ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Use of urine as an adjunct specimen for the diagnosis of Pulmonary Tuberculosis in people living with HIV in Addis Ababa

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This is to certify that the thesis prepared by Wendwessen Zewdie, entitled:use urine as an adjunct specimen for the diagnosis of pulmonary tuberculosis in people living with HIV in Addis Ababa andsubmitted in partial fulfillment of the requirements for Master of Sciencedegree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complieswith the regulations of the University and meets the accepted standards with respect to originality andquality.

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List of abbreviations

AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
DOTS	Direct Observed Therapy Strategy
EMU	Early Morning Urine
EPTB	Extra pulmonary TB
HIV	Human Immunodeficiency Virus
LJ	Lowenstein-Jensen
MDR	Multidrug-Resistant
MGIT	Mycobacterium Growth Indicator Tube
MTC	Mycobacteria Tuberculosis Complex
PCR	Polymerase Chain Reaction
TB	Tuberculosis
PTB	Pulmonary Tuberculosis
WHO	World Health Organization
XDR-TB	Extensively-drug resistant TB
ZN	Ziehl-Neelsen
TST	Tuberculin Skin Test
HTLV	Human T LymphotropicViruse
IPT	Isoniazid Preventive Therapy
rRNA	Ribosomal Ribo Nucleic Acid
LAM	LipoArabinoMannan
BMI	Body Mass Index
DOB	Difficulty of Breath

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Abstract

Background: Mycobacterial tuberculosis is a microorganism which causes a pulmonary disease called tuberculosis, and it enhances the disability and death of an immune compromised person. The diagnosis of it is done by ZN smear, culture, LAM, GeneXpert and radiology. The part of the bacterial antigen (Lipoarabinomannon) (LAN) is excreted by renal, due to this we have a long walk to go on tuberculosis samples to diagnose. To utilize urine as an additional samples.

Objective: Use of urine as an adjunct specimen for the diagnosis of pulmonary tuberculosis in people living with HIV.

Methods: A study done on immune compromised persons was a cross-sectional study designwho were suspected PTB from MAY 2022 to May 2023. A total of 800 samples (200 sputa & 600urine) were collected. GeneXpert,MGIT,LJ, LAM and ZN smear methods were employed to analyze the samples. Drug susceptibility test were done by MGIT and GeneXpert/RIF.

Result: Out of 200 PTB suspected HIV patients, [33 %(66/200)] were sputum culture positive, of which 15 were sputum and urine culture positive. 51 were urine culture negative and sputum positive, 3 samples were become urine culture positive but sputum culture and GeneXpert negative. The risk factors of the study participants smoking was [24%(48/200,OR=1.464)], family history of Tb was [7.9%(16/200,OR=0.917)], crowded house style were [78.2%(158/200,OR=4.9)].Drug susceptibility pattern out of 72 geneXpert/RIF positive patients [19.4%(14/72) were RIF resistance and [4.1%(3/72) were RIF-indeterminate, from MGIT out of 66 MTB positive patients [21.2%(14/66)] were RIF resistance. On LAM out of 18 urine positive patients [16.6%(3/18)] were RIF resistance.

Conclusion: Even if culturing specimens is the golden method to investigate pulmonary tuberculosis, GeneXpert is the fastest and also detect the non-alive mycobacteria. In HIV patients to increase the outcome of being positive of MTB the utilization of urine as an additional specimen is a wise choice.

Key words: MTB. PTB, GeneXpert, LAM, Tuberculosis, smear, culture

1. Background

1.1 Introduction

Among the deadliest diseases of the world is tuberculosis, it is the earliest disease which attack human. Mycobacterium TB group M. bacteria are the culprits behind this illness. TB is caused by thin, aerobic bacteria. *M. tuberculosis* and other mycobacteria are frequently inert to Gram staining. Mycobacteria are classified as acid-fast bacteria because once pigmented, they cannot be destroyed by acidic alcohol. The high concentration of mycolic acids, fatty acids, and another cell wall composition lipid is primarily responsible for the acid resistance. The lungs are commonly affected. Almost all instances of drug-sensitive TB can be cured with the right care. In 50–65% of cases, the disease has a five-year mortality rate if untreated. Typically, infected pulmonary tuberculosis patients produce airborne droplet germs that transmit the disease.[1] Fatigue, anorexia, low body mass index and persistent cough with purulent sputum lasting longer steady on more than 21 days are typical indications for pulmonary tuberculosis patients. Patients with tuberculosis frequently have symptoms like hemoptysis, anorexia, night sweats, chest pain, and shortness of breath. Adultshaving pulmonary tuberculosis co-infection with HIV mostly present with atypical clinical symptoms, including concavity disease, inferior lobe infiltrates, Hilary lymphadenopathy, and pleural effusion, especially when their HIV infection is advanced. In Early HIV infection the upper lobe infiltrates and cavitation are most common in post-primary tuberculosis. Reports of tuberculosis are rising in spite of the challenging diagnosis. [1, 2].

Different samples (sputum, body fluids, urine and blood) can be used to diagnose tuberculosis using a variety of techniques, including AFB microscopy, culture, nucleic acid amplification, radiographic approaches, and the tuberculin skin test (TST). [1]

Human Immuno deficient Viruse, alentivirus subfamily of the human retrovirus family.Other domestic animal and primates are infected by non-oncogenic lentiviruses, which can also be contagious. The 4 identified human retroviruses included in two groups: Human T-lymphotropic viruses (HTLV)-I and HTLV-II, transforming retroviruses, and the human immunodeficiency viruses HIV-1 and HIV-2, which either directly or indirectly have cytopathic effects. [2] HIV is spread through heterosexual and homosexual interactions, through blood and blood products, during childbirth, throughout the perinatal period, or through breast milk when an infected mother delivers a baby to a child .After more than 25 years of study, it has been determined that there is no proof that HIV can spread by causal contact or through insect bites, such a mosquito. [2]

The symptoms of tuberculosis vary depending on the stage of HIV infection. Pulmonary tuberculosis typically manifests as infiltrates and cavitations in the upper lobes and without considerable adenopathy or pleural effusion when CMI is only partially abnormal. A tuberculosis-like primary appearance with diffuse interstitial or miliary infiltrates, little to no cavitation, and intrathoracic lymphadenopathy is more typical in the latter stages of HIV infection. [1]

As a result, diagnosing tuberculosis can be highly challenging, especially considering the diversity of HIV-related lung disorders that mimic tuberculosis. In general, sputum smears may be less commonly positive in retroviral infected tuberculosis cases thanin non-TBpatients. [1]

In developing nations, tuberculosis—which takes around forty percent of symptoms noticed on immune compromised persons, is the most prevalent opportunistic infection and the main cause of death in patients.HIV/AIDS contributes to tuberculosis epidemics in a number of ways, including by weakening the immune system and boosting the progression of latent tuberculosis to active tuberculosis, raising the likelihood of latent tuberculosis reactivating, and increasing the risk of tuberculosis infection following exposure to *Mycobacterium tuberculosis* [3]. HIV and tuberculosis co-infected individuals had a 29-fold increased risk of developing active tuberculosis [4].HIV-infected patients with immunosuppressed (low CD4 count) present with aberrant clinical characteristics and test results [5]. Co-infections with HIV and tuberculosis present unique diagnostic and treatment difficulties and create a significant strain on healthcare systems in heavily afflicted nations like Ethiopia [6].

Sputum smear has historically been employed as an early diagnostic technique for PTB, although smear-negative PTB is more frequent in patients with HIV infection, which delays detection. In these situations, sputum culture is a more accurate way to diagnose PTB, but the process can take up to 8 weeks. Naturally, the patient's condition deteriorates during this time. The fact that patients with HIV-related PTB are more likely to have abnormal or normal chest x-rays is another factor that prolongs the diagnosis process.

Additionally, this delayed diagnosis raises the expense of the healthcare system and the number of hospital admissions. Besides postponing of commencing the anti-tuberculosis could hasten retroviral infection [7].

1.2 Statement of the problem

A considerable rise in tuberculosis has been attributed to HIV infection, which is still the public health concern. Every year, it affects millions of people's health and ranks second after HIV in terms of infectious disease-related fatalities. According to the statistics, there were 8.6 million new cases of tuberculosis in 2012, or 122 cases for every 100,000 people. Asia (58%) and Africa (27%) accounted for the majority of the projected cases, whereas the eastern Mediterranean (8%) and Europe (4%) and the Americas (3%), respectively, saw lower numbers of cases.China and India together accounted for 12% and 26% of all cases worldwide, respectively. About 0.5 million and 2 million of the 8.6 million involved children.4 million in 2001. The expected number of AIDS fatalities also declined around the same time, from 2.3 million in 2005 to 1.6 million in 2012, in part because more individuals are receiving antiretroviral therapy.

Although the HIV/AIDS pandemic impacts every region of the world, sub-Saharan Africa continues to be the most afflicted. The worldwide burden of HIV infection is highest in developing nations.According to estimates, sub-Saharan Africa is home to 69% of all HIV-positive people worldwide and has a prevalence of nearly 5% (1 in 20) among adults [10]. While HIV-1 is responsible for a sizable portion of HIV infections, HIV-2 is also present in some West African nations, including Senegal, Cape Verde, Guinea-Bissau, the Gambia, and the Ivory Coast, as well as in European nations with long-standing ties to these African nations, such as Portugal and France[11].

According to estimates, 14 million people worldwide have both HIV and Mycobacterium TB infections [12], and tuberculosis continues to be the top killer of HIV-positive individuals. In nations with high HIV incidence, it is thought that having HIV doubles one's risk of developing TB by a factor of twenty [9].Around 1.1 million (13%) of the 8.7 million cases of tuberculosis that occurred globally in 2012 were also HIV-positive. Twenty percent of the 2.8 million tuberculosis patients who underwent HIV testing in 2012 proved positive, including 42% of those in sub-Saharan Africa. Only ten nations (Ethiopia, India, Kenya, Mozambique, Nigeria, South Africa, United Republic of Tanzania, and Zimbabwe, Uganda, Zambia and Zimbabwe) are projected to have more than 75% of the world's HIV-infected tuberculosis cases. [9]

The population of sub-Saharan Africa is in favor of younger age groups, and it is thought that more than one third of those between the ages of 15 - 49 have Mycobacterium TB infections, making them more susceptible to developing active tuberculosis [13]. Due to their high risk of contracting HIV, these young adults experience overlapping epidemics [14].

One of the nations with the highest rates of HIV and TB is Ethiopia. Ethiopia was placed 11th by the World Health Organization out of 22 nations having a high global burden of HIV and tuberculosis [15]. Up to 65% of active tuberculosis (TB) infections in Africa have HIV co-infection [16].

In this class of patients, TB-related mortality is highest. Extrapulmonary and disseminated tuberculosis (EPTB) is more common when HIV-associated immunosuppression worsens.[17,18]. 75% of cases are faced difficulty producing sputum, which reduces the efficacy of sputum microscopy [19]. As a result, diagnosis is challenging and sometimes delayed, and autopsy studies show that hospitalized HIV-infected people have a high prevalence of undetected tuberculosis [20, 21, 22]. Early tuberculosis treatment can lower mortality, according to recent studies [23].

There is a definite need for novel, precise, and quick tuberculosis diagnostic that would be helpful for patients who are unable to produce sputum. [24] Because it can be collected easily and painlessly, urine can be used as of option, as this study was demonstrated. Although the majority of tuberculosis cases in Ethiopia occur in HIV patients, there is no proof that using different tests makes it easier to diagnose MTB in individuals who are co-infected with PTB and HIV who are unable to produce sputum. Therefore, the current investigation objective was to assess the utilization of urine as a sample in the detection of pulmonary tuberculosis in HIV-positive individuals using culture, smear, and GeneXpert.

1.3. Significance of the study

Tuberculosis co- infection with HIV becomes more challenging for diagnosis of tuberculosis particularly in patients of weak immune status expecting disseminated tuberculosis, because they produce less sputum samples or unable to produce sputum, which increases the likelihood of a negative sputum microscope result. Having this fact there should be another sample site of collection to increase the likelihood of diagnosing Tb.

