RESEARCH AND EVALUATION REPORT

Evaluating the incremental value of using the Urine TB LAM test in Intensified Case Finding for TB in People Living with HIV in Swaziland

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FOREWORD

Tuberculosis (TB) is the major cause of mortality in people living with HIV (PLHIV) in Swaziland and, due to well recorded difficulties in its diagnosis in this population group, often goes undiagnosed. Intensified TB case finding (ICF) is a key intervention in reducing the burden of TB among PLHIV, and is a gatekeeper to the other TB control measures that form the 'Three Is', including TB infection control (TB IC) in congregate settings, and isoniazid preventive therapy (IPT). However, ICF among PLHIV remains a challenge because TB presentation in HIV is atypical, and the available diagnostic methods are less accurate or delay disease identification and appropriate initiation of therapy.

TB diagnosis among PLHIV improved greatly following the widespread roll-out of the Xpert® MTB/RIF assay which is able to detect more TB cases regardless of HIV status, unlike sputum smear microscopy which has limited sensitivity among PLHIV. In spite of these advances, the need for a simple and inexpensive point of care assay has been gathering momentum. One promising phenomenon is the recent demonstration of moderate sensitivity and high specificity of a TB LAM test among PLHIV, which may show potential for complementing the existing ICF efforts. This commercially available TB LAM test detects the lipoarabinomannan (LAM) antigen from *Mycobacterium tuberculosis* in urine of patients with pulmonary and extra-pulmonary TB.

The Ministry of Health (MoH) and partners have a goal of improving TB case detection in PLHIV and based on scientific evidence. In 2015, we jointly implemented an evaluation of an incremental added value of the urine TB LAM test into routine TB screening among PLHIV attending ART clinics for care and treatment in Swaziland. Through this implementation research we generated evidence to help inform timely diagnosis and treatment, and to improve TB case detection among presumptive TB patients who are unable to submit sputum. The research also evaluated utility and issues surrounding nationwide-scale implementation of Urine TB LAM test when used in conjunction with the TB symptom screening tool.

This report describes a prospective observational cohort study of consecutive HIV positive patients attending the ART clinics at Mbabane, Hlatikulu Government, and Raleigh Fitkin Memorial Hospitals for treatment and care.

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ABBREVIATIONS TABLE

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ASSIST	Applying Science to Strengthen and Improve Systems
AUC	Area under curve
CI	Confidence Interval
DST	Drug susceptibility testing
HIV	Human Immunodeficiency Virus
IC	Infection Control
ICF	Intensified Case Finding
IPT	Isoniazid Preventive Therapy
IQR	Interquartile range
LAM	Lipoarabinomannan
LPA	Line Probe Assay
MDR	Multi-Drug Resistant
MGIT	Mycobacterium Growth Indicator Tube
MOTT	Mycobacteria other than tuberculosis
MTB	Mycobacterium tuberculosis
NERCHA	National Emergency Response Council on HIV and AIDS
NPV	Negative Predictive Value
NTCP	National Tuberculosis Control Programme
OR	Odds Ratio
PLHIV	People Living with HIV
POC	Point of Care
PPV	Positive Predictive Value
RIF	Rifampicin
ROC	Receiver operating characteristics
SEC	Swaziland Scientific and Ethics Committee
SNAP	Swaziland National AIDS Programme
SOP	Standard Operating Procedures
TB	Tuberculosis
TBIC	TB Infection Control
URC	University Research Co., LLC
USAID	United States Agency for International Development
UNAIDS	United Nations Joint Programme on HIV and AIDS
WHO	World Health Organization
XDR	Extensively Drug Resistant

EXECUTIVE SUMMARY

Background: Tuberculosis (TB) is the leading cause of death in patients infected with HIV. With a TB/HIV co-infection rate of 73% in Swaziland, the Swaziland National Tuberculosis Control Programme (NTCP) and Swaziland National AIDS Programme (SNAP) have a joint goal of improving TB case detection in People Living with HIV (PLHIV). As a result, the evaluation of urine TB LAM test as an additional test along the TB screening algorithm in order to scale up intensified case finding (ICF) including extra-pulmonary TB in PLHIV was conducted. The urinary TB LAM test, which detects the lipoarabinomannan (LAM) antigen in urine specimens, has the potential of rapidly detecting TB. In addition to the advantage of not requiring a sputum sample, thus no airborne infection risks, the other benefit of this test is point of care feasibility and ability to rule-in TB disease among PLHIV sooner than traditional methods thus enabling earlier treatment. The objectives were as follows:

1. To measure the incremental value of incorporating urine TB LAM testing into the national TB screening algorithm for PLHIV. This is a calculation of number of confirmed TB cases detected by urine TB LAM test among patients who screen positive for TB but with no cough or who had sputum induction due to inability to spontaneously produce sputum.
2. To determine the predictive value of non-cough symptoms in the screening tool (fever, night sweats and loss of weight) as predictors of TB disease among PLHIV without a cough
3. To conduct a sensitivity analysis of the urine TB LAM test by CD4 category relative to Expert® MTB/RIF, measured against Conventional Sputum Culture (MGIT) in the study population.
4. To determine factors associated with a true positive TB LAM test among patients screening TB positive but without cough (CD4, ART status, Age, Gender)
5. To use evidence generated to review the TB screening algorithm and screening tool among PLHIV to maximise TB case detection.

Methodology: The study was a prospective cohort observation of 417 consecutively enrolled HIV positive patients with presumptive TB attending ART clinics for treatment and care at Mbabane Government Hospital, Hlathikhulu Government Hospital and Raleigh Fitkin Memorial Hospital. All HIV positive patients were eligible for enrolment in the study, regardless of their ART status, provided they had not been diagnosed with TB or taken any form of anti-TB treatment in the previous two months during the enrollment period from April 2015 to September 2015. Each participant provided urine sample for the urine LAM test, blood samples for CD4 cell count testing and two sputum samples for Xpert MTB/RIF and culturing for MTB.

Results: Thirty participants were confirmed to have TB using culture as the reference test giving a calculated TB prevalence of 8.2% (95% confidence interval (CI):5.6-11.5). Of these, 6 were correctly identified by urine TB LAM test and 15 by Xpert MTB/RIF. Among the 30, 4 had sputum induction due to dry cough or inability to produce sputum spontaneously. TB LAM correctly detected TB in 1 among the 4 participants. Generally the sensitivity for non-cough symptoms among the study population was greater than 90%, except for weight loss at 68.4% (95% (CI): 51.3-82.5). However, all had specificities that were less than 35%. The positive predictive values of non-cough symptoms: fever, night sweats and weight loss were 11.9%, 11.8 and 10.7% respectively. The negative predictive values for fever, night sweats and weight loss were 95.9%, 96.7% and 90.4% respectively.

For the total cohort, sensitivity of TB LAM was 20% (95% CI: 7.7-38.6) but increased to about 55.6% (95% CI: 21.2-86.3) for those with CD4 cell count below 100 cells/mm3. Similarly the specificity was 96.4% (95% CI: 93.8-98.1) but decreased to 90.9% (95% CI: 75.7-98.1) for those with CD4 cell count below 100 cells/mm3. For Xpert MTB/RIF the sensitivity and specificity was 50% (95% CI: 31.3-68.7) and 99.7% (95% CI: 98.3-100), respectively for the entire

cohort. True positive TB LAM test was strongly associated with CD4 cell count <100 cells/mm3 (OR: 30.7, 95% CI: 3.1-303.9; p-value=0.01). TB LAM results showed a peak sensitivity of 55.6% (95% CI: 21.2-86.3) and a specificity of 91% (95% CI: 75.7-98.1) among those who had CD4 count less than 100 cells/mm3.The prevalence of TB among those unable to serve sputum spontaneously was 6.8% (95% CI: 1.9-16.5) and the median turnaround time for culture positive results was 32 days (IQR: 18-48) days.

Discussion and recommendations: The overall sensitivity of the TB LAM test as a diagnostic test for pulmonary TB was 20% but increased to about 55.6% for those with CD4 cell count below 100 cells/mm3. A true positive TB LAM was significantly associated with CD4 cell count controlling for ART status, age and gender. TB LAM test was found to be 30 times more likely to be truly positive if CD4 cell count is less than 100 compared to a positive TB LAM test if CD4 cell count is greater than 100 cell/mm3. However, the evaluation of Xpert MTB/RIF in this study showed a sensitivity of 50% although the specificity was consistently high above 99% regardless of CD4 cell count.

The study demonstrated that TB LAM use is feasible in HIV clinics and the report provides the following recommendations. TB LAM may be used to aid TB diagnosis for HIV positive patients presumed to have TB and have a CD4 cell count <100. TB LAM should be used as an add-on test to the existing TB screening algorithm for patients presumed to have TB and should not replace existing TB screening procedures. In addition, using one sample for Xpert MTB/RIF testing is not sufficient to guarantee accurate ruling out of TB disease and culture should be prioritized for all presumptive patients who test negative for Xpert MTB/RIF, more especially PLHIV with CD4 cell count of more than 200 cells/mm3.

1. INTRODUCTION

1.1 Background

Tuberculosis (TB) is one the most common serious opportunistic infections in HIV positive patients and is the manifestation of AIDS in more than 50% of cases in developing countries (1). TB can occur at any time during the course of HIV infection. The World Health Organisation (WHO) estimates that around 1.2 million people living with HIV (PLHIV) developed TB in 2014, of which 0.4 million died (2).

