _____

11.2 Annex- II. Questionnaire

ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCE SCHOOL OF MEDICAL LABORATORY SCIENCES UNIT

Quantitative Questionnaire

1. ID No: _____

2. Date of interview: _____

3. Name of the interviewer: _____

4. Code of the institution: _____

Participant Code		
Part one: socio-demographic characteristic		
S.N	Question	Answer
1	Age in year	
2	Sex	
3	Residence	**1.** Urban **2.** 2. Rural
4	Marital status	**1.** Married **2.** Single **3.** Divorced **4.** Widowed
5	Education status	1. Illiterate 2. Primary (grade1-8) 3. Secondary (grade9-10) 4. Preparatory (grade11-12) 5. Higher level 6. Read and write only
6	Occupation	**1.** House wife

		2. Government employee **3.** Merchant **4.** Student **5.** jobless
Part two Questions designed to assess tuberculosis symptom screening		
	Question	**Answer**
1	Do you know what Tuberculosis is?	1. Yes 2. No
2	Have you experienced a productive cough more than 30 weeks	1. Yes 2. No
3	Have you experienced unexplained weight loss	1. Yes 2. No
4	Have you experienced fever, chills, or night sweets for no known reason	1. Yes 2. No
5	Have you experienced Chest pain	1. Yes 2. No
6	Have you had contact with any one with active tuberculosis disease in the past year?	1.

1. Date of specimen collection: _____

2. Type of specimen: _____

:

Result

Tb culture Positive ☐ Negative ☐

Gene expert Positive ☐ Negative ☐

AFB Positive ☐ Negative ☐

TB rapid test Positive ☐ Negative ☐

11.3 Annex- III. Principle and procedure of LIODetect TB urine Tuberculosis Rapid test

Principle of the test

It is a membrane-based test for the rapid detection of cell wall antigens in urine. This innovative and sandwich-type rapid test is based on lateral flow immune chromatography. After the sample is pipette into the sample well together with the diluents buffer it passes through the gold marked protein (Conjugate) if any antigen is present in the sample it attaches to the conjugate. This antigen-conjugate complex then flows through the membrane. To the point where the capture antibodies to MTB antigens are immobilized (test line) resulting in a red band. The reaming conjugate complex then passes through the membrane until it reaches the control zone. Again a red band appears, indicating that the test has been performed properly.

Specimen labeling and request submission

Using the laboratory protocol, each sample will be marked with all the necessary information.

Rejection criteria:

- ✓ If there is evidence of contamination in the specimen and any material used for specimen collection.
- ✓ If the specimen is too old and if there is a sign of leakage
- ✓ If the collection time and Date have not been provided
- ✓ Samples that are not collected properly
- ✓ If the Samples are not labeled

Test procedure

1) Pipet 60µL (or 2 drops using the pipet for single use) of the sample into the sample well
2) Add 3 drops of diluent buffer into the sample well
3) Adjust the timer to 20 min
4) Read the results after 20 min

Interpretation of results

Negative: one pink/purple line appears in the control area "C", there is no other line appear.

Positive: two pink/purple bands appear one in control area "C" and the other one in the test area "T".

Invalid: no line appears or in the control area or line appears only test area

Limitation of test

LIODetect TB urine rapid test has been developed to detect cell well antigen from M. tuberculosis in human urine, using this test for other fluids has not yet been validated and can yield incorrect results. The test is specific for lipoarabinomannan (LAM) from the Tuberculosis complex. Is not used to other Mycobacteria spp.

Principle of the test

It is a membrane-based test for the qualitative detection of IgG, IgA, and IgM antibodies to M. tuberculosis in serum, plasma, or whole blood. This test strip consists of a special antibody-binding protein, coupled to colored particles (conjugate), and a membrane with two test lines and one control line. The test line contains TB antigens; the control line consists of an antibody-binding protein. After the sample is pipetted into the sample well(S) followed by the diluent, the diluent sample passes through the conjugate, and the antibodies in the sample bind to the conjugate. The antibody-conjugate complex migrates due to the capillary action to the site of the membrane where TB antigens are immobilized. If antibodies against tuberculosis are present in the sample, these bind to the test lines.

Then one or two colored lines appear in the test zone”T”. The remaining complex migrates further across the membrane to the control zone “C”.

Test procedure

Serum or plasma

1) take the test cassettes from packing and remove the aluminum bag and place the cassette on a clean, non-absorbent flat surface.
2) Pipette two drops of the sample into the sample well(S) on the cassette. Use the single-use pipet contained in the aluminum bag or use an 80µL pipet.
3) Add 2 drops of test diluent into the sample well(S).
4) Start stopwatch after addition of the diluent and read the results after 20 minutes.