On this regard this study done on the utility of urine in addition of sputum to diagnose tuberculosis in HIV patients, based on some advantages like in very sick patients of scarce sputum its ease of collection and lack of harm to the patient, urine can be used as a substitute sample,does not produce harmful infectious aerosols, LAM and other mycobacterial antigens can be tested with it. As a result, I conducted the current investigation for assessing of importance of urine for substitute sample to diagnose PTB in HIV-infected patients of faced sputum scarce, by microscopy, MGIT & LJ methods.

2. LITERATURE REVIEW

In a prospective research carried out in India, 81 patients were suspected of having PTB, and 56.8% had a positive sputum culture. This study used urine as an extra sample for the diagnosis of active pulmonary tuberculosis, assessing tuberculosis load in urine, also to compare the sample to culture and PCR. A positive urine culture for *Mycobacterium tuberculosis* was found in 26.1% of these individuals. 8.6% of the 35 individuals with negative sputum cultures had positive urine cultures.52.2% of patients with bacteriologically verified PTB and 28.6% of patients with bacteriologically negative PTB had positive results for the tuberculosis complex-specific PCR (cfp32). [26]

About 62.3% of the 77 patients in a case-control study using PCR urine testing to identify pulmonary tuberculosis in Iran who had sputum positive culture result. ZN- smears and urine cultures both came up negative. 48.0% of patients had positive urine PCR-TB results. In 56.2% of positive and 34.4% of negative cultures from PTB patients, the particular TBPCR complex was positive. [27]

A study was done in India to compare the recovery of *Mycobacterium tuberculosis* from urine and susceptibility profile with the traditional centrifugation method, the virulence of guinea pig urine and phage isolates with matched isolates, and sputum samples from cases of pulmonary tuberculosis and *Mycobacterium tuberculosis*. By using customary centrifugation and filtration, urine samples from 236 patients with pulmonary tuberculosis were cultured. While filtration produced 12 (12.6%), centrifugation produced 27 (11.6%).95 filtered samples, or 6% of them. The contaminants were more significant when using filteration methods. Comparing the biological characteristics of M. tuberculosis isolates from sputum and urine from the same patients indicated variations in the virulence or susceptibility profiles of the test subjects in 13 of 25 (52%) isolate pairs. Additionally, in 4 out of 11 cases, the major and minor phage types of the typed phage pairs varied. [28]

Urine samples from 328 patients with generalized tuberculosis were consistently cultured for *Mycobacterium tuberculosis*, and 33 patients (10%) had a urine culture for *Mycobacterium tuberculosis*, according to a Michigan study the prevalence of Mycobacterium tuberculosis in urine cultures from patients with generalized tuberculosis was studied. Surprisingly, urine cultures for Mycobacterium TB revealed positive results in 22 patients (7%). Upon examination, it was discovered that all 22 patients had normal urine values, 58% had no current urogenital symptoms, and 3% had normal intravenous pyelorograms. [29]

The detection rates of *Mycobacterium tuberculosis* in the urine of patients suspected of having MTB were investigated in a retrospective cross-sectional investigation of increased urinary detection rates in patients suspected of having miliary tuberculosis in Japan. The findings revealed that 45 culture-confirmed MTB suspects and PCR data for *M. tuberculosis* in urine and sputum specimens were investigated among the 687 hospitalized tuberculosis patients. Urine samples produced 78.6% of PCR positive cases and 57.1% of culture positive cases. [30]

A retrospective study was done at Kyungpook National University Hospital in South Korea to compare the performance of urine cultures in solid medium (ogawa solid media) and liquid medium (BACTEC MGIT 960) for the diagnosis of military tuberculosis. 3 (12%) were positive in both medium, while 8 (32%) were positive in either liquid or solid culture. On liquid culture media alone, five specimens (20%) confirmed positive. Compared to solid medium (12%), liquid medium cultures had a substantially higher positive yield (32%) [31].

Out of 412 urine specimens, 210 were different clinical specimens (sputum and CSF) by PCR from AIDS patients; Nearly identical levels of PCR positivity (14-17%) were observed in all samples tested, according to a study conducted in Italy at the Insassari Hospital to detect *Mycobacterium tuberculosis*. After that, the outcomes were contrasted with those attained using Bactec 460 TBand AFB. Additionally, 230 additional samples, including 190 urine samples, were examined using PCR, Bactec460 TB, and acid-fast staining on seronegative patients at the Sassari hospital's. Less than half as many urine samples tested positive by PCR (6.3%) and Bactec 460 TB (2.1%) as samples from AIDS patients. All methods showed an increase in the quantity of positive sputum samples, as was to be expected. [32]

Research on Clinical Samples for Direct Molecular Detection of *Mycobacterium tuberculosis* Complex carried out in Nigeria - Two hundred clinical specimens were sent to tuberculosis laboratories in addition to the diagnosis of tuberculosis by culture. The standard Polymerase Chain Reaction sample processing technique was used to process the samples. The Ziehl-

Nelseen staining technique was used for smear microscopy on sputum samples. The results showed that in M. tuberculosis complex positive samples, 96 (48%) samples were found by PCR using a combination of boiling and vortexing, and 72 (36%) positive MTB samples were found under a microscope. The polymerase chain reaction was shown of higher sensitive (75.5%) and specific (94.5%) in comparison to the gold standard, the culture method. when identifying *M. tuberculosis* complex from clinical specimens as opposed to microscopy (sensitivity 48.5% and specificity 85.7%). [33]

Urine specimens from immune compromised and non- immune compromised persons having pulmonary tuberculosis and extra pulmonary tuberculosisthat underwent testing in Burkina Faso and had another illness (control patients) were prospectively analyzed. Three patient groups were divided into microbiologically positive and negative categories for pulmonary tuberculosis and extra pulmonary tuberculosis based on symptoms and culture findings. The test had a sensitivity of 40.5% (88/217), 66.7% (20/30), and 57.1% (48/84) for culture positive pulmonary tuberculosis, culture negative pulmonary tuberculosis and extra pulmonary tuberculosis, respectively. With 98.2% specificity. The two populations, those with and without HIV infections, were shown to differ from one another. [34]

The high diagnostic yield of Tuberculosis from screening urine samples from HIV-infected patients with advanced immunodeficiency using the Xpert MTB/RIF assay was a study done in South Africa. The sensitivity of urine Xpert among patients with CD4 cell counts of 50 and 100 cells per microliter was 44.4%, when compared to the gold standard of sputum culture. Rapid tuberculosis detection is made possible by urine Xpert testing in individuals with severe immunodeficiency and a dismal prognosis. [35]

In addition to the method of laboratory diagnosis of tuberculosis by culture, the results of a study conducted in Nigeria for direct molecular detection of Mycobacterium tuberculosis Complex of clinical samples showed that 200 clinical samples were sent to tuberculosis laboratories and performed on sputum samples using the Ziehl-Nelseen staining technique; in culture, 96 (48%) samples were positive for M. tuberculosis complex by polymerase chain and 72(36%) were positive for smear microscopy. Taking the culture as gold standars, the polymerase chain reaction was shown to be more specific (94.8%) and sensitive (75.5%) than microscopy (48.5%

sensitivity and 85.7% specificity) in identifying M. tuberculosis complex from clinical material. [36]

According to a study conducted in Addis Abeba, Ethiopia, sputum culture yielded 33 (28.2%) more mycobacterial isolates than a urine sample alone (17.5%) did in 117 patients suspected of having pulmonary tuberculosis (PTB) who also had HIV infection. Thirteen patients had positive urine cultures out of the 33 patients with positive sputum cultures. Four (4.8%) of the 84 individuals who had negative sputum cultures also had positive urine cultures, making the sensitivity and agreement between the two tests 39.4% and 0.49, respectively.[37]

The status of the urine globally using it for the diagnosis of PTB is as seen of from the literature reviews is looks to give more attention for it and using supplementary to sputum in the near future. In our country there is vastly using of urine LAM kit in addition to using sputum and urine by GeneXpert.

Conceptual frame work

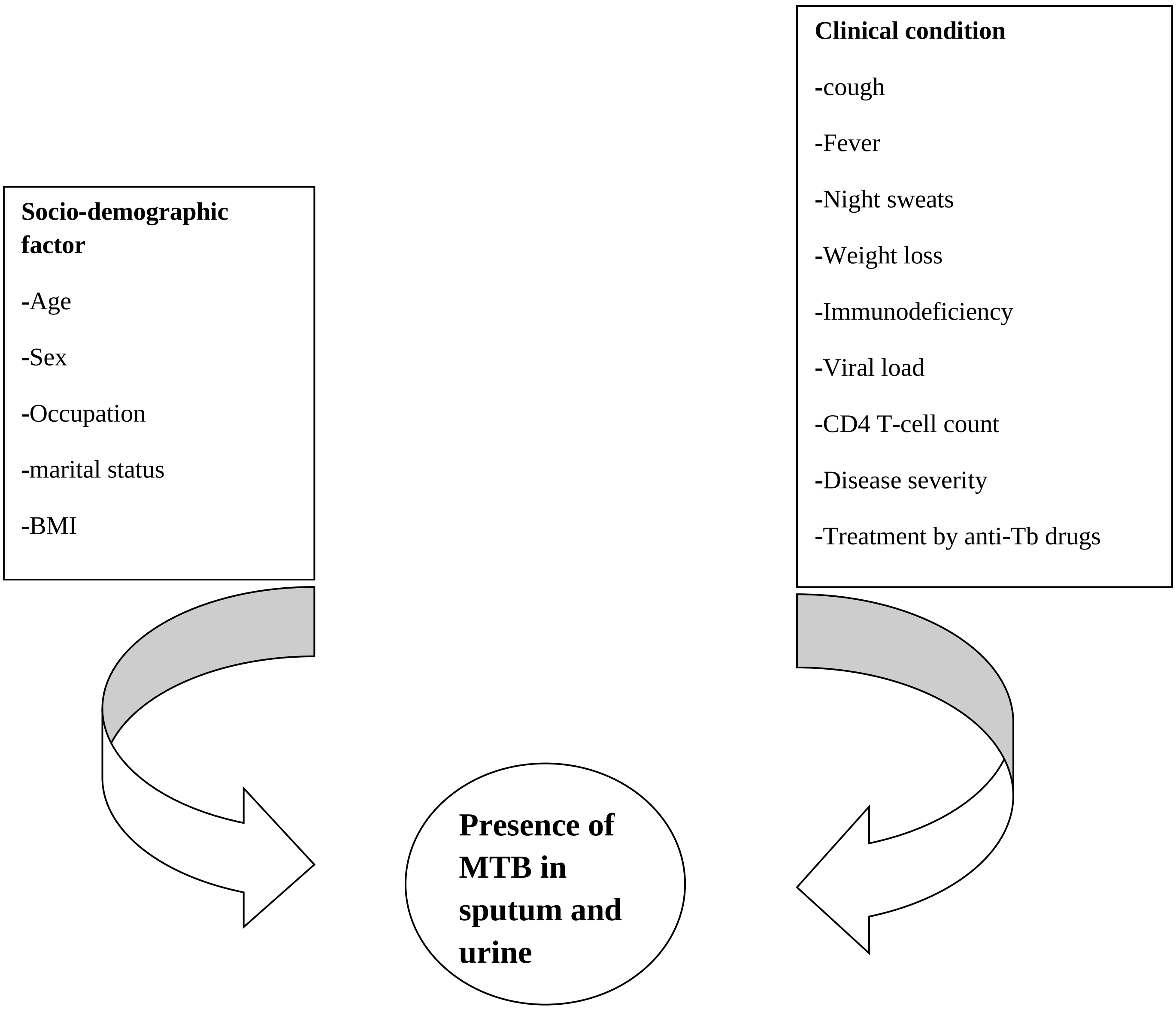


Figure 1: Conceptual framework to show relation of different variable

3. OBJECTIVES

3.1. General Objectives

➢To evaluate *Mycobacterium tuberculosis* from urine to use it as an adjunct specimen for the diagnosis of pulmonary tuberculosis by various methods in people living with HIV.