In Africa, TB represents a serious threat in countries severely affected by the HIV epidemic. In 2014, the African region accounted for 28% of the global TB cases, but the most severe burden relative to population (281 incident cases per 100,000 people, more than double the global average of 133) (2). While the burden significantly reduced in other regions, the situation in Africa worsened during the period 1991 to 2006, driven by a generalized HIV epidemic and compounded by weak health care systems, inadequate laboratory capacity and conditions that promote transmission of infection (3). The African region accounted for 74% of global HIV-positive incident cases and an estimated 250,000 TB deaths among PLHIV in 2015 (2). There exists a synergistic relationship between TB and HIV. The interface between TB and HIV is increased in developing countries where both TB and HIV infections are maximally prevalent in the 15-49 year age category. The association between HIV and TB presents an immediate and extensive public health and socioeconomic threat in developing countries (1).

In Southern Africa, TB is the leading cause of death among HIV-infected patients and approximately 50% of TB patients are HIV co-infected (4). The TB incidence among PLHIV in Swaziland was 464 per 100,000 population annually in 2015 (5), and is among the highest in the world with 41% of antenatal care (ANC) mothers in 2010 (6), 26% of the population aged 15-49 years in 2006/7 (7) and 32% of adults aged 18-49 years infected with HIV I 2013 (8). Moreover in 2015, 73% of incident TB cases in Swaziland were HIV co-infected (2). The prevalence of multidrug resistant (MDR) TB among new patients was 7.7% and 33.8% among previously treated cases with cases of extensively drug resistant (XDR) TB being reported in the country hence the on-going epidemic of HIV-TB co-infection being further complicated by operational challenges in effective TB control in 2010 (9).

Since 2006, Swaziland has being registering remarkable improvements in TB and HIV programme performance especially TB treatment outcomes. For instance, the treatment success rate increased from 42% in 2006 to 60% in 2008 and to 72% in 2012 (10), although these improvements still fall short of the WHO-recommended target of 85% by 2015. Against the background of a rising estimated TB incidence rate in Swaziland, there has been concern with declining TB case notification as well as case detection rates. For instance, the case detection rates dropped from 68% in 2010 to 53% in 2011 and 38% in 2013 (11), and then increased to 60% in 2014 (5) but is still lower than 68%. Available diagnostic tools and health seeking behavior impact both case detection and treatment success. Moreover, for a long time in TB control, early TB treatment has been hindered by the lack of access to rapid and accurate diagnostic tools in resource-constrained settings. While mycobacterium culture is regarded as the gold standard for diagnosis of active TB, it is expensive, not universally accessible and requires access to specialized laboratories and it takes weeks to provide results. Nonetheless, with its short comings, in resource constrained settings it is still a useful test for confirming the presence of the disease.

Through joint planning, the Swaziland National Tuberculosis Control Programme (NTCP), the Swaziland National AIDS Programme (SNAP) and partners have been implementing collaborative TB/HIV activities as a response to the dual HIV and TB epidemic in order to scale up evidence based recommendations from WHO on the implementation of the Three "I"s (ICF, IPT, TBIC) and integration of TB/HIV collaborative activities to other healthcare services as well as early ART initiation in TB/HIV co-infected patients. However, TB diagnosis using conventional methods continues

to be a challenge among PLHIV. Currently, ICF is implemented through routine TB screening conducted in over 183 health facilities in Swaziland (12), including ART sites, prison clinics, public health units and hospital out-patient departments. A standard TB screening tool (which uses symptoms of cough of any duration, fever, night sweats and unintended weight loss) is administered to identify presumptive TB cases.

The confirmation of TB is conventionally bacteriological through either phenotypic tests, like smear microscopy and culture, or genotypic tests, such as Line Probe Assay (LPA) and Xpert® MTB/RIF assay. In 2010 WHO endorsed the Xpert® MTB/RIF assay (Cepheid) as the recommended TB diagnostic test for TB in PLHIV, with sensitivity ranging from 44% to 75.9% from one sputum sample (13). This fully automated molecular assay simultaneously detects presence of *Mycobacterium tuberculosis (MTB)* and rifampicin (RIF) resistance, providing results in less than 2 hours. However, all these sputum based examinations preclude the presence of cough during the screening process. For patients who are unable to serve a sputum sample, or presumptive TB cases who may not be exhibiting respiratory symptoms, diagnosis is a challenge and there is a need to consider use of non-sputa requiring diagnostic tests in the strive towards ICF.

Swaziland adopted, and currently implements, a diagnostic algorithm that utilizes Xpert® MTB/RIF (GeneXpert) technology as a first line diagnostic tool for sputum analysis for TB. However, about 40% of extra-pulmonary TB cases are attributable to tuberculous lymphadenitis(14). Therefore, these patients are not beneficiaries of the sputum-based diagnostic methods. These patients are diagnosed by exclusion, delayed to be put on treatment and as a result carry poorer treatment outcomes.

The Alere Determine TB LAM test is an antigen (Ag) detection test which detects the presence of mycobacterial lipoarabinomannan (LAM) in urine samples. It provides an opportunity for rapid TB detection using non-sputa samples, with real potential for being a point-of-care (POC) test for TB irrespective of the site of the disease. The use of urine for TB diagnosis, rather than sputum, is highly advantageous as urine is simple to collect from all patients without generating hazardous bio-aerosols, is safe to handle and has relatively few bacterial contaminants and therefore sample quality is unlikely to be highly variable.

The effectiveness of this test has been considered in a number of studies, with evidence suggesting that the test is able to effectively rule in the presence of disease. Moderate sensitivity and high specificity have been reported in HIV-infected individuals with advanced immunodeficiency, the very patients for whom TB is especially difficult to diagnose (15). The test was found to be highly specific and sensitivity was found to increase with advanced immunosuppression and surpass that of smear microscopy among patients with CD4 cell count of less than 100 cells/mm^3. Interestingly, evidence suggests that, although sensitivity of the assay if used alone is not clinically useful, when combined with Xpert MTB/RIF testing, sensitivity increases to 83.3% in patients with CD4 cell count below 50 cells/mm^3 (13, 16). This was confirmed in a more recent study conducted in Uganda which concluded that the addition of urine TB LAM to TB diagnostic algorithms for HIV-infected individuals was highly cost-effective compared with usage of either sputum smear-microscopy or Xpert® MTB/RIF alone (17).

1.2 Problem Statement

Despite efforts to control the TB epidemic, there are still high incident cases of TB worldwide. The HIV epidemic and the emergence of anti-TB drug resistance represent serious threats for achieving the Stop TB Partnership's goal of eliminating TB as a public health problem by 2050. HIV co-infected patients are more likely to develop active TB compared to the HIV negative individuals. Even though antiretroviral therapy (ART) for HIV reduces this risk, TB remains 5 times more frequent in PLHIV than among the HIV negative population. Swaziland has a very high

incidence rate of TB with 733 cases per 100,000 people (5); compared with the global average of 133 per 100,000 (2) and hence the country has a large pool of people with TB in the community that likely perpetuate community transmission. The TB burden in Swaziland is predominantly HIV driven as exemplified by a TB/HIV co-infection rate of 73% (2). The incidence of TB among the PLHIV is 464 per 100,000 population annually (5). Moreover, Swaziland also has one of the worst HIV epidemics in the world with an HIV prevalence of 41% among women attending ANC clinics (6) and 26% in the population aged 15-49 years (7) and 32% of the adults aged 18-49 years being HIV positive (8). Swaziland is among the 41 high TB/HIV burden countries (2) and the co-epidemic has put a serious strain on the health system which is already grappling with shortages in healthcare personnel thus implementation of quick and accurate POC tests as a platform for rapid TB diagnosis is long overdue. The study evaluated incremental utility of the TB LAM test as an added tool to intensified case finding for TB in PLHIV due to its advantages such as easy handling procedures of less infectious specimen, less sophisticated and affordable laboratory instruments, and quick results turnaround time within 25 minutes as compared to the other diagnostic tests.

1.3 Significance of the Study

The study ought to benefit several areas of health in addition to providing evidence to healthcare personnel to inform decisions regarding TB diagnosis using TB LAM test among PLHIV. The study demonstrated the time of receipt of culture results from the laboratory which can be used as a proxy to the start of TB treatment although delays in the patient reporting back to the facility are possible. As a result, the evidence generated from the study provided the Ministry of Health with information needed in making informed decisions on issues around nationwide scale-up of implementation of Urine TB LAM test when used in conjunction with the TB symptom screening tool.

Specifically, the evidence based information directly benefited the TB and HIV programs in the country to design and implement strategies aimed at controlling the TB epidemic. The ultimate goal was to provide scientifically sound evidence to the Ministry of Health to influence the review and implementation of effective TB screening algorithms, in an effort to increase timely detection of true TB cases among PLHIV. Therefore, through early detection and early treatment of TB, the burden, morbidity and mortality due to TB is likely to be reduced. Evidence generated from this study also added to the body of literature that is available on TB LAM assisting international organizations such as WHO in the review of TB and HIV guidelines and their operationalization in resource-limited settings.

1.4 Research Question

What is the incremental value of using the TB LAM test in ICF for TB among PLHIV?