Whole blood

1) take the test cassettes from packing and remove the aluminum bag and place the cassette on a clean, non-absorbent flat surface.
2) Pipette one drops of the sample into the sample well(S) on the cassette. Use the single-use pipet contained in the aluminum bag or use a 40µL pipet.
3) Add 3 drops of test diluent into the sample well(S).

Start stopwatch after addition of the diluent and read the results after 20 minutes.

Interpretation of results

Negative: one pink/purple line appears in the control area "C", there is no other line appear.

Positive: two pink/purple bands appear one in control area "C" and the other one in the test area "T".

Invalid: no line appears or in the control area or line appears only test area

Limitation of test

LIODetect TB blood rapid test has been developed to detect IgG, IgA, and IgM antibodies to M. tuberculosis in serum, plasma, or whole blood. Using this test for other fluids has not yet been validated and can yield incorrect results. The test is specific for lipoarabinomannan (LAM) from the Tuberculosis complex. Is not used to other Mycobacteria spp.

11.4 Annex IV. (LJ) Lowenstein–Jensen media for TB Culture

PURPOSE

This procedure provides instructions for preparation of plain egg based media.

ABBREVIATION

LJ ---------- Lowenstein-Jensen Medium

PRINCIPLE

Egg-based media are widely used for the isolation and cultivation of mycobacteria since they can be prepared locally and offer several other advantages such as low cost, stability of the chemicals needed, long shelf-life, and characteristic morphology and luxurious growth of tubercle bacilli.
Egg mass of whole eggs is mixed with ingredients needed for the growth of tubercle bacilli. Malachite green in the medium helps to suppress the growth of contaminating bacteria and fungi. Egg mass provides fatty acids and protein necessary for the metabolism of mycobacteria. Glycerol in the medium is a source of carbon and energy; favors the growth of *M.tuberculosis* while the medium without glycerol but containing pyruvate encourages the growth of *M.bovis*. Monopotassium phosphate acts as a buffer. Magnesium sulfate provides the magnesium ion required in a large variety of enzymatic reactions including DNA replication. Asparagine is a source of nitrogen and acts as a growth stimulant. The coagulation of the egg albumen during sterilization gives a solid medium for inoculation purposes.

Preparation of materials used for the media preparation

- ✓ The day before the media preparation, all necessary equipment need to autoclave at 121^0C for 15 min and sterile by dry oven at 190^0C for 12 min
- ✓ Materials that need to autoclave are:-
  - Gauzes need to be covered with aluminum foil
  - Metal forceps and disposable forceps(covered with aluminum foil)
  - McCartney bottle screw cups(cover with separate autoclavable bag)
  - 1000ml bottle(covered by aluminum foil at the top and the lead also should be covered)
  - Dispenser(all parts should be separated and should be autoclaved by covering a separate autoclavable bag)
  - blender bowl(covered with aluminum foil)
- ✓ Materials which need to be sterile by dry oven are:-
  - Washed McCartney bottles
  - Thick-walled conical flask of 2500 ml

- ✓ Fill the insspitator with distilled water up to the tank mark and also the front drawer of the inside of the insspitator then switch on the night before the media preparation day.

procedure

1. Clean and disinfect the work area
2. Clean the eggs carefully with plain soap and water, soak them in 70% ethanol for 15 minutes and let them dry on an absorbent pad
3. Crack each egg with a sterile knife into a sterile petri dish to check its freshness
4. Transfer the whole egg into the blender bowl
5. Add sufficient eggs for the volume needed; about 20-25 eggs will be needed per liter of egg mass(up to the label of 32OZ or 4cups of the blender bowl)
6. Aseptically homogenize the egg mass by vigorous swirling using a blender for 3 minutes
7. Filter the homogenized egg mass into a sterile conical flask through 2 layers of sterile cotton gauze into a 2000ml conical flask