3.2.Specific objectives

➢To compare the prevalence of MTB in specimens of sputum as well as urine taken from suspicious HIV-positive cases with PTB.

➢To evaluate the effectiveness of several investigating techniques for finding Mycobacterium tuberculosis in samples of sputum and urine taken from HIV patients.

- ➢ To examine the correlation between CD4+ count and the existence of MTB in urine.
- ➢ To determine the risk factors of pulmonary tuberculosis in people living with HIV.

4. HYPOTHESIS

The detection rate of MTB from urine sample in HIV positive patients was the same to the sputum on different methods.

5. MATERIALS AND METHODS

5.1. Study area

This research was done in St. Peter specialized hospital which is located around shiro meda, Addis Ababa has an average of 65 Tb patients per day, Yekatit 12 teaching hospital located around sidist killo, Addis Ababa has an average of 37 Tb cases per day, Tikuranbessa specialized hospital around tekile haymanot, Addis Ababa has an average of 97 Tb cases per day, Nefas silk health center located around saris, Addis Ababa has an average of 21 cases per day, Churchil health center located around piassa, Addiss Ababa has an average of 16 cases per day and Kazanchis health center which is located around kazanchis, Addiss Ababa has 23 cases per day, for the reasons of all have enough number of patients, ART center, have geneXpert and geographically placed properly for the study.

5.2. Study design and period

Cross-sectional study was done from May 2022 and May 2023.

5.3 Populations

5.3.1 Source populations

All PTB cases that visit the outpatient department of St.Peter hospital, Yekatit 12 teaching Hospital, Tikuranbesa specialized hospital, Nefas silk health center, Churchil health center and Kazanchis health center during the investigating time.

5.3.2 Study Populations

All HIV positive individuals who visited the outpatient department of St. Peter Hospital, Yekatit 12 teaching hospitals, Tikuranbessa specialized hospital, Nefas silk health center, Churchil health center and Kazanchis health center and were suspected of having pulmonary TB

5.4 Inclusion and Exclusion criteria

5.4.1 Inclusion Criteria

- HIV patients with three or more PTB-suggestive characteristics, such as fever, weight loss, or a cough lasting greater than fifteen days, night sweat, Difficulty of breath, fatigue
- HIV positive patients suggestive of PTB who are inpatient or outpatient department who faced scarce sputum and able to give urine
- Who consented to join the study
- Age above 18 years.

5.4.2 Exclusion criteria

- Patients that are in critical condition
- Patients receiving (IPT) Ionized Preventive Therapy
- Patients taking anti-TB medication
- Suspicious patients having renal TB.
- HIV positive individuals who were on ART.

5.5. Study variables

5.5.1Dependent Variables

Presence of MTC in sputum and urine

5.5.2 Independent Variables

Sex, Sign & symptom, Age, CD4 count, viral load, disease severity, occupation, marital status.

5.6. Sample size calculation and Sampling method

5.6.1 Sample size calculation

The sample size was calculated using a 95% confidence interval and a 5% level of precision for the previous prevalence from previous research on the same subject. According to one study from Adama, pre-ART HIV + patients have a 7.5% higher prevalence of PTB [41]. With this knowledge, sample size was approximately estimated below:

$$n = \frac{(Z\alpha/2)^2 PQ}{d^2}$$

$\frac{(1.96)2 \times 0.075(1-0.075)}{(0.05)2}$ = 107 where, sample size together with 10% contingency 117

5.6.2. Sampling Method

To choose samples from the source population, convenience sampling techniques were used.

5.7 Measurement and Data collection

5.7.1 Data collection procedure and methods

A pre-tested questionnaire was applied for the collection of data. For the purpose of gathering data on the sociodemographic nature, clinical characteristics, and risk factors of the research attendants, structured and standardized questionnaires were used. From each health facility, a nurse and laboratory staff member were chosen, and they got training on the goal of the study, its advantages, people's rights, informed consent, and interviewing techniques for the collecting of data and specimens.

5.7.2 Laboratory Analysis

5.7.2.1 Data collection, Transportation and analyzing

About 50 ml of urine and 10 ml of sputum from three morning specimens were taken from suspicious patients of pulmonary tuberculosis in people living with HIV from the chosen health facilities after interviewing using a questionnaire and taking written information provider consent from each investigation attendant. On the day of collection, urine and sputum samples were carried in ice box to the TB laboratory of the Ethiopian Public Health Institute (EPHI) after being refrigerated. Then within a week of collection were processed.

5.8. Samples Processing

5.8.1. Urine processing

The patients provided a 50 ml sample of early morning or spot urine that was taken in a sterile, open-mouthed container. Each patient's urine sample was centrifuged for 20 minutes at 3200 rpm. An equal amount with the urine sediment,of 4% NaOHadded (with 1:1 volume) which was used to disinfect the resulting pellet. Mixed well with vortex. The mixture (urine sediment and NaOH) was incubated for 15 minutes, neutralized with phosphate-buffered saline (PBS; pH 6.8), and then centrifuged once more at 3200 rpm for 15minutes. The supernatant discarded and 2 ml PBS was added to the tube and mixed well, and smears were prepared for Ziehl-Neelsen staining. 0.2 ml was then inoculated on Lowenstein- Jensen (L-J) slants, and 0.5 ml was injected in MGIT 960 tubes (mycobacteria growth indicator tubes) for culture isolation.The suspended pellets' remaining aliquots were kept chilled to keep the viability of the mycobacteria, between 2 and 8 Oc. To prevent any cross-contamination, sputum and urine samples were handled separately.

5.8.1. Sputum processing

A falcon tube with NALC and 4% NaOH was applied to digest a sputum sample with a 1:1 volume.vortexed it to ensure thorough homogeneity. Centrifuge it for 15 minutes at 3200 rpm and 4-12 oC after mixed with PBS to neutralize the sediment. After discarding the supernatant 2ml PBS was added to the sediment. The remaining sediment was then preserved after being promptly injected onto culture medium (MGIT and LJ) and Smear preparation. This method of processing all sputum samples was used to create smears, MGIT and LJ cultures.

5.8.2. Ziehl-Neelsen staining for urine and sputum

At the processed site (In a biological safety cabinet),direct microscopic investigations of the sputum for AFB were carried out after Ziehl-Neelsen staining. After vortexing the decontaminated sediment to mix thoroughly a subset of the specimens were smeared with an area of 1x2 cm(the wideness or narrowness of the area has effect on the positivity and negativity of the slide) by taking approximately two drops /~100 microliters by using a transfer pipette of mixture, fixed by heat and air dried. After being stained with Ziehl-Neelsen (ZN) staining methods, the specimens were examined under a microscope. The outcomeswere rated and classified as positive or negative for AFB.

5.8.3. Preparation of Smears from Positive Cultures

If examining a positive MGIT culture: mix the tube well, and sample an aliquot of broth using a disposable pipette. If examining colonies from solid medium dispense ~100 µl of distilled water on a glass slide with pipette and mix with the colony from the solid medium. Air-dry the smear. Fix the sample by heat.

5.9. LIQUID CULTURE – MYCOBACTERIA GROWTH INDICATOR TUBE (MGIT)

5.9.1. Inoculation of MGITTubes

Label the side of the MIGIT tubes after preparing the antibiotic supplement (PANTA). Add 0.8 ml of PANTA and after processing the sputum/urine for culturing take 0.5 ml sputum with disposable pipette and add it to each MIGIT tube. Mix it well and then put the tubes in to the machine after scanning.

5.10. SOLID CULTURE: LOWENSTEIN JENSEN (LJ) MEDIA

5.10.1. InoculationandIncubation

After labeling the LJ tubes, inoculates the tube with 200 µL of processed urine or sputum, and wipe of the outside of the tubes with a paper towel soaked in tuberculocidal disinfectant (baciloll). By leaving the tube in slanted position with loosened cap in 37 ^{O}C incubator and then after about a week tighten the cap and incubate in upright position. Examine and record the cultures weekly for eight intervals. The MTB growth usually looked like as buff-colored, dry colony.

5.11. AN IMMUNOCHROMATOGRAPHIC ASSAY (TBAg MPT64/capilia)

Principle

A rapid immunochromatographic assay will be used to differentiate MTB and MOTT (Mycobacteria Other Than TB). The BD assay detects MPT64 antigen, while Capilia detects MPB64 antigen, a mycobacterial protein that is specifically secreted from MTB cells during culture. When a sample is added to the test device, MPT64/MPB64 antigen binds to anti-MPT64/MPB64 antibodies conjugated to colloidal gold particles present on the test strip, forming an antigen-antibody complex. This antigen-antibody complex then migrates across the test strip to the reaction area, where it is captured by a second specific MPT64/MPB64 antibody fixed to the membrane. If MPT64/MPB64 antigen is present in the sample, a color reaction is produced by the labeled colloidal gold particles and is visualized as a pink (or purple) to red line. An internal positive control is included to validate proper test performance. The test will detect the following species of MTB complex: M. tuberculosis, M. bovis, M. africanum, and M. microti.

Bring to room temperature and remove the foil pouch. Add 100μL of sample either from MGIT or bacterial suspension from LJ slant in to the specimen well. Read the result after 15 minutes.
Positive: Pink/purple to red lines form on the reading areas labeled Test [T] and Control [C] of thedevice.
Negative: A pink/purple to red line forms on the reading area labeled [C] of the device, but not[T].
Invalid: If no line is observed on the reading area labeled [C], technical errors or product damage has occurred.

5.12. DRUG SUSCEPTIBILITY TESTING: MGIT SYSTEM

First prepare the drug stock of the first line drugs. Then prepare the susceptibility testing tubes for each drug. Take an inoculum from either positive MGIT culture or positive LJ culture and prepare it for MGIT DST. Vortex the MGIT tube or diluted inoculums from LJ, mix well and let the suspension settle for 5-10 minutes. Then aseptically add 0.5 ml of the organism suspension from the supernatant in to all drug containing tubes (STR, INH, RIF, EMB, PZA). Tightly recap the tubes and mix it by inverting 3-4 times, then inter the AST sets in the MGIT 960 machine.

5.13. Quality Control

Pre-analytical, analytical, and post-analytical phases all ensured the data's quality. **Pre analytical:** The patient's request form was used to identify the patient, and data from the questionnaire was gathered by skilled laboratory staff. Senior physicians then gave the patients thorough instructions on how to collect sputum and urine samples for culture, ZN-smear and GeneXpert. Technician was appointed.The specimens were gathered in Falcon tubes, and all laboratory testing were carried out in accordance with operating standards. Specially for urine collection all the urine were collected was the morning urine to increase the probability of positivity, besides this the collection method should be in clean way to minimize the load of bacterial contaminants, the amount of the urine should not be less than 50 ml.

Analytical phase: According to the operating procedure standards for smear microscopy, all the reagents were tested on known positive and negative control samples before the patient's sputum and urine smear and control slides were made. For sputum and urine culture, the quality of the Medias should be checked and the starting and end control should be run in all through the process, the incubation temperature and time was mandatory. In order to check for contamination, cares were given when preparing the media by placing it at 37°C for 48 hours. All AFB-smear and sputum and urine culture processes were conducted simultaneously with positive and negative controls. Cross contamination was prevented by processing urine and sputum samples separately.

Post Analytical: The outcomes were registered using the distinct attendant I/D number, and data entry problems were prevented by frequent inspection and storage of the remaining samples at -20°C.It is important for all laboratories that diagnose tuberculosis to conduct a thorough quality control.

5.14. Data Analysis and Interpretation

Excel was being used to enter the data, which was then sent to SPSS version 23 for analysis. The prevalence data were computed for the entire research population and then performed a descriptive analysis before being displayed as tables.

5.15. Operational Definition

ZN-smear negative patients: are TB patients but their zn-smear is negative for sputum with various reasons, but they will become culture positive.