1.5 Rationale

In Swaziland, the NTCP and SNAP have the joint goal of improving TB case detection in PLHIV and, based on scientific evidence, one potential means of achieving this goal is through the routine use of TB LAM test on urine specimens during the TB screening process. In addition to the advantage of not requiring a sputum sample, the major benefit of this test is its ability to rule-in TB disease among PLHIV sooner than traditional methods, providing results in 25 minutes thus enabling earlier diagnosis and initiation of treatment for such patients. Currently the process of diagnosing TB in HIV positive patients can take up to a week and a large proportion of patients who screen positive for TB are not investigated at all in Swaziland. The urine TB LAM test has the potential of making immediate diagnosis and early initiation of anti-TB treatment a reality, especially in TB/HIV co-infected patients either presenting with extra-pulmonary disease or unable to produce sputum; for whom late diagnosis is associated with high rates of mortality. Urine TB LAM is a true POC test, which expedites clinical decision making in the management of TB/HIV co-infected patients, allowing for prompt treatment of TB thus hopefully leading to better outcomes in this population group.

Evidence supports the TB LAM test's potential role in scaling up of Intensified TB Case Finding, one of the 'Three I's' to reduce the burden of TB among PLHIV (the other two being Isoniazid Preventive Therapy (IPT) and TB Infection Control in Congregate and HIV care settings (TBIC)). By detecting TB early in PLHIV and initiating appropriate treatment early, the TB LAM test therefore has potential to decrease the incidence of TB among PLHIV thereby contributing towards a reduced TB burden in Swaziland. In view of the high HIV/TB burden in the country, TB LAM use requires exploration.

1.6 Aims and Objectives

The aim of this implementation research was to generate evidence required by the Ministry of Health to improve diagnostic options for patients who are unable to produce sputum for the diagnosis of TB and to evaluate whether a new urine based TB test (TB LAM) will contribute to the increase of the proportion of TB cases detected among PLHIV and shorten the length of time from TB screening to TB treatment for patients unable to produce sputum spontaneously.

1.6.1 Hypotheses
- The urine TB LAM test is associated with increased detection of, otherwise missed, true TB cases among presumptive TB patients without a cough.
- The performance of the urine TB LAM test is consistent with existing evidence.

1.6.2 Primary Objectives:
- To measure the incremental added value of incorporating urine TB LAM testing into the national TB screening algorithm for PLHIV. This is a calculation of number of confirmed TB cases detected by urine TB LAM test among patients who screen positive for TB but with no cough or who had sputum induction due to inability to spontaneously produce sputum.
- To determine the predictive value of non-cough symptoms in the screening tool (fever, night sweats and loss of weight) as predictors of TB disease among PLHIV without a cough
- To conduct a sensitivity analysis of the urine TB LAM test by CD4 category relative to Expert® MTB/RIF, measured against Conventional Sputum Culture (MGIT) in the study population.
- To determine factors associated with a true positive TB LAM test among patients screening TB positive but without cough (CD4, ART status, Age, Gender)

1.6.3 Secondary Objective:
The secondary objective of this study was to use evidence generated to review the TB screening algorithm and screening tool among PLHIV to maximise TB case detection.

2. METHODOLOGY

2.1 Study design
The study was a prospective observational cohort study introducing urine TB LAM testing as a sequential step along the current national TB screening algorithm.

2.2 Study Setting
Consecutive recruitment of participants was conducted at HIV care clinics in Swaziland at three hospitals providing HIV care and treatment services, namely Mbabane Government Hospital, Hlatikulu Government Hospital and Raleigh Fitkin Memorial Hospital from April 2015 to September 2015. All samples from participants were transported to, and processed on the same day at the national TB reference laboratory for quality control and standardization purposes.

2.3 Study sample
The target population was HIV positive patients with TB presumptive symptoms attending the HIV testing centers in the three mentioned hospitals.

2.4 Inclusion criteria
All adults who willingly consented to be part of the study, aged 18 years and above living with HIV attending the 3 HIV care centres regardless of ART status were eligible for inclusion in the study.

2.5 Exclusion criteria
The following patients were excluded from the study:
- Patients that had recently been diagnosed with active TB and had been on TB treatment within the past 60 days.
- Patients who were currently on IPT (or any other form of anti-TB medication) or had received IPT within the last 60 days.
- Children aged less than 18 years
- Any patient who was unwilling or unable to provide all samples

2.6 Study variables
2.6.1 Socio-demographic and clinical predictor variables
Variables under investigation in this study were derived from the literature review. Table 1 shows variables which were collected from every participant who was part of the study. Primary sources of data were patient treatment cards or records and participant interviews. Data was either extracted from patient treatment cards or provided by the participants if the treatment cards were not available.

Table 1: Socio-demographic and clinical variables considered in the study

Variable	Type of variable	Source of variable
Age/DOB Patient treatment ca	Continuous	Patient treatment card
Sex	Dichotomous	Patient treatment card
Marital status	Categorical	Self-reported
Educational level	Categorical	Self-reported
Occupational level	Categorical	Self-reported
Region of residence	Categorical	Self-reported
Type of residence	Dichotomous	Self-reported
Weight	Continuous	Patient treatment card
Height	Continuous	Patient treatment card
ART Status	Dichotomous	Patient treatment card
Previously treated for TB?	Dichotomous	Self-reported
Months since patient finished treatment	Continuous	Self-reported
Presence of TB symptoms: cough, fever, night sweats and loss of weight	Dichotomous	Self-reported
Duration of each TB symptom	Continuous	Self-reported

2.6.2 Diagnostic variables
Reference variable (Gold test)
Sputum culture: Indicator for true state of disease. Results reported as culture positive or negative to indicate the presence or absence of TB disease respectively. A positive culture result had a first-line drug-susceptibility testing (DST) report.

Classification variables (Tests evaluated)
TB LAM: Determine urine TB LAM test was reported as positive or negative.
Xpert MTB/RIF: results of Xpert ® MTB/RIF were recorded as MTB detected or MTB not detected. The rifampicin resistance status was also recorded for all the positive results.

2.7 Sample size calculation

Epi Info software (Epi Info™ 7) was used to calculate sample size using a margin error of 5%, a confidence level of 95% with an estimated population of 20,000 and TB prevalence among PLHIV of 17% (1). Using the above assumptions, the minimum sample size required was 377 participants.

2.8 Enrollment of participants

Eligible participants were consecutively enrolled from the three centers during normal working hours for a data collection period of 5 months.

2.8.1 Participants enrolment and data collection techniques
From April 2015 to September 2015, 417 adults aged 18 years and above, HIV positive irrespective of ART status and screened positive for TB using the national TB screening tool, were consecutively enrolled in this clinic-based study from three study sites: Mbabane Government Hospital, Hlathikhulu Government Hospital and Raleigh Fitkin Memorial Hospital. Patients underwent TB screening using the established patient flow systems at the study sites. Those who screened positive for TB where referred to a qualified and trained study nurse research assistant (study nurse) stationed within the ART clinic who then administered the process of informed consent in a private room. Patients who were able to provide a written consent and were willing to provide all required specimens were enrolled

into the study by the study nurse. A structured questionnaire was used to record required clinical and demographic patient data which was obtained from the patient through interview as well as transcription of data from the TB screening tool and patient ART booklet. The patient was then provided with specimen bottles for urine and sputum collection. Blood samples were collected by the study nurse. All samples were labelled according hospital laboratory sample labelling procedures. All data files were kept in a locked cabinet at the facility.

All samples were transported to the National TB Reference Laboratory for processing on the same day. Results for TB LAM, Xpert MTB/RIF and CD4 cell count were delivered to the study nurse the following working day from the date the sample was sent. Results for culture were delivered to the study nurses as soon as authorized results are available. Patients were called through telephone by facility nurses to come back for their results upon receipt of results by facility staff. Figure 2 below demonstrates patient flow during the study.
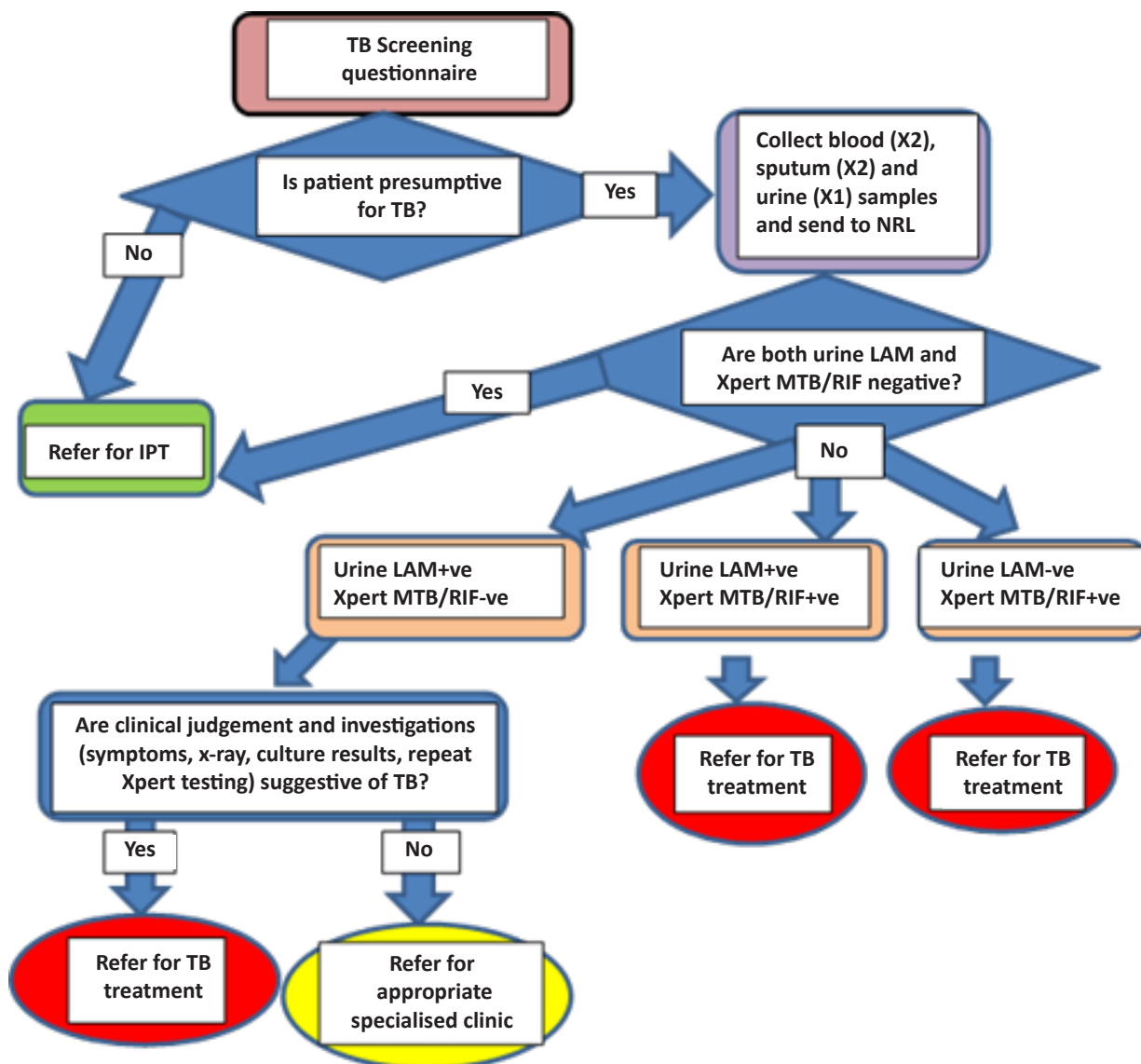