8. Transfer 600ml of salt solution into a sterile 2000ml conical flask with filtered egg
9. Slowly add 20ml of 2% Malachite green solution to the mixture of salt solution and egg mass. Avoid creating bubbles
10. Mix gently by swirling the conical flask to obtain a homogenous mixture.
11. Transfer 800ml of the homogenous mixture into a clean sterile 1000ml screw-cap bottle
12. Screw the dispenser device onto the screw cap bottle containing homogenous
13. Open the Bunsen burner flame and flame the top of the McCartney bottle well
14. Dispense 8ml volume into each sterile screw-cap McCartney bottle and tighten screw caps. Dispense all media
15. Repeat Steps 12 to 14 for the remaining 800ml homogenous mixture.
16. Place the tubes on the racks of the inspissator to achieve appropriate slopes; each tube should be in contact with the surface of the inspissator
17. Immediately take the tubes out of the inspissator. Allow the media to cool at room temperature and away from direct sunlight
18. Inspect all media tubes; discard tubes containing discolored or disintegrated media
19. Keep the prepared LJ media in an upright position at 2-8°C in the refrigerator for up to 3 months. Label storage trays with batch data; the name of media, lot number, date of preparation, date of expiration, and initial of technologist

11.5 Annex V. Detection of TB Using Gene Xpert MTB/RIF Test

Purpose

This procedure provides instructions on how to use the Gene Xpert instrument in detecting TB and rifampicin resistance from sputum samples.

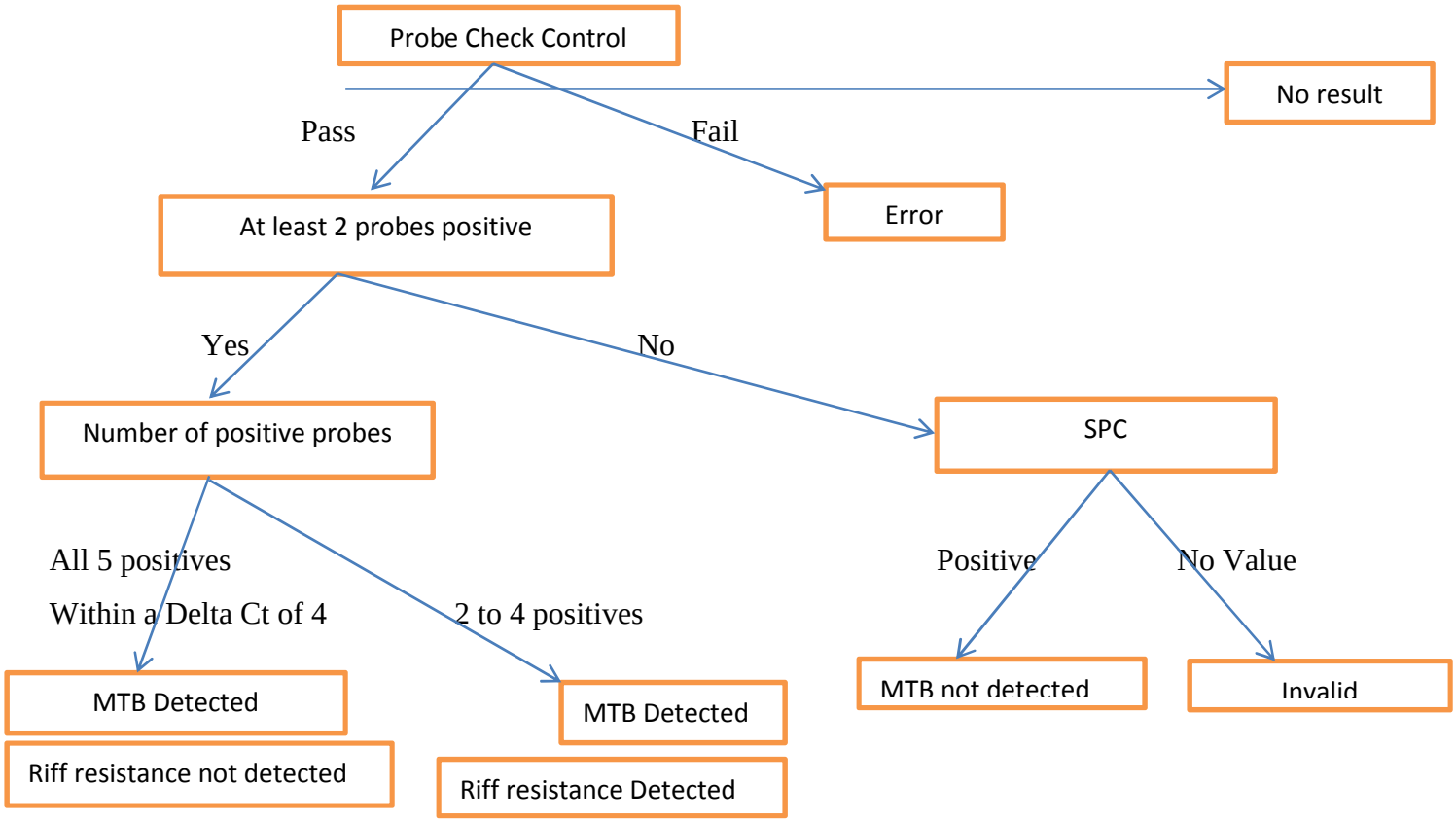
Reagents preparation: Ready to use

Reagents stability and storage:

The reagents should be stored at 2-30 °C and should be used before the expiry date

1. Quality Control

- Gene X-pert test uses the inbuilt internal quality system and the lab control and document every day IQC performance.
- The following diagram shows how to interpret IQC for the gene X-pert test.



2. Procedure

1. Check the sample integrity. If food particles are present – Proceed but be careful not to add food particles to the Xpert cartridge
2. Visually inspect and estimate the volume of sputum in the collection container
3. Open the lid of the collection container and pour SR buffer (supplied in kit) to the sputum specimen in a 2:1 (SR buffer: specimen) volume ratio
4. Close the lid of the container tightly and vortex for 15 seconds.
5. Allow the mixed specimen to stand for 15 minutes at room temperature

6. Shake the container once during the 15-minute incubation i.e. after 10 minutes
7. At the end of 15 minutes if the sample is still viscous, shake the sample again and leave it for another 5 min until it is properly liquefied
8. This mixture can be kept for up to 8 hours at 2-8ºC, in case repeat testing is required
9. Remove an MTB/RIF X-pert cartridge from its wrapper, taking care NOT TO TOUCH the back of the cartridge. Once the foil wrapper is removed, the specimen must be added to the cartridge within 30 minutes
10. Label the side of the X-pert cartridge with the sample ID. Do not write or place a sticker over the cartridge barcode
11. Use the pipette to aspirate >2ml of the specimen (just above 2ml mark on pipette) and slowly dispense it into the open port of the X-pert MTB/RIF cartridge. Do not add less than 2ml of mixed specimen to the cartridge
12. Take the X-pert cartridge to the bench with the Gene X-pert instrument. NOTE: the test must be started within 30min of adding the specimen to the cartridge.

Result Interpretation

- MTB DETECTED – MTB target DNA is present hence the patient is TB positive
- MTB NOT DETECTED – MTB target DNA is was not detected

Expected Values

- RIF Resistance DETECTED - MTB present is resistant to Rifampicin
- RIF Resistance NOT DETECTED – MTB present is sensitive to Rifampicin

Limitations

- INVALID – repeat test
- ERROR – repeat test
- NO RESULT – repeat test

Principle

The Gene X-pert (Cepheid) is a closed, self-contained platform for the extraction, amplification, and detection of the *Mycobacterium tuberculosis* (*Mtb)* complex from unprocessed samples. The Gene X-pert system can generate a result within 2 hours.

The X-pert MTB/RIF assay allows for the rapid detection of *Mtb* and Rifampicin (Rif) resistance by combining automated extraction, amplification, and detection on a single system. Rif is one of

the first-line anti-TB drugs and is also a surrogate marker for multi-drug resistant TB (MDR-TB). The assay amplifies a portion of the "rifampicin resistance determining region" of the *rpoB* gene, the most common site for Rif mutations, in real-time, using two sets of primers. Fluorescent probes are then used to differentiate between wild-type and mutant strains so that if one or more probes do not bind, this indicates the presence of a mutation and therefore Rif resistance.

A sample processing control (SPC) consisting of spores from *Bacillus globigii*, is included in the assay as an internal control to ensure adequate processing of the sample as well as to monitor the presence of PCR inhibitors. A probe check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

11.6 Annex VI. Ziehl-Neelsen staining technique.

Principle

The DOTS strategy is based on the detection of AFB by microscopy as the first recommended technique for diagnosis of tuberculosis as well as follow-up of patients during treatment.

Negative- no AFB found in at least 100 fields

Exact figure/100-1 to 9 AFB per 100 fields

1+-10 to 99 AFB per 100 fields

2+-1 to 10 AFB per field (count at least 50 fields)

3+-more than 10 AFB per field (count at least 20 fields)

12. Declaration

I the undersigned declare that this M.Sc thesis is my original work, has not be presented for a degree and it has not been submitted to any institutes as it is or modified form. Any literatures used in this study are duly acknowledged.

Name of the student:

Mekdes Bekele (BSc)

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Date of submission: _____

Name of the advisors:

Kassu Desta Signature _____

Asegedech Asmamaw signature _____