Presumptive Tuberculosis: a patient having cough for more than three weeks, but in bacteriology laboratory not confirmed for pulmonary tuberculosis.

HIV/TB Co-infection: A person infected by HIV and Tubercuosis.

People living with HIV: Those people who have HIV viruses in their blood.

Immunecompromised: a person whose immune status is low.

5.16. Ethical consideration

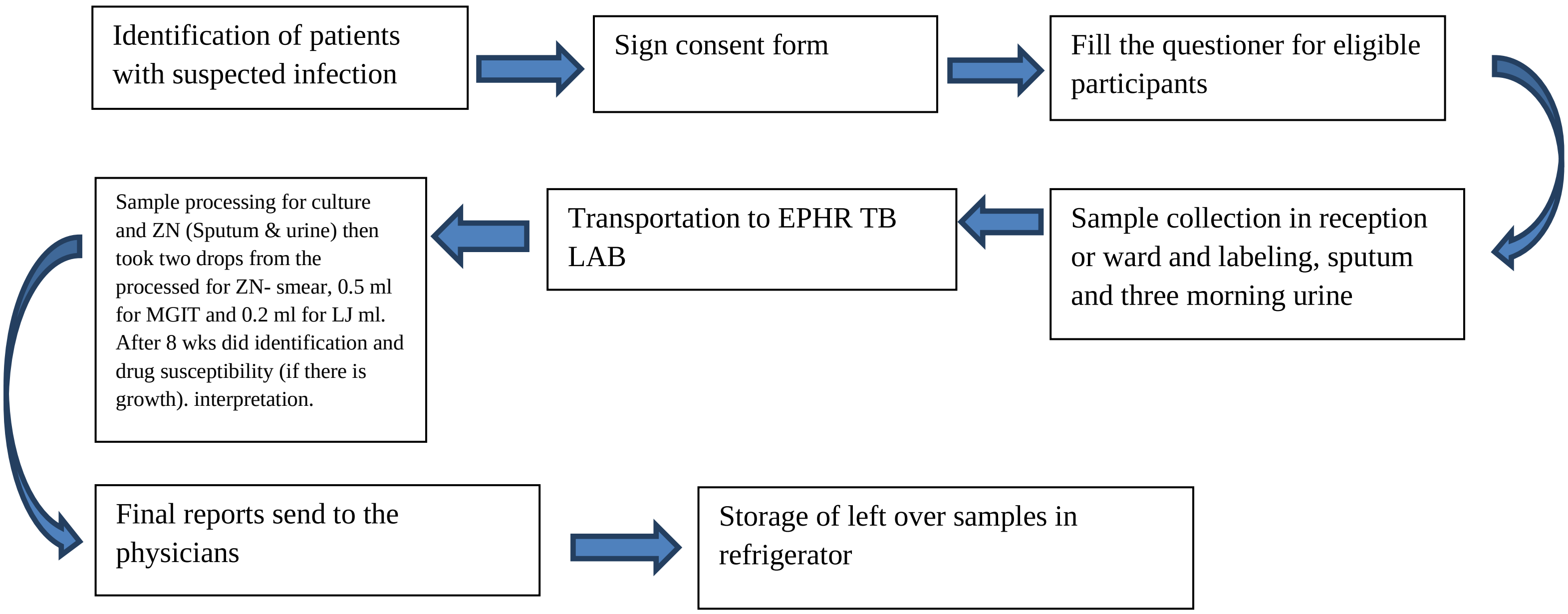
Ethical approval for the current investigation was get from the Departmental Research and Ethics Committee (DRERC) on Ref.No. MLS/113/22 of the College of Health and Medical Sciences, Addis ababa University. Besides, a letter was obtained from the medical laboratory science department on Ref.No. MLS/186/14/22 to the Ethiopian Public Health Institution (EPHI), St. peter specialized hospital, Tikuranbessa hospital,Yekatit 12 Hospital, Churchil health center and kasanchis health center. Moreover, respondents were fully informed about the purpose of the study and signed their consent. Information obtained during the study was kept confidential.

5.17. Dissemination of results

The Federal Ministry of Health, Addis Abeba University, the College of Health Sciences, hospitals, and health facilities that provided support for the study's findings, as well as other institutions, received the study's findings. The study will be presented at various conferences, workshops, meetings with stakeholders, and meetings.

6. Work flow

Fig. Work flow that shows the laboratory process



7. RESULTS

7.1 Socio-demographic characteristics of the attendants

Totally 200 eligible patients were attended in the research from May 2022 to May 2023. All of them gave their urine and sputum properly for research investigation. The participants' age ranges from 22-89 and most of the participants age category [(27.7%), 56/200] were <30. The dominant number of the attendants was female [(68.3%), 138/200]. Majority of the patients [(60.4%), 122/200] were married, [54/200, (26.7%) were single. And [108/200, (53.5%)] came from urban. Those who were employed are [48/200, (23.8%), house wife were [56/200, (27.7%), farmer [27/200, (13.4%)], student [24/200, (11.9%)] and pension [2/200, (1.0%)]. About [76/200, (37.6%)] were non-educated and [63/200, (31.2%)] were joined higher education. (Table 1).

Table 1 Socio-demographic characteristics of the study participants (N=200)

VARIABLES		FREQUENCY	PERCENTAGE
AGE	<30	56	27.7
	30-39	42	20.8
	40-49	49	24.3
	50 & above	53	26.2
SEX	Male	62	30.7
	Female	138	68.3
PLACEOF RESIDENCE	Urban	108	53.5
	Rural	92	45.5
MARIETAL STATUS	Single	54	26.7
	Married	122	60.4
	Divorced	15	7.4
	Widowed	9	4.5
OCCUPATION	Employed	48	23.8
	House wife	56	27.7
	Daily laborer	18	8.9
	Merchant	25	12.4
	Farmer	27	13.4
	Student	24	11.9
	Pension	2	1.0
EDUCATIONAL	Non educated	76	37.6

STATUS	Elementary	25	12.4
	High school	36	17.8
	Higher education	63	31.2

7.2 Clinical data

A total of 200 participants underwent questionnaire-based interviews, and all had their medical records evaluated for additional clinical information. Among the suspected pulmonary TB patients, cough was the most prevalent symptom, with a prevalence of [172/200, (86.0%)], followed by DOB [(159/200, (78.7%)], weight loss [(157/200, (77.7%)], night sweat [(141/200, (70.5%)], and fatigue [(139/200, (69.5%). The percentage of participants who had pulmonary TB when they had a cough was [63/172, (36.8%), while [108/172, (63.1%] patients had no pulmonary TB but had cough, and [54/141, (38.3%] patients had pulmonary TB had night sweat. Participants with no cough but pulmonary TB diagnoses included [3/28, (10.3%)] and their urine result showed culture and smear negative and their CD4 results were 100-149 cells/mm3. A statistical analysis showed that clinical symptoms such as cough, night sweat and fever have significant association with PTB ($p<0.05$), whereas the rest of the clinical symptoms have no significant association with PTB ($P>0.05$) (Table 2)

Table 2 compares the clinical characteristics of PTB cases to those of non-PTB cases in the group whose PTB status was confirmed by AFB smear test, culture, and GeneXpert.

Clinical symptoms	Frequency	Pulmonary TB positive	Pulmonary TB Negative(onculture,smear & GeneXpert	X^2	P-value
Cough	172	63(36.8%)	108(63.1%)	7.410	0.004
Fever	131	50(38.1%)	81(61.9%)	7.744	0.002
Nausea & vomiting	79	25(31.6%)	54(68.4%)	0.072	0.789
DOB	159	52(32.7%)	107(67.3%)	0.022	0.881
Chest pain	135	50(37.0%)	85(63.0%)	3.184	0.074
Night sweat	141	54(38.2%)	87(61.8%)	7.325	0.003
Chills	84	29(34.5%)	55(65.4%)	0.202	0.202
Fatigue	139	41(29.4%)	98(70.5%)	2.425	0.119
Weight loss	157	53(33.7%)	104(66.3%)	0.213	0.644

7.3 Laboratory results

A) Findings of sputum samples from Xpert MTB/RIF, MGIT, L-J and Smear

Out of 200 sputum samples collected from participants for evaluation a total of [66/200, (33%)] were become positive for pulmonary TB which was proven by the gold standard method [culture (MGIT/LJ)]. Among the positive sputa [62/66, (93.9%)] were positive on liquid culture media which is mycobacterial growth indicator tube (MGIT) while [48/66, (72.7%)] were positive on L-J solid culture media, surprisingly [47/66, (71.2%)] were smear positive. On Xpert MTB/RIF out of 200 sputum samples [72/200, (36%)] were positive and of this [6/72, (8.3%)] samples were culture (MGIT/LJ) negative. The Xpert MTB/RIF method outperformed the culture and smear procedure, according to the results. Every culture and smear positive results were also genexpert positive. Although Xpert MTB/RIF were identifies further Pulmonary TB in [6/134(4.4%)] sputum culture negative. Xpert MTB/RIF's likelihood of correctly diagnosing a patient with pulmonary tuberculosis is significantly higher than the culture. (p=0.01)

Table 3: Finding of PTB from sputum samples of Xpert MTB/RIF, MGIT, LJ and Smear

Methods	Frequency	PTB positive on culture	PTB negative on culture
GeneXpert	72/200, (36.0%)	66/72, (91.6%)	128/200, (64.0%)
MGIT	62/200, (31.0%)	62/66, (93.9%)	138/200, (68.3%)
LJ	48/200, (24.0%)	48/66, (72.7%)	152/200, (75.2%)
Smear	47/200, (23.5%)	47/66, (71.2%)	153/200, (75.7%)

B) Findings of urine samples from LAM, MGIT, LJ and Smear

A total of 572(U1=200, U2=183, U3=189) Urine samples were collected from 200 participants for evaluation (each patient should give three morning urine but there was a miss) and Evaluated on LAM, MGIT, LJ and smear. Out of 200 participant's urine [18/200, (9%)] were positive on LAM [out of 18 LAM positive samples U1=18(100%), U2=18(100%), U3=18(100%) were positive]. Out of 200 participants' urine [15/200, (7.5%)] were become positive for pulmonary TB on culture (MGIT/LJ)]. Among the positive PTB urine samples [15(100%)[[U1=15(100%), U2=15(100%), U3=15(100%] were positive on liquid culture media which is mycobacterial growth indicator tube (MGIT) while [15(100%)[[U1=15(100%), U2=15(100%), U3=15(100%) were positive on L-J solid culture media and [12/200, (6%)[[U1=6(50%), U2=3(25%), U3=3(25%) were smear positive.

Out of 200 participants' urine samples [15/200, (7.5%)] were positive on culture(MGIT/LJ) and their sputum were also positive for PTB on culture(MGIT/LJ) and Xpert MTB/RIF, but three (3) urine results were positive on LAM but negative on urine culture, besides were negative for sputum PTB which were analyzed by culture (MGIT/LJ) and GeneXpert.