Figure 1: Study flow diagram

Specimen collection procedures

The nurse research assistants were trained on sample collection methods for urine, sputum (including sputum induction) and blood in accordance to phlebotomy, urine collection and sputum collection national standard operating procedures (SOP). Sputum samples were collected either spontaneously or by sputum induction using 5% hypertonic saline nebulisation for those with dry cough or without cough. To minimise harm to patients, blood sample collection was deferred until the patient completed consultation with the clinician so that all samples for additional blood tests that were to be ordered by the clinician were collected at once by the study nurse. Labelled sputum and urine samples were placed in cooler boxes with temperatures according to the sample storage and transportation SOP. Labelled blood samples were placed in a sample collection tray at room temperature and out of direct sunlight. These samples were picked from the health facilities by a study dedicated sample transport driver on a daily basis and taken to the National Reference Laboratory.

Laboratory procedures and results

At the national reference laboratory, a laboratory research assistant was responsible for receiving the samples. During this process, the laboratory research assistant acknowledged receipt of the samples using a sample tracking form. All the samples were processed according to the sample processing SOPs for TB culture, TB LAM and Xpert MTB/RIF testing. Available laboratory results were printed and returned directly to the study nurse or through the driver in a sealed envelope. Necessary infection prevention and control measures were implemented in each step.

2.9 Validity and reliability

For content validity in the research tool, principal investigators and co-investigators assessed the tool and reliability in the research tool.

2.10 Training of study personnel and pretest of study tools

The research staff consisting of two study nurses, laboratory research assistant and study sample driver were trained for two days on the rationale, aims, objectives, design and methodology of the study. The session aimed to enhance the knowledge of the research staff regarding this specific study as well as detail the research ethics and expectations of the research staff. Orientation to the data collection tools was also done during the training. The study nurses explained the process, requested for their consent and interviewed the laboratory research assistant and the driver. The findings of the pre-test were discussed with the nurse research assistants and appropriate changes were made through recommendations and approval by the research investigators.

2.11 Data Management

Weekly site visits were conducted to check data regularly for quality assurance, control measures as well as timely resolution of on-field challenges. The laboratory was visited on a weekly basis and a result tracking spreadsheet updated. To validate the results, the laboratory DISA machine was used to go through all the available results. Weekly meetings with the research team members namely, the Study Coordinator, Senior Research Advisor, Co-investigators and Principal Investigators were conducted as part of quality improvement. Regular verification and validation of data was conducted with all inconsistencies being checked and resolved.

2.12 Data processing and analyses

Upon completion of data collection, all data (demographic, socio-economic, clinical and laboratory) from the paper-based completed questionnaires was entered into Epidata version 3.1 software (EpiData Data Entry, Data Management and Basic Statistical Analysis System. Odense Denmark, EpiData Association, 2000-2008). Data were later exported into excel and then to STATA version 12.1 (© 1985-2011 StataCorp LP, Texas USA) for final cleaning and analyses. Results for Xpert MTB detected cases were sub-classified to whether rifampicin resistance was detected or not detected by creating another variable. For culture results, MTB positive and mycobacterium other than TB (MOTT) positive were separated and the true state of being infected with MTB was only reflected if and only if culture results were MTB positive.

Descriptive analyses using proportions and medians were conducted. Sensitivity, specificity, positive and negative predictive values analyses for TB symptoms and TB diagnostic tests were performed. The area under curve for diagnostic tests were estimated using non-parametric receiver operating characteristics (ROC) analysis. A true positive TB LAM test result was deduced from a positive urine LAM test when the culture was also positive for MTB. Logistic regression was done to determine factors associated with true TB status as well as true TB LAM status. A p value ≤ 0.05 was considered statistically significant using 95% confidence intervals. Mantel-Haenszel methods were applied to compare the odds ratios between strata of CD4 cell count and between ART statuses.

2.13 Ethical consideration

The study protocol was submitted to, and approved by, the Swaziland Scientific and Ethics Committee (SEC). A permission letter to gain entry to health facilities was obtained from the Principal Secretary (PS) in the Ministry of Health. Health care workers and management at participating study sites were sensitized on the study for awareness and to improve their understanding on its objectives and procedures. Study procedures were fully explained to potential participants who signed consent forms for voluntary participation or withdrawal from the study. There were no major foreseen risks to participants associated with participation in the study as the procedures were done routinely except for the discomfort from collecting blood for CD4 cell count and nebulization for those unable to serve sputum. There were no remuneration or incentives provided to participants.

Each participant was assigned a unique study identifier linked to the patient medical record. The study identifiers were consecutively assigned and were specific for each facility (nomenclature: HH001, HH002; RF001, RF002 and MH001, MH002 etc.). All patient identifying information was removed from the electronic dataset generated from the questionnaires. Laboratory request forms also included the unique identifier code for the participant as well as the participant name as required for laboratory requests. However, only the unique identifier code was entered by study nurses into the results collection form for data analysis. Thus, those analysing the data were blinded to the identity of the patients.

3. RESULTS

3.1 Descriptive statistics

The enrolment period took longer than otherwise anticipated. No data was collected to determine the response rate. A total of 417 participants were enrolled from April to September 2015. The median age was 42 years (IQR: 33.0-55.6) and 68.1% were females. The majority (91.1%) were already on ART whilst 77.2% had CD4 cell count more than 200 cells/mm3. There were 32.4% participants who were previously treated for TB and had completed anti-TB treatment more than 2 months prior to the study. The description of the study participants is shown in Table

Table 2: Description of study participants

Variables	Number of participants n (%) N=417
Gender	
Males	133(31.9%)
Females	284(68.1%)
Region of residence	
Hhohho	38(9.1%)
Manzini	138(33.1%)
Shiselweni	233(55.9%)
Lubombo	8(1.9%)
Type of residence	
Urban	93(22.3%)
Rural	324(77.7%)
Marital status	
Single	174(41.7%)
Married	205(49.2%)
Separated	10(2.4%)
Divorced	1(0.2%)
Widowed	27(6.5%)
Education	
No formal education	63(15.1%)
Primary	161(38.6%)
Some secondary education	161(38.6%)
Secondary graduate	16(3.8%)
Tertiary	16(3.8%)
Occupation	
Unemployed	230(55.1%)
Employed	155(37.2%)
Self-employed	32(7.7%)

Variables	Number of participants n (%) N=417
WHO stage	
Stage 1	334(80.1%)
Stage 2	68(16.3%)
Stage 3	15(3.6%)
Pre-ART	37(8.9%)
ART	380(91.1%)
Previous TB treatment	
Yes	135(32.4%)
No	282(67.6%)
CD 4 count	
<100	47(11.3%)
100-200	48(11.5%)
>200	321(77.0%)
Missing	1(0.2%)
*Age	42.2 (IQR: 33.0-55.6)
*BMI	22.4 (IQR: 13.8-26.3)
* Median and interquartile range for continuous variables	

Four hundred and fifteen participants were coughing. Three hundred and twenty two participants had sputum spontaneously collected, 65 had sputum induction done and 31 did not have any sputum collected. Among those who had sputum induction done, 4 failed induction. TB LAM test was performed on 414 patients of which 20 were positive, Xpert MTB/RIF was performed on 347 samples and MTB was detected in 18 samples and culture was performed on 367 samples from which 30 were positive for MTB and another 8 were positive for mycobacterium other than TB species. The results for TB LAM test and Xpert MTB in relation to sputum culture are shown in the following 2X2 tables (*Table 3* and *Table 4)*. TB prevalence was significantly higher in pre-ART patients (19%) compared to 7% in ART patients (Chi2: p-value=0.022). The median turnaround time for receiving TB culture positive results was 32 days (IQR: 18-48) days.