Table 4: Findings of Urine samples from LAM, MGIT, LJ and Smear

Methods	Frequency	Sputum & urine positive	Urine(U1,U2,U3) positive & sputum negative	Urine positive for MTB			Urine negative for MTB		
				Urine1 (U1)	Urine2 (U2)	Urine3 (U3)	Urine1 (U1)	Urine2 (U2)	Urine3 (U3)
LAM	18(9%)	15	3	18(9%)	18(9%)	18(9%)	182(90.1 %)	165(82.1%)	171(85.1%)
MGIT	17	15	2	16(7.9 %)	18(8.9%)	15(7.4%)	184(91.1 %)	165(83.6%)	174(85.1%)
LJ	16	15	1	14(6.9 %)	18(8.9%)	15(7.4%)	186(92.1 %)	165(83.6%)	174(86.1%)
Smear	15	12	3	7(5.4%)	4(5.9%)	4(5.9%)	193(91.6 %)	174(86.1%) 179(90.4%)	183(93.6%)

7.5 MTB isolation from sputum and urine samples of HIV patients by diagnostic methods of Xpert MTB/RIF, culture, smear and LAM

We got the biggest figure on the identification of MTB by combination of different diagnostic methods like Xpert MTB/RIF, culture, smear and LAM from sputum 72/200(34.2%) and urine 18/200(25.6%). Of 72/200(34.2%) MTB identified from sputum by Xpert MTB/RIF method and of which 66/72(91.6%) were culture positive (gold standard). Smear detected MTB in 47(27.5%), in addition Xpert MTB/RIF also identifies 6/72(8.3%) from sputum of which culture were negative. Plus to this, 18/200 (9%) MTB detected from LAM urine samples and of which 15/18(83.3%) were positive MTB on liquid and solid culture media and three were gives culture negative. Out of 18/200 urine LAM positive samples, 15/18(83.3%) were become positive by smear. (Table 5)

Table 5: MTB isolation from sputum and urine samples of HIV patients by diagnostic methods of GeneXpert, culture, smear and LAM

Sample	GeneXpert		Culture		LAM		Smear		Total	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Urine	-	-	15	185	18	182	14	186	18	172
Sputum	72	128	66	134	-	-	47	153	72	128

7.6 The relation of urine MTB positive and CD4+T cells count

From 66 PTB patients investigated, 51/66(77.2%) had low urine bacterial load or there were no mycobacterium in urine, (the majority were urine culture negative and smear negative). On the contrary 15/66(21.7%) which were urine culture and smear positive had high urine bacterial load. As we seen from our study, patients less than 100 cells/mm3 (lower) CD4 count, had high urine mycobacterial load, which means out of 15urine culture positive MTB 14 were smear positive which indicates that there were a high probability of high bacterial load in urine. Statistical analysis revealed that, there is a robust correlation between low CD4 levels and the identification of MTB in urine smear, r (198)=0.68, p=0.001 (Table 6)

Table 6: The relation of urine MTB positive smear and CD4+T cells count(N=200)

CD4	Frequency	Urine culture positive	LAM	Smear
< 100 cells/mm3	15	15	15	14
100-149 cells/mm3	8	3	3	3
150-199 cells/mm3	14	0	0	0
>= 200 cells/mm3	32	0	0	0

7.7 Risk factors of the investigation attendants for Pulmonary Tuberculosis

To find the risk factors related to PTB, 200 individuals with probable pulmonary tuberculosis were investigated. Smoking, alcohol use, diabetes, and family history of TB cases were the risk factors for PTB that we focused on, with respective rates of 24.0%, 6.5%, 5.4%, and 7.9%. In comparison to individuals who were TB negative, an increased percentage of participants had smoking ($P<0.001$) and lived in crowded homes with mud walls ($P<0.001$). (Table 7)

Table 7: Risk factors of the study participants

Risk factors		Frequency (N=200)	%	GeneXpert (N=72)	Culture (N=66)		Smear (N=47)	OR
Smoking		48	24.0	19	19		9	1.464
Alcohol consumption		13	6.5	5	5		17	1.291
Family history of Tb		16	7.9	5	5		4	0.917
Past History of Tb		0	0	0	0		47	
DM		11	5.4	7	7		4	3.856
House Style	Crowded	158	78.2	81	64		37	4.9
	Ventilated	42	20.8	33	23		10	0.9

7.8 **Drug susceptibility test of sputum samples subjected to GeneXpert and MGIT**

Out of 200 sputum samples that were analyzed by Xpert MTB/RIF and 72/200 became positive for MTB, of which 14 were RIF resistance, 55 were not RIF resistance and three sputa became RIF indeterminate. Of 200 sputa, pulmonary TB positive on culture were 66 i.e the gold standard. for Rifampicin resistance out of 66 sputa positive for MTB 14 were RIF resistance and 52 were RIF not resistance and three sputa which were RIF indeterminate were now became RIF resistance. 200 participants' urine samples were analyzed by LAM and of which 18 were became positive. Out of 18 LAM positive samples 3 were RIF resistance and 12 were RIF-not resistance.

(Table 8)

Table 8: Drug susceptibility pattern of GeneXpert and MGIT

Methods	MTB positive frequency	RIF Resistance	RIF-Not Resistance	RIF-Indeterminate
GeneXpert	72/200, 36%	14/72, 19.4%	55/72, 76.3%	3/72, 4.1%
MGIT	66/200, 33%	14/66, 21.2%	52/66, 78,7%	-
LAM	18/200, 9%	3/18, 16.6%	12/18, 66.6%	-

8. Discussion

The most frequent cause of death among people living with HIV is tuberculosis,especially among individuals in underdeveloped nations where diagnostic resources are scarce. Due low tendency of AFB smear on the diagnosis of TB and sputum scarce patients who are living with HIV leave the diagnosis difficult for decades. There were a push to develop a new diagnostic method for PTB, which in addition will be more sensitive and specific, has an answer for sputum scarce patients. On this regard science trying to solve the problem by developing new methods on TB which analyzes sputum and urine, as well as easy to apply in rural area and scarce resource settings.

In our current investigation, 56 (27.7%) of the 200 eligible participants were aged 18-29, and 46.9% of the confirmed PTB cases were discovered in this age range. The majority of studies on HIV-infected patients around the world, where PTB/HIV was shown to be increased among this age [38], confirm this conclusion. This could be explained by in this age group definitely exhibits a strong interest in sexual activity, which is the primary method of HIV/AIDS transmission. According to statistical analysis, clinical symptoms like fatigue, night sweat and a cough in HIV-positive individuals had significantly correlate with pulmonary TB infection ($p<0.005$). This result was consistent with a research that evaluated a WHO recommendation to enhance TB diagnosis in ambulatory HIV-positive people [39].

The prevalence of pulmonary TB among HIV-positive individuals in this study was 72/200 (36%) and agrees to that of a study done in Nigeria, where the prevalence was 37% [40]. Our results, however, were significantly better than those of studies carried out in various regions of Ethiopia [41, 42]. The diagnostic technique and further sample (urine) we employed in our study may be responsible for this discrepancy.

The capability of ZN-smear detection under resource limited area determines the prevalence of pulmonary tuberculosis. According to our findings, the ZN smear 47 (23.5%) capacityto detect MTB in pulmonary tuberculosis is sufficient to classify patients and start early treatment.

However, this result was nearly identical to that of a study done in Rwanda, where the detection rate was 26% [44].

The main issue in diagnosing and treating tuberculosis in HIV-infected patients is the rate of smear-negative pulmonary tuberculosis. One choice in such a situation is to engage in culture. Out of 200 PTB suspected patients, the culture method wasdetecting 66 (33%) cases. This result revealed a little variation from the research done in Singapore, where the probability of being positive rate of culture was 27.1%, but was not put in comparison to the study conducted in India, where the probability of being positive rate of culture was 56.8% [45]. Xpert MTB/RIF molecular approaches are currently the best hope for identifying pulmonary tuberculosis in immunocompromised patients. In the current study, 72 (36%) patients living with HIV had PTB cases that were detected by Xpert MTB/RIF. Our results were consistent with the research conducted byAlemu chemeda in Addis Ababa (37.6%) [37], and a research also conducted in Iran (35.8%) is compatible with our findings.[48]. This result has shown a slight variation with a research conducted in Brazil (41.2%).[47]

There is little information on the use of urine as a clinical specimen for the diagnosis of PTB in developing as well as industrialized nations. But it has gained interest since the HIV epidemic and offers hope for people who faced sputum scarce or smear negative results in the future. In the current investigation, sputum culture discovered 66 (33%) more mycobacterial isolates than urine specimen which was 15 (7.5%). This outcome was significantly varied with a research done by AlemuChemedain Addis Abeba, which found 28.2% and 14.5% of mycobacteria in sputum cultures and urine culture respectively. It also not comparable with a study done in India, which was sputum culture 56.8% and urine culture 19.7%.[37, 45]. This discrepancy could be explained by the sample size of the studies or the clinical status of the studies participants. But once more, our outcomes were somehow the same with those of a research conducted in Bangalore that found MTB in 19 (8.8%) of PTB patients' urines [49]. However, three (1.5%) of the 134 individuals with negative sputum cultures also had positive urine cultures. This outcome is somewhat identical to a study conducted in Indonesia where 2.6% of urine cultures that were positive [45]. And a study conducted by AlemuChemeda where (4.8%) urine cultures were positive, this has somehow in line with our findings. This statistic could incline us to believe that collecting urine samples in addition to sputum samples will improve the likelihood of identifying pulmonary TB, particularly in cases of sputum scarce patients or disseminated tuberculosis.

Some studies attempted to show the value of urine as a clinical specimen in diagnosing pulmonary TB in both HIV and non- HIV cases to get around the problem of sputum smear's subpar performance. In this study, 66 (33%) of HIV patients had pulmonary TB that was confirmed by sputum culture, and 18 (9%) of these individuals also had positive urine cultures and urine LAM tests. Our findings shown that PTB can be identified using a readily available urine specimen supported by LAM and culture.

Urine Mycobacterium TB identification rate is significantly correlated with base line CD4 count. Pulmonary tuberculosis in HIV patients is highly correlated with low CD4 count. The frequencies of isolation from culture, Xpert MTB/RIF and smear for patients under 100 cells/l CD4 count were 21.9%, 25.2% and 21.2 % respectively. However in those patients who had a CD4 count more than 200 cells/l, mycobacterium tuberculosis detected by culture, GeneXpert and smear were only 12.1%, 15.9% and 6% respectively. For positive culture time, urine has longer culture time than Sputum culture in the diagnosis of tuberculosis because the acidity of the urine kills most of the mycobacterium. This gives a flush light to conclude that the bacterial burden in sputum is higher than urine. But when we came to the LAM assay it basically identifies an antigen which is the part of the mycobacterium called lipoarabinomanan which does not affected by the acidity of the urine. The patient's CD4 T cells, which are negatively correlated with bacillary load, influence the patient's urine and sputum samples. Which means the amount of bacteria burden in the urine sample marginally increased as the CD4 T cell count decreased [54]. The correlation between the CD4 count and the AFB technique for PTB diagnosis in immunocompromised patients was adversely linked (-0.462) with the rate of MTB detection in urine samples. Our findings confirm that having an active TB infection while also having a low CD4 cell count in an HIV person.

Being diabetic increased the risk of TB in those with HIV, according to our study. People who had been Diabetic have 3times to develop PTB than non -diabetic. (95%CI OR=3.85). This result in line with data from South Africa, which found that MTB detection in HIV-infected patients, is significantly correlated with all WHO screenings [53]. Cigarette smoking in those of HIV patients the risk to develop PTB is 1.4 times than the non-smoker, a research done by Alemu Chemeda and another research which was done in India do not support this conclusion. [37, 55].

Those who live in crowded house had a probability of a two-fold increased risk of developing PTB compared to those who live in vented housing (95% CI).

The drug susceptibility patterns of the different methods were more or less similar and have no any discrepancy. The samples which were analyzed by Xpert MTB/RIF had 72 positive MTB, of which 14 were RIF resistance, 59 were RIF not resistance and three were became indeterminate. The Xpert MTB/RIF analyzer has four modules and only detects RIF resistance. While we were observe the MGIT drug susceptibility pattern out of 200 sputa samples 66 were became PTB positive and of which 14 were RIF resistance and 52 were RIF not resistance. The three samples which were RIF-indeterminate on GneXpert were became RIF resistance on MGIT.

9. Conclusion and Recommendation

In Ethiopia and other nations where TB and HIV are common, diagnosing and treating PTB in people who are immune compromised continues to be a challenge. In order to better understand how MTB excretes through urine in HIV-positive individuals, this study has been conducted. According to the current research, from bacteriologically isolated and confirmed pulmonary mycobacterium tuberculosis positive sputum specimens, urine culture and LAM can be used to detect 7.5%. The above figure which indicates us that generally mycobacterium tuberculosis excreted through urine in HIV patients, so urine LAM should be run congruently with sputum culture and smear to diagnose MTB in this group of patients, this makes the diagnosis of tuberculosis in HIV=positive individuals. For sure, when we conjugate LAM and urine (the common specimen to get easily) in addition to the familiar diagnostics methods in our algorism, we were increase the detection possibility of disseminated pulmonary tuberculosis in 1.5%.This result is an eye opening for the appropriate persons to think twice to use urine as an alternative specimen for the diagnosis of pulmonary tuberculosis for sputum scarce in immune suppressed individuals.