Table 3: Cross tabulation of TB culture and TB LAM

TB LAM	TB culture		Total
	Positive	Negative	
Positive	6	12	**18**
Negative	24	324	**348**
Total	**30**	**336**	**366**

Table 4: Cross tabulation of TB culture and Xpert MTB/RIF

Xpert MTB/RIF	TB culture		Total
	Positive	Negative	
MTB detected	15	1	**16**
MTB not detected	15	334	**349**
Total	**30**	**335**	**365**

Figure 2 represents the outcomes at each step following enrollment. The prevalence of TB in this study as determined by sputum culture was 8.2% (95% CI: 5.6-11.5).

Figure 2: An illustration of outcomes after enrollment

- To determine the predictive value of non-cough symptoms in the screening tool (fever, night sweats and loss of weight) as predictors of TB disease among PLHIV without a cough

3.2 The sensitivity and predictive values of TB symptoms

The national TB screening tool is a signs and symptoms based questionnaire. The proportion of reported signs and symptoms were: cough 99.5%, and night sweats, fever and weight loss were 84.4%, 79.6% and 66.7% respectively. The sensitivity of the cough symptom was 100% whether or not the duration was greater than 2 weeks or any duration. However, specificity was very low; 0.3% (95%CI: 0.0-1.7) for a cough of any duration and still very low at 0.65% (95%CI: 0.0-3.5) for a cough of more than 2 weeks. Except for weight loss (sensitivity 68.4% (95% CI: 51.3-82.5) , cough, night sweats and fever each had a sensitivity greater than 90%. However, all had specificities that were less than 35%. The predictive values of TB signs and symptoms were deduced and the results are shown in *Table 5* below.

Table 5: Predictive value of TB symptoms

Screening symptom	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Any Cough	100% (90.7-100)	0.3% (0.0-1.7)	10.4% (7.5-14)	100% (2.5-100)
Cough >2 weeks	100.% (82.4-100)	0.65% (0.0-3.5)	11% (6.7-16.6)	90.2% (2.5-100)
Night Sweats	94.7% (82.3-99.4)	17.9% (13.9-22.5)	11.8% (8.4-15.9)	96.7% (88.7-99.6)
Fever	92.1% (78.6-98.3)	21.3% (17-26.1)	11.9% (8.4-16.2)	95.9% (88.5-99.1)
Weight loss	68.4% (51.3-82.5)	34.3% (29.2-39.8)	10.7% (7.1-15.3)	90.4% (83.8-94.9)

- To conduct sensitivity analysis of the urine TB LAM test by CD4 category relative to Expert® MTB/RIF, measured against Conventional Sputum Culture (MGIT) in the study population.

3.3 Accuracy of diagnostic tests

The sensitivity of TB LAM was 55.6% (95% CI: 21.2-86.3) and a specificity of 90.9% (95% CI: 75.7-98.1) among those who had CD4 cell count less than 100 cells/mm^3 compared to 20% (95% CI:7.7-38.6) and 96.4% (95% CI: 93.8-98.1) respectively, for the overall study population. The sensitivity and specificity of Xpert MTB/RIF results was 77.8% (95% CI: 40.0-97.2) and 100% (95% CI: 89.4-100) among those who had CD4 cell count less than 100 cells/mm^3. For both TB LAM and Xpert MTB/RIF tests, the sensitivity was lower for CD4 cell count categories 100-200 cells/mm3 and ≥200 cells/mm^3 as shown in Table 6.

The area under curve (AUC) for TB LAM ranged from 0.49 for CD4 cell count ≥200 cells/mm^3 to 0.73 for CD4 cell count <100 cells/mm^3. AUC for Xpert MTB /RIF was 0.63 for CD4 cell count ≥200 cells/mm^3 and 0.89 for CD4 cell count <100 cells/mm^3. For the overall study population, TB LAM and Xpert MTB/RIF each had an AUC of 0.49 and 0.75 respectively.

Table 6 a: Diagnostic accuracy of TB LAM test disagregated by CD4 Cell count category against TB culture.

CD 4 category	LAM			
	<100	100-<200	>=200	Overall
Sensitivity	55.6% (21.2-86.3)	16.7% (0.4-64.1)	0.0% (0-21.8)	20% (7.7-38.6)
Specificity	90.9% (75.7-98.1)	97.3% (85.8-99.9)	97.0% (94.2-98.7)	96.4% (93.8-98.1)
PPV	62.5% (24.5-91.5)	50.0% (1.26-98.7)	0.0% (0-36.9)	33.3% (13.3-59)
NPV	88.2% (72.5-96.7)	87.8% (73.8-95.9)	92.3% (88.5-95.2)	94.5% (91.1-96.9)
ROC	0.73	0.57	0.49	0.49

Note: Confidence intervals are in parenthesis

Table 6 b: Diagnostic accuracy of Xpert MTB/RIF disagregated by CD4 cell count category against TB culture.

CD 4 category	Xpert MTB/RIF			
	<100	100-<200	>=200	Overall
Sensitivity	77.8% (40.0-97.2)	66.7% (22.3-95.7)	26.7% (7.8-55.1)	50% (31.3-68.7)
Specificity	100% (89.4-100)	100% (90.5-100)	99.6% (97.9-100)	99.7% (98.3-100)
PPV	100% (59-100)	100% (39.8-100)	80.0% (28.4-99.5)	93.8% (69.8-99.8)
NPV	94.3% (80.8-99.3)	94.9% (82.7-99.4)	96.0% (93.0-98.4)	95.7% (93.0-97.6)
ROC	0.89	0.83	0.63	0.75

Note: Confidence intervals are in parenthesis

MTB was detected in 18 patients using Xpert MTB/RIF and rifampicin resistance was not detected in all the 18 MTB detected specimens. Sputum culture was positive for MTB in 13 of the 18 and in all the 13 specimens, DST indicated that all were susceptible to rifampicin.

The Venn diagram below indicates the relationship of TB test results among all those with confirmed culture results.
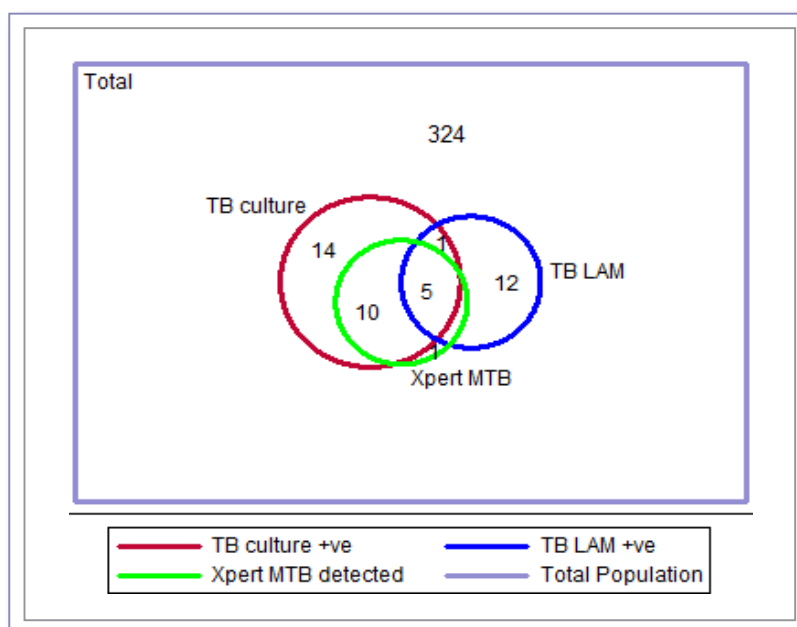
Figure 3: Relationship of TB diagnostic test results

3.4 Utility of TB LAM test

3.4.1 TB LAM test performance as a screening test to identify TB

TB LAM test performance and culture were compared by CD4 cell count categories. **Table 7** shows the TB LAM results relative to culture classified by CD4 category.

Table 7: TB LAM test as a predictor of positive sputum culture

CD4 cell count category (cells/mm³)	TB LAM	TB Culture		Total	OR [95% CI] (for positive culture)	P-value
		Negative	Positive			
<100	Negative	30	4	34	1 (ref. Negative) 12.5 [2.1- 73.5]	0.005
	Positive	3	5	8		
100- <200	Negative	36	5	41	1 (ref. Negative) 1.6 [0.2-13.5]	0.649
	Positive	1	1	2		
≥200	Negative	258	15	273		
	Positive	8	0	8		
	Total	**336**	**30**	**366**		

At CD4 count <100, a positive TB LAM test was significantly associated with MTB culture positive (i.e. TB disease): odds ratio (OR) =12.5; 95% CI: 2.1- 73.5; p-value=0.005. The OR for a positive culture when urine TB LAM test is positive for CD4 cell count ≥100 cells/ mm³ was found not to be statistically significant: OR= 1.6; 95% CI: 0.2-13.5; p-value=0.649.

- *To determine factors associated with a true positive TB LAM test among patients screening TB positive (CD4, ART status, Age, Gender)*

3.4.2 Determinants of a true positive TB LAM test

True positive TB LAM test was significantly affected by ART status and CD4 cell count on univariate analysis. The odds ratio (OR) that TB LAM was truly positive was 11.36 (95% CI: 1.21- 58.55; p-value <0.01) for those who were on pre-ART when compared to those on ART. The OR of having true TB LAM positivity was 43.57 (95% CI: 4.97-381.86; p-value < 0.01) if CD4 cell count was <100 cells/mm^3 when compared to those with CD4 cell counts above 100 cells/mm3. However, after controlling for age, gender, CD4 cell count and ART status, only CD4 cell count <100 (reference to CD4 cell count ≥100) remained a significant predictor of true positive TB LAM (OR=30.68; 95% CI: 3.10-303.89; p-value <0.01). *Table 8* below shows detailed results of univariate and multiple logistic regression model.