The strength and limitation of the research

- All the sputum were analyzed for pulmonary TB by Xpert MTB/RIF, culture (MGIT/LJ), and Smear which increases the positivity of the mycobacterium.
- All urine samples were analyzed for pulmonary TB by LAM, culture(MGIT/LJ), and smear
- The samples were transported from the collection sites to EPHI within one week which maintain the liability of the bacteria
- From this research we can measure the prevalence of disseminated TB and also we can identifies the risk factors for TB
- It give a firm evidence that we can diagnose pulmonary TB from immune compromised patients from urine samples

The limitations of the study

- It may not give any assurance to use urine for the diagnosis of TB from the non-disseminated HIV patientsalso asymptomatic urogenital might have an impact on the result

- ➢ The sample size were not satisfactory for more analysis
- ➢ The resource limitation

The outcomes of our investigation have led to the following recommendations:

✓ Urine must not be overlooked for the diagnosis of PTB in immune compromised persons, despite the higher weight of mycobacterium and higher rates of culture positivityin sputum than urine specimen.

✓ When possible, doing urine culture and LAM could be utilized as a diagnostic tool for PTB suspected patients when sputum samples are scarce.

✓ Urine culture should also be performed besides to sputum culture to obtain a more precise diagnosis of PTB in immune compromised patients

- ✓ It should be recommended specially for the diagnosis of disiminated Tuberculosis suspected patients
- ✓ Applying a molecular tests for diagnosis of tuberculosis is mandatory
- ✓ Better to study on the genitourinary tuberculosis

10. References

1. Harison's.principles of internal medicine. In: Fauci, Braunwald, Kasper, Hauser, Longo, Jameson, Loscalzo. 17th edition. Mcgraw-Hill's:2008.
2. Nelson: text book of pediatrics. In: Kliegman, Stanton, ST. Geme, Schor, Behrman. 19th edition. Elsevier:2011.
3. Harries AD, HargreavesNJ, Kemp J, Jindani A, Enarson D, Maher D, Salaniponi F, Deaths from tuberculosis in sub-Saharan African countries with a high prevalence of HIV-1. Lancet, 2001. 357: p. 1519+1523.
4. WHO, Global Tuberculosis report, in WHO Report. 2014.
5. Affusim, CC, E. Kesieme, and VO Abah, The pattern of presentation and prevalence of tuberculosis in HIV-seropositive patients seen at Benin City, Nigeria. International Scholarly Research Notices, 2012.
6. Federal Ministry of Health (FMOH), HIV Prevention and Control Office: Single Point HIV Prevalence Estimate. Addis Ababa, Ethiopia. 2007.
7. Asgedom TT, Gunnar B, Getachew A, Clinical and Radiological Features in Relation to Urinary Excretion of Lipoarabinomannan in Ethiopian Tuberculosis Patients.Scand J Infect Dis 34: 167–171, 2002.
8. WHO. 2013. Global tuberculosis report. WHO, Geneva.
9. WHO. 2015. Global tuberculosis report. WHO, Geneva.
10. UNAIDS. 2012. Report on the global AIDS epidemic. United Nations, New York.
11. Camacho RJ., Special aspects of the treatment of HIV-2-infected patients. Intervirology 55: 179–183,2012.
12. Getahun H, Gunneberg C, Granich R, Nunn P. HIV infection-associated tuberculosis: Clin Infect Dis 50: S201–S207,2010.
13. Styblo K. Overview and epidemiologic assessment of the current global tuberculosis situation with an emphasis on control in developing countries. Rev Infect Dis 11: S339–346,1989.
14. Girardi E, Raviglione MC, Antonucci G, Godfrey-Faussett P, Ippolito G. Impact of the HIV epidemic on the spread of other diseases: The case of tuberculosis. AIDS 14: S47–S56,2000.

15. WHO. 2019. Global tuberculosis report. WHO, Geneva.
16. WHO (2011) WHO Report 2011: Global tuberculosis control. Geneva: World HeathOrganisation. ISBN 978 92 4 156438 0.
17. Reid MJ, Shah NS, Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings. Lancet Infect Dis 9: 173–184,2009.
18. Jones BE, Young SM, Antoniskis D, Davidson PT, Kramer F, et al. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with human immunodeficiency virus infection. The American review of respiratory disease 148: 1292–1297,1993.
19. Peter JG, Theron G, van Zyl-Smit R, Haripersad A, Mottay L, et al. Diagnostic accuracy of a urine LAM strip-test for TB detection in HIV-infected hospitalized atients. The European respiratory journal: official journal of the European Society for Clinical Respiratory Physiology,2012.
20. Cox JA, Lukande RL, Lucas S, Nelson AM, Van Marck E, et al. Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa andcorrelation with clinical diagnoses. AIDS Rev 12: 183–194, 2010.
21. Rana FS, Hawken MP, Mwachari C, Bhatt SM, Abdullah F, et al. Autopsy study of HIV-1-positive and HIV-1-negative adult medical patients in Nairobi, Kenya. J Acquir Immune DeficSyndr 24: 23–29, 2000.
22. Cohen T, Murray M, Wallengren K, Alvarez GG, Samuel EY, et al.Theprevalence and drug sensitivity of tuberculosis among patients dying in hospital in KwaZulu-Natal, South Africa: a postmortem study. PLoS Med 7, 2010.
23. Holtz TH, Kabera G, Mthiyane T, Zingoni T, Nadesan S, et al. Use of a WHO recommended algorithm to reduce mortality in seriously ill patients with HIV infection and smear-negative pulmonary tuberculosis in South Africa: an observational cohort study. The Lancet infectious diseases11:533–540, 2011.
24. Peter JG, Theron G, Muchinga TE, Govender U, Dheda K. The Diagnostic Accuracy of Urine-Based Xpert MTB/RIF in HIV-Infected Hospitalized Patients Who Are Smear-Negative or putum Scarce. PLoS ONE 7, 2012.
25. WHO. 2016. Global tuberculosis report. WHO, Geneva.

26. Krishnamoorthy G., Sarman S. Urine as an adjunct specimen for the diagnosis of active pulmonary tuberculosis, International Journal of Infectious disease 13:374-379, 2009.
27. Ali Ah ;Masood Reza Md; Kiarash G, Urine PCR Evaluation to Diagnose Pulmonary Tuberculosis, Jundishapur J Microbiol. 2014 March.
28. WHO. 2017. Global tuberculosis report. WHO, Geneva
29. Rodney RB, Donald GD, Yuan Yu, Pang Y, Hairong H, The incidence of urine cultures positive for mycobacterium tuberculosis in a general tuberculosis patient population.
30. Toshinobu Yokoyama, Takashi Kinoshita, Masaki Okamoto,et al. High Detection Rates of Urine *Mycobacterium tuberculosis* in Patients with Suspected Miliary Tuberculosis.(Intern Med 56: 895-902, 2017)
31. Chang Ho Kim, Eun Jin Kim, Seung Ick Cha and Jaehee Lee, The Diagnostic Yield of Urine Cultures with Liquid Media in Cases of MiliaryTuberculosis.Intern Med Advance Publication.
32. Leonardo A. Sechi,Maria Piera Pinna,*1* Alberto Sanna, Detection of Mycobacterium tuberculosis by PCR analysis of urine and other clinical samples from AIDS and non-HIV-infected patients.*Molecular and Cellular Probes (1997)* 11, *281–285*
33. Alli OAT, Ogbolu OD, Alaka OO. Direct molecular detection of *Mycobacterium tuberculosis* complex from clinical samples – An adjunct to cultural method of laboratory diagnosis of tuberculosis. North Am J Med Sci2011; 3: 281-288
34. Gabriela Torrea, Philippe Van de Perre, PCR-based detection of the Mycobacterium tuberculosis complex in urine of HIV-infected and uninfected pulmonary and extrapulmonary tuberculosis patients in Burkina Faso. Journal of Medical Microbiology (2005), 54, 39–44.
35. Stephen D. Lawn, Andrew D. Kerkhoff, High Diagnostic Yield of Tuberculosis From Screening Urine Samples From HIV-Infected Patients With Advanced Immunodeficiency Using The Xpert MTB/RIF Assay.JAcquir Immune DeficSyndr 2012;60:289–294
36. Cox JA, Lukande RL, Lucas S, Nelson AM, Van Marck E, Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa and correlation with clinical diagnoses. AIDS Rev 12: 183–194, 2010.

37. Mr. Chemeda A , Abebe T, Mihret A, Utility of Urine as a Clinical Specimen for the Diagnosis of Pulmonary Tuberculosis in People Living with HIV in Addis Ababa, Ethiopia, Journal of Clinical Tuberculosis and Other Mycobacterial Diseases (2019)

38. Wood, E. Kerr, Thoma, Tyndall, Mark w, Montaner, Julio SG , A review of barriers and facilitators of HIV treatment among injection drug users, Lippincott Williams and wilkins(2008)
39. Koole, O., et al., Evaluation of the 2007 WHO guideline to improve the diagnosis of tuberculosis in ambulatory HIV-positive adults. PLoS One, 2011. **6**(4): p. e18502.
40. Jffusim F, D Kellen, and S Abraham, The presentation and prevalence of tuberculosis in HIVpositive patients, International Scholarly Research Notices,2012.34:126

41. G Ferede, G Yismaw, Y Wondimeneh, Z SisayThe prevalence and antimicrobial susceptibility pattern of bacterial uropathogens isolated from pregnant women - Eur J ExpBiol, 2012.13:78-83

42. Agersew A, Feleke M, Yitayal S, Ketema T, Afework K, Belay A & Abebe A, Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at university of Gondar teaching hospital, North west Ethiopia, springer (2012).18:63

43. RK Singh, D Rai, D Yadav, A Bhargava. Synthesis, antibacterial and antiviral properties of curcuminbioconjugates bearing dipeptide, fatty acids and folic acid- European journal of 2010 – Elsevier.46:78
44. Batungwanayo J, Taelman H, Dhote R, Bogaerts J, Allen S, Van de perre P, Impact of human immunodeficiency virus infection on clinical and radiographic presentation of PTB in Kigali, Rwanda. American Review Respiratory Disease, 1992. 146: 53-56.
45. Gopinath, K. and S. Singh, Urine as an adjunct specimen for the diagnosis of active pulmonarytuberculosis. International Journal of Infectious Diseases, 2009. 13(3): 374-379.