Table 8: Factors associated with a true positive urine TB LAM test

Variable	Univariate		Multiple	
	OR [95% CI]	P-value	OR [95% CI]	p-value
Gender				
Male	1 [---]		1 [---]	
Female	0.94 [0.17-5.17]	0.94	3.14 [0.35-28.33]	0.31
ART status				
ART	1 [---]		1 [---]	
Pre-ART	11.36 [2.21- 58.55]	**<0.01**	4.82 [0.60-38.45]	0.14
Age (years)	0.91 [0.84- 1.00]	0.06	0.96 [0.86- 1.07]	0.41
CD4 cell count				
≥100	1 [---]		1 [---]	
<100	43.57 [4.97-381.86]	**<0.01**	30.68 [3.10-303.89]	**<0.01**

- **To determine factors associated with a true positive TB LAM test among patients screening TB positive <u>but without cough</u> (CD4, ART status, Age, Gender)**

Two patients were not coughing, 1 had a successful sputum induction procedure done and cultured negative for TB. Since 415 (99.5%) patients were coughing, the study could not analyse a subgroup of those who were not coughing. However, a sub-analysis of those who had induced sputum induction (i.e. those who would normally be unable to produce sputum at that instance) revealed that only one patient out of the total of 65 in this group had a true positive TB LAM test and therefore could only fall on one category of any classifier of interest. Prediction of the above factors in determining association with true positive TB LAM was therefore not possible due to perfect prediction. The split *Table 9* shows the frequency of true positive TB classified by CD4 cell count category, ART status, age and gender.

Table 9: Status of true positive TB LAM among those unable to serve sputum spontaneously classified by CD4 cell count, ART status and Gender

TB LAM test	CD4 cell count category		ART status		Gender	
	<100	**≥100**	**Pre-ART**	**ART**	**Male**	**Female**
True positive	0	1	0	1	0	1
False positive + Negative	5	62	7	60	18	49
Total	**5**	**63**	**7**	**61**	**18**	**50**

- **To measure the incremental added value of incorporating urine TB LAM testing into the National TB screening algorithm for PLHIV. Calculate the number of confirmed TB cases detected by urine TB LAM test among patients who screen positive for TB but with no cough.**

3.5 Incremental value of using TB LAM on patients screening positive but not coughing

Using dry cough as a proxy to "no cough" which is characterized by inability to obtain a biological specimen for TB diagnosis, TB LAM test was evaluated on 59 patients who had successful sputum induction as well as confirmation of the true TB status by culture. The prevalence of TB among those with successful sputum induction was 6.8% (95% CI: 1.9-16.5). From the tree diagram in **Figure 4**, TB LAM would detect 1 case out of 4 that would otherwise be missed or not confirmed due to lack of specimen for bacteriological confirmation. Using the tree diagram below, 1.8% of the additional TB cases (i.e. those that would have been otherwise missed) would be correctly identified by use of TB LAM as part of an algorithm for those not spontaneously producing sputum or with no cough symptoms.
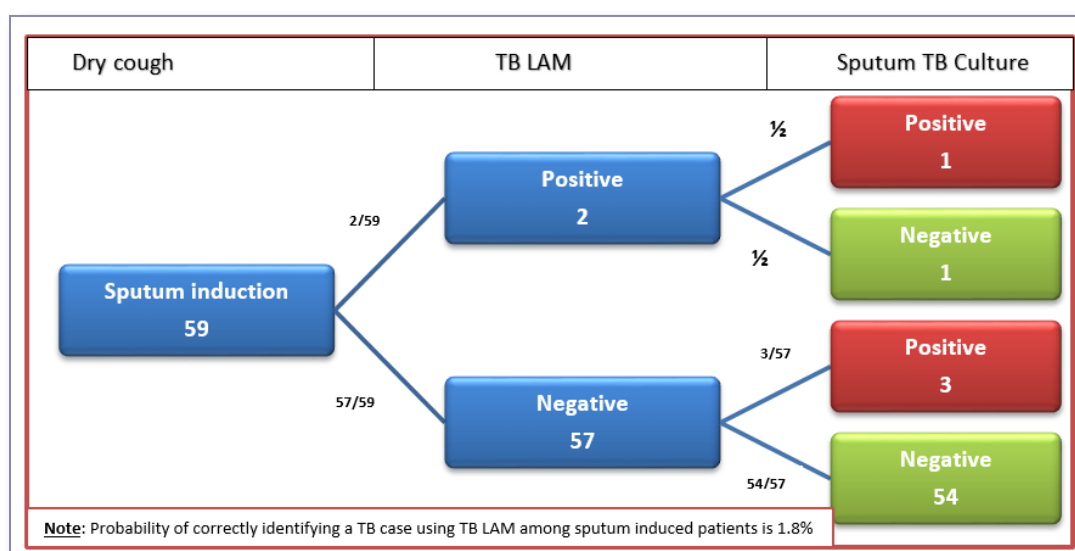

Figure 4: Value of TB LAM test among those unable to produce sputum

The sensitivity, specificity, PPV and NPV of TB LAM test among patients with dry cough who had successful sputum induction are shown in **Table 10** below.

Table 10: Performance of TB LAM among those who had a dry cough

TB LAM	(95% CI)
Sensitivity	25% (0.63-80.6)
Specificity	98.2% (90.3-100)
PPV	50% (1.26-98.7)
NPV	94.7% (85.4-98.9)
ROC area	0.62

3.6 Significance of urine TB LAM in increasing number of true TB cases in those without cough or without productive cough

The utility of TB LAM could not be evaluated on patients with no cough due to lack of sample in this category. However, TB LAM performance was evaluated on 59 patients with dry cough. Under routine settings, patients with dry cough would not normally serve sputum for bacteriological examination. Therefore, those with dry cough were used as a proxy to no cough and thus would not be able to spontaneously provide sputum for bacteriological testing routinely. There was an 18X higher odds of getting an additional TB case if TB LAM was positive compared to if it was negative among patients with dry cough but this was not statistically significant (OR=18; 95% CI= 0.89-363.63; p-value=0.059). There was a significant association between TB culture and TB LAM after controlling for CD4 count (OR=25; 95% CI= 1.11-561.28; p-value=0.043). Further analysis for factors associated with true positive TB LAM in the context of CD4 cell count level could not evaluated due perfect prediction of TB LAM result if CD4 count >100.

4. DISCUSSION

4.1 Overview of the study findings

A total of 417 patients participated in the study with a mean age of 42 years and 367 had bacteriological testing for TB. The prevalence of TB in this study setting was 8.2% [95% CI: 5.6-11.5] which is expectedly lower than the prevalence of TB among newly enrolled HIV patients in high HIV/TB burden countries (9.1%) including South Africa (10%) (2) considering that this study included both newly enrolled and existing ART experienced patients. The prevalence of 8.2% provides a reliable estimate for the prevalence of TB in HIV settings in Swaziland considering the gains achieved over the years in TB and HIV control in Swaziland.

TB remains disproportionately high among HIV positive patients not on ART compared to those already on ART, as exemplified by the contrasting TB prevalence among the two, 19% versus 7.2% respectively. Additionally those who were TB LAM positive and not on ART were 11 times more likely to have TB disease than those who were TB LAM positive and were on ART. Therefore prompt diagnosis of TB (and early initiation of TB treatment) remains critical to prevent further transmission of the disease within ART clinics, specifically among the HIV positive clients that are not yet on ART regardless of their CD4 cell count. The lack of a comparison group for those without cough symptoms as well as small numbers for true TB LAM positive patients affected the analysis, precision and inference of TB LAM utility to this group of interest.

4.2 Diagnostic accuracy for TB LAM & Xpert MTB RIF

The overall sensitivity of the TB LAM test as a diagnostic test for pulmonary TB was 20% but increased to about 56% for those with CD4 cell count below 100 cells/mm^3. These findings are consistent with findings in other settings (13, 18, 19). Other investigators argued that the increasing sensitivity of urine TB LAM testing with progressive HIV immune suppression as reflected by falling CD4 cell counts is the major distinguishing feature from other TB diagnostics (20). TB LAM evaluation remains valuable for all HIV positive patients with CD4 cell count below 100 cell/mm3 regardless of ART status. Adding TB LAM as part of TB screening is reasonable under the above two scenarios in which case those testing negative by TB LAM test are unlikely to have TB. The specificity of the TB LAM test has remained consistently high above 90%. The study results do not support health sector wide implementation of TB LAM across all HIV patients but rather a targeted approach identifying patients where the maximum benefit is realized without cost inefficiencies.