46. Aceti A, Zanetti S, Mura MS, Sechi LA, Turrini F, Saba F, et al., Identification of HIV patients with active pulmonary tuberculosis using urine based polymerase chain reaction assay. Thorax, 1999. 54: 145-6.
47. Sechi LA, Pinna MP, Sanna A, Pirina P, Ginesu F, Saba F, et al., Detection of Mycobacterium tuberculosis by PCR analysis of urine and other clinical samples from AIDS and non-HIV-infected patients. Molecular and cellular probes, 1997.44: 281
48. Heydari AA, M.R.M. Danesh, and K. Ghazvini, Urine PCR evaluation to diagnose pulmonary tuberculosis. Jundishapur Journal of Microbiology, 2014. 144.
49. Challu, VK, Recovery of tubercle bacilli from urine of pulmonary tuberculosis patients and its comparison with the corresponding sputum isolates. Indian J TB, 1989. 36: 107-111.
50. Bentz, RR, Dimcheff DG, Nemiroff MJ, Tsang A, Weg JG, The Incidence of Urine Cultures Positive for Mycobacterium tuberculosis in a General Tuberculosis Patient Population 1, 2. American Review of Respiratory Disease, 1975. 111(5): 647-650.
51. Torrea G, Van de perre P, Ouedraogo M, Zougba A, Sawadogo A, Dingtoumda B, et al., PCR-based detection of the Mycobacterium tuberculosis complex in urine of HIVinfected and uninfected pulmonary and extrapulmonary tuberculosis patients in BurkinaFaso. Journal of medical microbiology, 2005. 54(1): 39-44.
52. Rebollo J, Garrido R, Folgueira D, Palenque E, Diaz-pedroche C, Lumbreras C, et al., Blood and urine samples as useful sources for the direct detection of tuberculosis by polymerase chain reaction. Diagnostic microbiology and infectious disease, 2006. 56(2): 141-146.
53. Hartung T, Maulu A, Nash J, Fredlund V, Suspected PTB in rural South Africa-sputum induction as a simple diagnostic tool?SAfr Med J, 2002. 92: 455-458.
54. Conde MB, et al., Yield of sputum induction in the diagnosis of pleural tuberculosis. Am J RespirCrit Care Med, 2003. 167: p. 723±725.
55. Jürgen R, Andriy V S, Manuela G,Robin R, Charles ,Knut, Jayadeep , Vladimir & Svetlana P, The association between alcohol use, alcohol use disorders and tuberculosis (TB). A systematic review,springer 2009.28: 344

11. Annexes

Annex I: Laboratory Test Procedures

1. Specimens collection

Sputum collection

With clean sputum container collect the sputum, not saliva, from the patient

Note: Any incoming specimen must be properly labeled, as a minimum with a unique identification number. This identification is also written on the request form and must correspond with the identification in the laboratory register.

Type of Specimen

Collect minimum 1ml of sputum. Do not accept specimens with obvious food particles or other solid particulates.

Storage of Specimens

Specimens should be held at 2–8 ^oC prior to processing whenever possible. If immediate processing is not possible, the specimens can be stored at a maximum of 4^{o}C for 4-10 days.

Processing Sputum for Smear Microscopy and Qualitative Culture

1. Label slides and tubes

2. Allow refrigerated specimens and reagents to come to room temperature before testing.
3. The ideal maximum volume of a sputum specimen is 10ml. If the volume is greater than 10ml, vortex the specimen and remove the excess volume with a pipette.
4. Using a transfer pipette, add to the sputum tube a volume of NaOH/NALC-Na citrate solution

that is equal to the specimen volume (1:1 volume) to the centrifuge tube.
5. Close tube tightly and vortex the suspension until liquefied (15-30 seconds). Invert the tube several times so the tube walls and cap are exposed to the NaOH/NALC-Na citrate solution.
6. Start the timer for 15 minutes when the digestion-decontamination solution is added to the first specimen. Place tube in rack on shaker platform to improve homogenization:
7. When time has elapsed, remove tube from shaker. Add phosphate buffer (pH 6.8) to the 50 ml mark on the centrifuge tube using pre-dispensed, 50 ml aliquots of buffer.

8. Continue to add phosphate buffer to all specimens at 30 sec or 1 minute intervals (as above), so that each specimen is ONLY exposed to digestion-decontamination solution for a maximum of 20 minutes.

9. Transfer tubes in a 50 ml tube rack to the centrifuge.
10. Place tubes in centrifuge buckets, ensuring they are equally balanced and the biosafety covers (lids).
11. Centrifuge at 3,000 rpm for 15 min at 4-12°C.

12. Carefully decant the supernatant into a splash-proof container containing a tuberculocidal disinfectant, taking care not to disturb the sediment at the bottom of the tube.
13. Add sterile phosphate buffer to the pellet to a
final volume of 2.5 ml, and resuspend the sediment using a pipette -- gently aspirating and expelling buffer.

14. Use the suspended pellet for culture in the MGIT BACTEC 960 TB System Liquid Culture – Mycobacteria Growth Indicator Tube (MGIT), and culture on LJ media
Solid Culture: Lowenstein Jensen (LJ) Media. The same pipette can then be used to prepare the smear for AFB microscopy.

15. When finished, dispose of the pipette into the biohazard discard bucket.
16. It is recommended to store any remaining sediment at 2-8°C, at least until the sputum smear is confirmed as acceptable.

2. Ziehl-Neelsen staining procedure

Principle

The Ziehl-Neelsen method uses a carbolfuchsin stain, acid alcohol decolorizer, andmethylene blue counterstain. Acid-fast organisms stain red, while the background of debrisstains blue. The ZN stain confirms the acid-fast property of mycobacteria.

Preparationofsmearsfromprocessedsputum

1. Label the frosted end of the slide in pencil with the laboratory accession number.

2. Working in a biological safety cabinet, vortex the decontaminated sediment to mix thoroughly.
3. Use a transfer pipette to place ~100 µl (2 drops) of well-mixed resuspended pellet from the digested-decontaminated specimen onto the slide, spreading over. Air-dry the smear.
4. Place the slides on a hot plate or slide warmer at a temperature between 65°C to 75°C for at least 2 hours (longer time is preferable), to heat-fix the samples.

PreparationofSmearsfromPositiveCultures

1. Label frosted end of slide in pencil with laboratory accession number

2. Work in a BSC.

3. If examining a positive MGIT culture: vortex tube well, unscrew MGIT tube cap and sample an aliquot of broth using a disposable pipette.

4. If examining colonies on solid medium dispense ~100 µl of distilled water on a glass slide withatransferpipette.

5. Air-drysmear.

6. Place the slides on a hot plate or slide warmer at a temperature between 65°C to 75°C for at least 2 hours (longer time is preferable), to heat-fix the samples.

PROCEDURE

1. Place slides on staining rack, and flood with carbolfuchsin.

2. Heat the slide to steaming with the flame from a Bunsen burner. Apply only enough additional heat to keep the slide steaming for 5 minutes

3. Washoffthestainwithwater.

4. Floodslideswith3%acid-alcohol.

5. Let stand for 2-3 min (more acid-alcohol should be used if the smear is heavily stained).

6. Wash off the acid-alcohol with distilled water and tilt the slides to drain.

7. Flood the slides with methylene blue and let stand for 1-2 minute.

8. Wash off the methylene blue withwater.

9. Tilt the slides to drain.

10. Allow slides to air dry in the slide rack.

3. LIQUID CULTURE – MYCOBACTERIA GROWTH INDICATOR TUBE (MGIT)

Principle

Mycobacteria multiply in a nutrient-rich medium, while contaminating bacteria are inhibited by the addition of a cocktail of antibiotics. Growth of bacteria, including mycobacteria, is indicated by fluorescence, which increases proportionally as oxygen decreases in the tube. The instrument detects this fluorescence in the medium using a UV light and complex computer algorithms.

Sputum specimens are processed (in different section, Processing Sputum for Smear Microscopy and Qualitative Culture), and inoculated into 7ml MGIT tubes, which are supplemented with

Growth Supplement and a cocktail of antibiotics (PANTA). The MGIT tubes contain a fluorescent compound embedded in the base of the tube, which is sensitive to the presence of oxygen dissolved in the broth. Initially, the large amount of oxygen quenches the emissions from the compound and little fluorescence is detected. Bacteria present in the concentrated sputum specimens metabolize oxygen in the culture medium, allowing the fluorescence to be detected. Blood samples are not suitable for the MGIT system.

PROCEDURE

1. While the specimens are being processed, prepare the antibiotic supplement (PANTA) for the MGITtubes.
2. Label the side of each MGIT tube.

3. Once dissolved, add 0.8 ml of the PANTA/growth supplement mixture to each MGIT tube using a micropipettor with sterile tip, taking care not to contaminate the tubes.
4. Using a sterile, graduated transfer pipette, add 0.5 ml of well-mixed sputum sediment to the MGITtube.

5. Tightly recap the MGIT tube and mix well by gently inverting the tube several times.

6. Enter tubes into the instrument as soon as possible. Always scan the MGIT barcode first; the instrument will assign the stations.

4. SOLID CULTURE: LOWENSTEIN JENSEN (LJ) MEDIA

Principle

Many different solid media are available for cultivating mycobacteria. Most are variationsof egg-potato base or albumin-agar base media. There is no general consensus on whichmedium is best for routine isolation. The advantages of egg-based media such as LJ are:1) it is easy and economical to prepare, 2) it is associated with lower contamination rates,and 3) isolated colonies with characteristic colony morphology for MTB can be observed.Disadvantages are: 1) when contamination occurs, it often involves total surface of medium,2) if contamination is slight, it is not evident when mycobacterial growth is confluent, and 3)drug susceptibility tests are more difficult to perform using egg-based media because somedrugs must be adjusted to account for their loss by heating or by interaction with certaincomponents of the egg.As with all media preparation, attention must be given to purity of chemical components,including quality of eggs; preparing and sterilizing medium and glassware; exposure of finalproduct to excessive heat or sunlight; and method and length of storage. All lab-preparedmedia must be tested for sterility and performance characteristics before being used.

PROCEDURE

1. Label LJ tube.

2. Remove any excess water in the slant using a sterile transfer pipette.
3. Inoculate the tube with 200 µl of the sample using a sterile graduated disposable pipette. Spread inoculum evenly over entire surface of medium. Take care to minimize aerosol generation when sampling from positive MGIT tubes as this broth will contain large numbers of M. tuberculosis.
4. Replace cap and ensure there are no droplets around the rim of the tube. Wipe off the outside of the tube with a paper towel soaked in tuberculocidal disinfectant.
5. Leave tube in slanted position with cap loosened until inoculum is absorbed (about a week), then tighten cap securely and incubate in upright position at 37°C (±1°C).

6. Examine and record results for the cultures weekly, for 8 intervals. Cultures can be read on the bench, as long as the caps are NOT loosened.
7. To observe fine growth, a strong direct light from the angle poise lamp must be shone onto the slant surface. M. tuberculosis usually grows as a buff-colored, dry colony, which is very distinctive.

5. RAPID IDENTIFICATION OF M. TUBERCULOSIS COMPLEX

USING AN IMMUNOCHROMATOGRAPHIC ASSAY

Principle

A rapid immunochromatographic assay will be used to differentiate MTB and MOTT. BD's MGIT TBc ID and Tauns' Capilia TB are both lateral flowimmunochromatographic assays. The BD assay detects MPT64 antigen, while Capilia detects MPB64 antigen, a mycobacterial protein that is specifically secreted from MTB cells during culture. When a sample is added to the test device, MPT64/MPB64 antigen binds to anti-MPT64/MPB64 antibodies conjugated to colloidal gold particles present on the test strip, forming an antigen-antibody complex. This antigen-antibody complex then migrates across the test strip to the reaction area, where it is captured by asecond specific MPT64/MPB64 antibody fixed to the membrane. If MPT64/MPB64 antigen is present in the sample, a color reaction is produced by the labeled colloidal gold particles and is visualized as a pink (or purple) to red line. An internal positive control is included to validate proper test performance. The test will detect the following species of MTB complex: M. tuberculosis, M. bovis, M. africanum, and M. microti. These tests have been shown to be highly sensitive (>95%) and specific (> 95%) in a number of studies conducted in clinical settings.

PROCEDURE

1. If devices are refrigerated, bring to room temperature in the foil pouch prior to testing.
2. Place the device on a flat surface inside the BSC. Remove the rapid ID device from its foil pouch immediately before testing.
3. Label one device for each specimen to be tested.

4. Place 100μL of specimen, either MGIT culture or bacterial suspension from LJ slant, into the specimen well of the test device. Change pipette tips between specimens.
5. Start timer for 15 minutes.
6. Examine the reading area of the test device after 15 minutes and record test results. Do not interpret test after 60 minutes.

Positive: Pink/purple to red lines form on the reading areas labeled Test [T] and Control [C] of thedevice.

Negative: A pink/purple to red line forms on the reading area labeled [C] of the device, but not[T].

Invalid: If no line is observed on the reading area labeled [C], technical errors or product damage has occurred.