Xpert MTB/RIF testing has proved to provide answers for diagnosing TB disease in HIV positive patients using a simple and rapid methodology, due to its automation and rapid turnaround time. In the Swaziland setting where 73% of TB cases are HIV associated, Xpert MTB/RIF has reduced the number of TB cases that were missed by the traditional smear microscopy. However, the evaluation of Xpert MTB/RIF in this study revealed a sensitivity of 50% although the specificity was consistently very high above 99%. The sensitivity of Xpert MTB would have been higher if repeated samples (1-2) were collected consecutively as acknowledged in WHO's Xpert MTB/RIF implementation manual although it recommends one sputum specimen testing due to cost implications (21, 22). Testing the second and third samples increased sensitivity by 20% and an additional 16%, respectively (23). The study therefore demonstrates that one sample for Xpert MTB/RIF is not sufficient to correctly rule out TB diagnosis/disease if the recommendations from WHO of using one sputum sample are to be considered (24). Swaziland should therefore adopt a two sputum sample collection and testing for Xpert MTB/RIF or at least evaluate the gains and cost implications from such an approach.

The Xpert MTB/RIF did not miss Rifampicin resistance when compared to the conventional phenotypic culture and drug susceptibility testing. The performance of Xpert MTB/RIF decreased as CD4 cell count increased and only 27% of true TB cases were correctly identified as infected with TB among those with CD4 cell count above 200 cells/mm^3 using Xpert MTB/RIF. The sensitivity of Xpert MTB/RIF dropped by more than 30% when comparing those not on ART (sensitivity= 67%) versus those on ART (sensitivity= 46%). This questions the use of Xpert MTB in populations with high CD4 cell count and on ART. Therefore the application of the Xpert MTB/RIF molecular technique may not be confidently generalized to all groups.

Culture still remains a valuable test to establish TB diagnosis (25). Algorithms should therefore incorporate an extended TB diagnostic process recommending the collection of sputum samples for TB culture for all suspected TB cases that are negative to Xpert MTB/RIF in order to reliably rule out presence of TB disease. This will also decrease the proportion of clinically diagnosed TB. In spite of the costs that may be involved, and the prolonged culture results' turnaround time, correct identification of all presumptive cases and initiating anti-TB treatment is a documented method of TB control and ending the epidemic (2).

4.3 Incremental value of using TB LAM among patients screening positive but not coughing

All patients presented with a history of cough thus the value of TB LAM among those not coughing could not be established. However, a sub-analysis of those who had dry cough and hence under routine practice would not have produced biological specimen for TB testing provided a proxy to the value of using TB LAM in differentiating TB and non-TB diseased patients. The study showed a small chance (1.8%) of correctly identifying additional TB cases that confirmatory TB diagnosis would have otherwise been missed due to lack of sputum sample. The chance is small mainly due to a single true positive LAM case identified in this group. It is expected that with correct targeting of those with CD4 cell count <100, the probability of correctly identifying the TB cases for those unable to serve sputum may be considerably higher. In addition, further exploration of population attribution of this small chance may shed more light to its clinical significance given the majority of morbidities among PLHIV are attributed to TB.

4.4 Factors associated with true positive TB LAM

Overall, a true positive TB LAM was significantly associated with CD4 cell count. In the multiple regression model, our study revealed that TB LAM positive patients with a CD4 cell count <100 cells/mm^3 were just over 30 times more likely to be truly identified with TB disease when compared to those who have CD4 cell count ≥100. Our findings affirm that the TB LAM test performs better on HIV infected presumptive TB cases with very low CD4 cell count.

The TB LAM was significantly affected by the CD4 cell count and ART status in the univariate logistic regression model. A positive TB LAM positive result was 11 times more likely to be truly (or culture) positive and hence confirmatory for TB disease in pre-ART patients compared to those who were on ART. The study findings place emphasis on the need for availability of HIV rapid testing and expanding availability of POC CD4 count testing to facilitate identification of the target population; very ill adults who have CD4 cell count <100 cells/mm. WHO recommends that for patients with HIV infection and with low CD4 counts or who are seriously ill, the TB LAM test can be used for diagnosis as well as a screening test for TB (26). The potential programmatic impact of TB LAM would be expected to be greatest in countries with high TB/HIV burden and low coverage or uptake of ART (27).

4.5 Predictive value of the TB LAM test in detecting MTB disease

Patients with a TB LAM positive result were 4.9 times more likely to be culture positive than those who were TB LAM negative in the unadjusted model [OR 4.9, 95% CI: 1.7-14.0, p-value<0.01]. Further analysis indicated that the probability of TB was very high (25 times) among pre-ART patients compared to ART patients whose odds ratio was 3.9. TB LAM missed 20 of the 21 culture positive TB cases whose CD4 cell counts were at least 100 cells/mm^3. The odds ratio of having a TB culture positive result given that TB LAM was positive compared to when TB LAM was negative was 1.6 and 12.5 if CD4 cell count was ≥100 and <100 respectively. These results challenge the use of TB LAM in HIV positive patients with higher CD4 count (>100) or who are already on treatment. Therefore the TB LAM test in patients with CD4 cell count greater than or equal to 100 cells/mm^3 remains unsubstantiated as also suggested by d'Elia et al (28). TB LAM test also performed poorly in a study by d'Elia et al, conducted in a population where >50% of the study sample had a CD4 cell count above 200 cell/mm^3 (28). Lawn et al also argued that the test should not be used to screen or investigate unselected patients in primary care settings as the positive predictive value is very limited among those who are HIV infected and have high CD4 cell counts (29).

4.6 Limitations

The small numbers for true positive TB LAM results affected the precision of the results. This is mainly attributable to the ART population wide approach to the application of the test. The timing of ART initiation was not collected hence it could not be determined whether the prevalent TB cases were mainly in the context of immune reconstitution syndrome among newly enrolled patients or TB events were mainly on ART patients with treatment duration over 6 months. Lack of sputum samples for confirmation of TB from some patients and hence their exclusion might have biased the results since any correct classification of TB status might have affected the sensitivity and specificity of TB LAM test and/or Xpert MTB/RIF.

4.7 Recommendations

Using the evidence from this study, the following recommendations to improve TB diagnosis and case detection were made:
1. TB LAM can be used in severely immunocompromised patients with CD4 cell count <100 to aid the diagnosis of TB *(the sensitivity and specificity of TB LAM is 55.6% and 90.9% in patients with CD4 cell count <100, without targeted selection of patients in this study)*.
2. Targeting the correct population, TB LAM may have a limited value in aiding the detection of TB in patients unable to produce sputum *(the probability of confirming additional TB cases using TB LAM among those without sputum who would have otherwise been missed is 1.8% without proper patient targeting)*.
3. Using one sample for Xpert MTB/RIF testing is not sufficient to guarantee accurate ruling out of TB disease. Therefore at least two samples for each patient should be sent for Xpert MTB/RIF *(the sensitivity of Xpert MTB/RIF is 50% and specificity is 99.5% using one sample)*.
4. Culture should be prioritized for all presumptive patients who test negative for Xpert MTB/RIF, specifically those PLHIV with CD4 cell count ≥200 cells/mm^3 *(generally 50% of those who actually had TB were negative on Xpert MTB/RIF and specifically 73% of those with CD4 cell count ≥200 cells/mm^3 who had TB were missed negative on Xpert MTB/RIF)*.

4.8 Conclusion

TB LAM use as part of the national TB screening algorithm is feasible and the additional step of collecting urine is acceptable to patients. However, TB LAM should be used in a targeted approach by applying the test to those with very low CD4 cell counts to maximize TB case detection benefits. There is limited benefit in detecting additional cases of TB among those patients who may be missed due to inability to provide sputum either due to lack of cough symptoms or who have a dry cough. Use of sputum culture should be prioritized in patients testing negative to Xpert MTB/RIF to confidently rule out TB especially among those patients without severe immunosuppression i.e. those with CD4 cell count ≥100 cells/mm3 where Xpert MTB/RIF sensitivity was 27% despite this group having 50% of the culture positive TB cases.

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6. APPENDICES

Appendix 1: Informed Consent Form

Informed Consent Form and Information Statement

Principal Investigators: Dr Munyaradzi Pasipamire, Dr Mazibuko Sikhathele

Participating Institutions: Swaziland Ministry of Health (Swaziland National AIDS Programme, Swaziland National TB control Programme, National TB Reference Laboratory, Mbabane Government Hospital, Hlatikulu Government Hospital and Raleigh Fitkin Memorial Hospital), and University Research Co. LLC (URC)

Sponsor: UNAIDS and USAID Assist project
This Informed Consent Form has two parts:
• Information Sheet (to share information about the study with you)
• Certificate of Consent (for signatures if you choose to participate)
You will be given a copy of the full Informed Consent Form.

Part I: Information Sheet

Introduction

My name is …………………………., and I am working on this study on behalf of the Ministry of Health, and University Research Co., LLC. I am doing research on a diagnostic test to tell whether or not you have tuberculosis. I am going to give you information and invite you to be part of this research. Before you decide, you can talk with anyone you feel comfortable with about whether or not to participate. As I go through this information sheet with you, there may be words or ideas that you are not familiar with. Please interrupt me at any time and ask questions. If you have questions later, you can ask them of me or another researcher involved in this study.

Purpose of the research

Tuberculosis or TB is more difficult to diagnose in people living with HIV and this is a problem for ensuring that co-infected patients are initiated on the right treatment at the right time. This study is investigating a new way to test for TB, using urine. The study will not involve taking any samples from you other than would normally be taken and the samples will be collected by myself and the regular hospital staff. The other part of the study involves me asking you some questions about your age, occupation, marital status and ART-status. We anticipate these questions will take less than 5 minutes.

Method of sample testing

The samples will be analyzed by different methods to determine whether or not you are likely to have TB. If the test is positive, you will receive the same treatment that you would have received whether you were part of this study or not.

Participant Selection

You have been invited to take part in this study because we are asking all patients attending the HIV clinics who do not know their TB-status if they can participate.