DRUG SUSCEPTIBILITY TESTING: MGIT SYSTEM

Principle

Susceptibility testing in the MGIT 960 system is based on the same principles as isolation from sputum (detection of growth). DST is performed using an AST (antibiotic susceptibility testing) set, which consists of a Growth Control tube and one tube for each drug, as well as a bar-coded tube carrier that holds the set. A known concentration of drug is added to a MGIT tube, along with the specimen, and growth is compared with a drug-free control of the same specimen. If the drug is active against the mycobacterial isolate (isolate susceptible), growth will be inhibited and fluorescence will be suppressed in the drug-containing tube; meanwhile, the drug-free control will grow and show increasing fluorescence. If the isolate is resistant, growth and its corresponding increase in fluorescence will be evident in both the drug-containing and the drug-free tube. The MGIT 960 system monitors these growth patterns and can automatically interprets results as susceptible or resistant. An isolate is defined as resistant if 1% or more of the test population grows in the presence of the critical concentration of the drug.

PROCEDURE

Preparation of SIRE

1. Label five 7 mL MGIT tubes for each test isolate with a study label that includes identifying information. In addition, label tubeswith one of each of the following: GC (Growth Control), STR (streptomycin), INH (isoniazid),RIF (rifampicin), EMB (ethambutol).

2. Place the tubes in the following sequence in the 5 tube AST set carrier, from left to right: GC, STR, INH, RIF, EMB.

3. Aseptically add 0.8 mL of BACTEC MGIT SIRE Supplement (provided in the SIRE kit) to eachSIRE tube. It is important to use the supplement supplied with the kit.

4. Aseptically pipette 100 µl of the appropriately reconstituted drug into the corresponding MGITtube; e.g., add 100 µl of the 83 µg/mL MGIT STR solution to the MGIT tube labelled “STR”,etc.

5. It is important to add the correct drug to the corresponding tube.

6. Do not add drugs to the MGIT GC tube.

Preparation of PZA

1. Label two 7mL MGIT PZA media tubes for each test isolate with a study label that includes identifying information. In addition,label tubes with one of each of the following: GC (Growth Control) or PZA (pyrazinamide).

2. Place the tubes in the following sequence in the 2 tube AST set carrier, from left to right: GC, PZA.

3. Aseptically add 0.8mL of BACTEC MGIT PZA supplement to each PZA tube.

4. Aseptically pipette 100uL of the 8000µg/ml MGIT PZA solution to the appropriately labelled MGITtube.

5. Do not add drugs to the MGIT GC tube.

Using an Inoculum from Positive MGIT Culture

1. Positive MGIT cultures must have pure growth of *M. tuberculosis* (ZN positive, BAP negative,MPT/MTB 64 antigen test positive; in order to be tested for drug susceptibility.

2. DST must not be set up on the same day a MGIT tube signals positive.

3. If the culture is worked up one or two days after signalling positive, it can be used directly to inoculate the DST MGIT tubes.

4. If the culture is used to set up DST between three and five days after signalling positive, dilute 1 ml of MGIT broth in 4 ml of sterile saline (1:5 dilutions).

5. If the culture has been positive longer than five days, subculture into a fresh MGIT tube:

• Vortex MGIT broth well to mix thoroughly. Leave 5-10 minutes to allow any large clumps to settle.

• Supplement a new MGIT tube with 0.8 ml Growth Supplement withoutPANTA.

• Remove inoculum from the supernatant broth and make a 1:100 dilution of the positive MGIT tube into sterile saline or 7H9 broth.

• Mix tube well by inverting gently several times.

• Inoculate new MGIT tube with 0.5 ml of the 1:100 diluted specimens.

• Cap tube tightly and mix well by inverting gently several times.

• Enter tube into MGIT 960 instrument and monitor until it becomes positive.

• Use new tube for DST tests from one to fi ve days of positivity as described above.

Using an Inoculum from Positive LJ Culture

1. DST can be performed with growth from positive LJ slants if the MGIT tube is contaminated or fails to grow *M. tuberculosis*.

2. Add 4 ml of Middlebrook 7H9 Broth (or BBL MGIT broth) to a 16.5 x 128 mm sterile tube With cap containing 6 – 10, 2 mm, glass beads (or use tube the same size as lab's McFarland Standards).

3. Using a sterile loop or applicator stick, scrape as many colonies as possible from growth no more than 14 days old. Do not remove any solid medium. Suspend the colonies in the Middlebrook 7H9 broth.

4. Vortex the suspension for 2-3 min to break up the larger clumps. The suspension should exceed a 1.0 McFarland standard in turbidity.

5. Let the suspension stand for 30 min without disturbing. Sediment should settle to the bottom of the tube.

6. Transfer the supernatant fluid to another 16.5 x 128 mm sterile tube with cap (avoid

transferring any of the sediment) and let the suspension stand for another 15 min.

7. Transfer the supernatant fluid (it should be smooth, free of any clumps) to a third 16.5 x 128 mmsteriletube.

8. Using 7H9 broth, adjust the suspension to a 0.5 McFarland standard. Do not adjust below or

9. Dilute 1 ml of the adjusted suspension in 4 ml of sterile saline (1:5 dilution) using a new 16.5 x 128 mm sterile tube. This diluted inoculum is used for preparation of the MGIT DST tubes.

Inoculation of Tubes Containing Test Drugs

1. Vortex original MGIT tube, diluted MGIT culture, or diluted inoculum from LJ as applicable, tomix well; let suspension settle for 5-10 minutes.

2. Aseptically pipette 0.5 ml of the organism suspension from the supernatant into all drug Containingtubes (STR, INH, RIF, EMB, PZA, any 2nd line drug tubes), using a micropipettor and sterile aerosol resistant tips – a separate tip must be used for each tube. Take care not to disturb the sediment.

3. Tightly recap the tubes. Mix tubes by gently inverting 3-4 times.

Annex II: Questionnaire

Questionnaire

Sex Male Female

Age 18-29

30-39

40-49

50 and above

Marital status Single Married Divorced Widowed

Educational statusNone educated

Elementary school

High school

Certificate and above

Occupation 1) Government employee

2) Housewife

3) Daily laborer

4) Merchant

5) Farmer

6) Student

7) Pension

Housing style 1) Crowded

2) Ventilated

Place of residence1) Urban

2) Rural

Weight (Body mass index (BMI)), kg/m2 ---------------------------

Symptoms Cough Yes No

Fever Yes No

Nausea or vomiting Yes No

Night sweats Yes No

Difficulty breathing Yes No

Chest pain Yes No

Weight loss Yes No

FatigueYes No

Chills Yes No

Rule out urogenita sign and symptoms

Frequency/urgency Yes No

Dysuria Yes No

Nocturia Yes No

Hematuria Yes No

Flank/abdominal pain Yes No

Burning micturition Yes No

Chronic cystitis Yes No

Chest radiograph with any abnormality Yes No

Previous history of TB Yes No

Does the client take ionized prevention therapy (IPT)? Yes No

HIV test result Positive Negative

WHO stage of HIV 1) I 3) III

2) II 4) IV

Does the client on antiretroviral therapy (ART) Yes No

If yes for how long?< 1 year > 1 year

CD4+ Lymphocyte count<50Cells/mm3

50–99 Cells/mm3

100–149 Cells/mm3

150- 199 Cells/mm3

≥200 Cells/mm3

Annex III: participants' Information Sheet

Date ------------

Title: Urine as an adjunct specimen for the diagnosis of active pulmonary tuberculosis in people living with HIV.

Background: Tuberculosis is the opportunistic infections which increases the mortality in human immunodeficiency virus infected individual. The diagnosis of pulmonary tuberculosis (PTB) is usually established by examination of three Zeihl-Neelsen stained smears but in HIV infected person show negative smear which negative results do not preclude active TB. In order to overcome this problem other attractive potential specimen that replaces the conventional method should be addressed. Since tubercle bacilli or their nucleic acids are also expected to be excreted through the kidneys, we will be interest to assess spot urine as a supplementary specimen for diagnosing PTB.

Objective of the study: The aim of this study is to evaluate urine as an adjunct specimen for the diagnosis of active pulmonary tuberculosis in people living with HIV. The sensitivity and specificity of PCR and ZN smear will be compared against culture method.

Organizations: The study will be conducted by Addis Ababa University, school of Graduate studies. Laboratory procedure will be carried out at Ethiopian public health Institute.

Procedure: Expert (in the area) Nurses willcollect urine and sputum for routine microscopic diagnosis. Laboratory method to be used includes; ZN smear, culture.

Participation: The procedure will be carried out after getting your willingness to participate. All volunteer patients with pulmonary problem, fulfilling inclusion criteria, will be included.

Risks associated with sample collection: No pain during specimen collection and to reduce the aerosol, you will be administered to use capped tube.

Benefit: As different study shows diagnosis of PTB in HIV patients is very difficult and end up with high mortality. You will be benefited from the study; because it will be part of your diagnosis and might be a key to your current and/or your future problem if it will come up with positive result.

Compensation: You will receive your result (only positive patients) through your physician. You will get treatmentforfreeif you become positive for PTB.

Confidentiality: From medical ethics point of view and research ethics, every part of your personal information will be kept confidentially. Information to be collected and variables expressing your identity will be coded secretly. The only responsible person to link your variables (important for your follow up and treatment) with the code number is the principal investigator. However, other researchers can see your clinical information, which is without your identity. Your result andinformation will be used only for thementioned purpose.

Sharing the Result: Eventually, the result, devoid of your identity, will be reported through publication or by other means. Have no suspicion on the confidentiality of your information, even at this time. We request your permission to use the result for reporting.

Right to refuse or withdraw: Thus, it is your right to agree or to refuse to participate in the study. Withdrawal from the study is also possibleatany time. Withdraw or refusing to participate will not have any impact on your normal diagnosis or medical follow up. You can address your problem orquestion through oneof the addresses given bellow.

Contact Addresses:

1. WendwessenZewdie, Address: Addis Ababa University, College of Health Science,

   Mobile: +251911893848, E-mail – amaroderba@gmail.com

2. Meronyohanes, Address: Addis Ababa University, College of Health Science,

   Mobile: +251925907169, E-mail- meritilove@gmail.com

3. KassuDesta, Address: Addis Ababa University, College of Health Science,

   Mobile: +251911107099, E-mail-kassudesta2020@gmail.com

Thank you for your patience and kindness

Annex IV: Informed Consent/ Ascent form

Informed Consentform

Name ---, Age ------------, Sex -------

Identification No -------------------------------, lab No ------------------

I read and/or well informed about the nature of the study, entitled "urine as an adjunct specimen for the diagnosis of active pulmonary tuberculosis in people living with HIV at selected Hospital Addis Ababa, Ethiopia." Finally, she/he told me that this will be certain if

I agree onthe following pointsandsigned bellow.

a) I understood the objective ofthe study.

b) I understood that the sample won't be used for farther study, and after completion of the whole procedure, the leftover sample will be discarded safely

c) I am aware of any information describing my identity, collected using questionnaire and , won't be disclosed.

d) I understood report ofmy result won'tinclude my name

e) I understood that I won't get money for being part of the study

f) I clearly informed as I have the right to refuse to participate and withdraw (if I change my idea) from the study at any time

g) I understood that my refusal to take part in this study won't have impact to the normal diagnosis and to my future medical follow up.

I have had enough time to think over it freely and I understood it well. I found it would have positive impact in the investigation of my case. My agreement to this consent is without anyexternal enforcement, andwillbeconfirmedbymy signature below.

The information sheet was given/ explained to me by: ---,

Signature ----------------------------, phone --

Name of participant: -------------------------------------, signature --------, phone ---------------------

Name of physician: --------------------------------------, signature --------, phone, --------------------

Declaration

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

M.Sc. candidate: WendwessenZewdie

Signature: ___________________________

Date of submission ___________________________

This thesis has been submitted with our approval as advisors.

Advisor:Meronyohanes, M.Sc., PhD candidate

Signature _________________________

Date ___________________________

Place Addis Ababa, Ethiopia

Advisor: KassuDesta, M.Sc., PhD candidate, Assistant professor

Signature: ______________________________

Date: ______________________________

Place: Addis Ababa, Ethiopia