Voluntary Participation

Your participation in this research is entirely voluntary and you will not receive any payment for agreeing to be part of the study. It is your choice whether to participate or not. If you choose not to participate, there will be no negative consequences to you. If you decide to participate, you may change your mind at any time and withdraw with no negative consequences.

Procedures

The samples will be collected by me and the medical and nursing staff at the hospital as they normally would. These include a urine, blood and sputum samples.

- To collect urine, you will be given two bottles to fill them in a private room.
- To collect blood, you will be pricked on your fore arm.
- You will also be pricked on your finger, to collect blood for haemoglobin.
- To collect sputum, you will be asked to cough out two bottles.

Duration

The data collection will last about 20 minutes. The time between when the samples are collected and when the results are available is 4 hours to 6 weeks. Preliminary results will be provided within 24 hours but you might be requested to receive additional results after six weeks.

Risks

There are no additional risks to you than there would normally be because we are only using samples that are normally collected in these types of cases.

Benefits

The samples that are obtained from you will be tested in several ways for TB at no cost to you or your family. The number and different types of test are probably higher than you would normally get, therefore you and the medical staff should know with a greater degree of certainty whether or not you have TB.

Reimbursements

There is no payment for your participation in the study.

Confidentiality

The results of the test will be completely anonymous for the researchers and anyone reading about this study once it is completed.

Sharing the Results

The results of this study will be shared widely, but your name will never be associated with the study.

Feedback

Although samples will be sent to the national laboratory, these will be provided within 24 hours to your doctor who will utilize existing systems to contact you for follow up, when required.

Right to Refuse or Withdraw

Even after you have agreed to participate, you can withdraw from the study at any time with no reason given and no negative consequences to you.

Who to Contact

This proposal has been reviewed and approved by Swaziland Ministry of Health Scientific and Ethics committee, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find out more about this study, contact Dr. Munyaradzi Pasipamire, PO Box 1404 Mbabane, Telephone 24047156. You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions? You are also free to ask questions later as well, not only when you are here. Here is the contact information to use if you have any other questions: 24027156. Like any other information in this study, any questions you ask or anything else you say will be kept confidential.

Part II: Certificate of Consent

I have been invited to participate in this research on TB diagnostic testing.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study

Print Name & Surname of Participant: _____

Signature of Participant: _____

Date _____Day/month/year

If the study participant is illiterate or unable to sign a document, thumb prints are an acceptable alternative.

Print Name & Surname of Participant: _____

Thumb print of participant

A witness is required to observe the consent process and sign below only if written informed consent is not possible and the patient has provided verbal consent. The signature of the witness below means that another person has observed the consenting of the participant. The witness must be impartial and not part of the evaluation staff.

Witness Signature: _____ **Date:** _____

EVALUATING THE INCREMENTAL VALUE OF USING THE TB LAM TEST IN INTENSIFIED CASE FINDING FOR TB IN PEOPLE LIVING WITH HIV

Participant's unique identifying code:_____   Enrollment date: _____(D/M/Y)

Participant's cell phone number: _____

Participant next of kin name: _____   Next of kin cell phone no: _____

SECTION A: SOCIO-DEMOGRAPHIC INFORMATION

1. Date of birth: _____   If not known, estimate age: _____

2. Participant's sex: Male ☐1   Female ☐2

3. Participant's marital status: Single ☐1   Married ☐2   Separated ☐3
   Divorced ☐4   Widowed ☐5

4. Participant's educational status: Primary ☐1   Some secondary school ☐2
   Secondary graduate ☐3   Tertiary ☐4   No formal education ☐5

5. Participant's occupational status: Employed ☐1   Unemployed ☐2   Self-employed ☐3

6. Participant's region of residence:
   Hhohho ☐1   Manzini ☐2   Shiselweni ☐3   Lubombo ☐4

7. Type of residence:
   Urban ☐1   Rural ☐2

SECTION B: CLINICAL INFORMATION

8. WHO stage of disease
   Stage I ☐1   Stage II ☐2   Stage III ☐3   Stage IV ☐4

9. Weight in kilograms _____   10. Height in centimeters _____   11. Temperature _____

12. Haemoglobin level_____

13. CD4 cell count determined by FACS Calibur _____
14. Participant's ART status   Pre-ART client ☐0   ART client ☐1

15. Previous TB treatment   Yes ☐1   No ☐2
16. If yes, how many months ago did patient finish treatment?_____

17. Does the patient experience the following?   (*Please tick on the right column and state the duration of symptoms)

SYMPTOMS	TICK HERE	DURATION OF SYMPTOM
Cough 1	☐	_____
Fever 2	☐	_____
Night sweats 3	☐	_____
Loss of weight 4	☐	_____

18. Method of sputum collection : Spontaneously ☐ 1   Induced ☐ 2

SECTION C: LABORATORY RESULTS

TEST	RESULTS		IF MISSING STATE REASON
19. Results of urine TB LAM test	Positive ☐ 1	Negative ☐ 0	_____ 99
20. Results of Xpert ® MTB/RIF	MTB detected ☐ 1	MTB not detected ☐ 0	_____ 99
21. RIF resistance if MTB detected by Xpert MTB/RIF	RIF resistant ☐ 1 RIF Indeterminate ☐ 2	RIF susceptible ☐ 0	_____ 99
22. Results of Bactec ® blood culture	Positive ☐ 1 ZN: AFB seen ☐ 1 AFB not seen ☐ 0	Negative ☐ 0 GRAM _____	_____ 99
23. Results of sputum culture	Positive ☐ 1 Contaminated ☐ 2	Negative ☐ 0	_____ 99
24. DST if sputum culture positive	_____		

For research assistant only: I hereby attest that the information provided above is valid and it has not been fabricated or falsified.

Signature: _____   Date: _____ (D/M/Y)

Appendix 3: Specimen collection, handling, transportation and sputum induction procedures

The purpose of the SOP is to explain and standardize the procedural methods for collecting, handling, transportation samples and performing sputum induction.

Preparing Laboratory Samples
General requirements for laboratory tests
Proper specimen quality, proper tube and proper quality

Specimens must be collected and preserved in the appropriate manner as detailed within this guide.

Lab requisition
Specimens must be accompanied by a completed requisition form or doctor's order signed by the requesting physician.

The form must contain all of the patient's identification details including:
- ✓ Brief history (clinical details and medication currently given)
- ✓ To prevent testing delays, all tests and panels ordered should be clear.
- ✓ Time of collection

Laboratory personnel will clarify unclear orders before collecting or processing samples.

The patient's full name must be clearly written on the specimen

Transport and handling
Particular care must be taken with considerations as; refrigeration, warming and priority as well as where special handling is required. Further details are outlined in this guide.

A. Blood collection
1. Collect all of the patient information required by the laboratory test requisition form and fill out the requisition form.
2. Other relevant information namely drug therapy and other clinical details should be indicated.
3. Ensure use of appropriate colour tubes for each requested laboratory test.
4. Patient should be comfortably seated.
5. Always clean venipuncture site with 70% alcohol and allow it to dry.
6. Avoid performing venipuncture using a syringe. This method is poor practice due to the increased risk of haemolysis and clotting of the sample
7. Blood collection should be from the arm opposite the site of any intravenous infusion.
8. Apply a tourniquet 10-15 cm above the puncture site. Do not leave the tourniquet for more than 3 minutes in place, as plasma proteins and protein bound constituents will be falsely elevated.
9. Collect blood using vacutainer needles and tubes. For infants and neonates, use butterfly winged needles. **DO NOT USE SYRINGES**: using a syringe for blood collection causes haemolysis which will greatly impede the accuracy of some laboratory investigations.
10. Remove the tourniquet before the needle is withdrawn
11. For tube with anticoagulant (purple ,green, blue) immediately and gently invert the tube 8 to 10 times

to ensure complete mixture of blood and anticoagulant. **DO NOT SHAKE THE TUBE** as this will cause haemolysis

12. Label tube with the patient name and time of blood draw
13. Arrange for prompt delivery of specimen of laboratory.

NOTES:
- Multiple sticks and patting at the venipuncture site can affect the quality of the sample
- If multiple specimens are collected, the anticoagulant should be collected into the second or third tube.
-

Please note:

Several changes occur in blood specimens following collection including:
- Glucose is converted to lactate due to glycolysis by red blood cells
- Potassium (K^+) and LDH pass through the RBC membrane into plasma leading to falsely elevated results
- Phosphate increases due to hydrolysis of organic ester phosphate in RBC
- Decrease in activity of labile plasma enzymes

B. Specimen requirements for URINE collection

Wash hands with soap and water; Open the container; be careful not to touch the inside edges of the container and its lid.

Urine collection technique for women
- Spread labia with one hand and hold apart
- Dry the area with sterile swab or gauze
- Void into the toilet for a few seconds and then stop
- Restart urine stream and collect into sterile container
- Cap and avoid touching inside or edges of container

Urine Collection Technique for men
- Retract foreskin if present
- Dry the area with sterile swab or gauze
- Void into the toilet for a few seconds and then stop
- Restart urine stream and collect in sterile container
- Cap and avoid touching the edges of the container.

Note: Label the container with patient's first name, last name, and time of collection and the ward/clinic hospital. Refrigerate container after collecting the specimen and deliver to the laboratory the same day.

C. Sputum collection procedures (Adapted from the National Tuberculosis Programme Manual):

Purpose: To collect a proper sputum sample for Xpert MTB/RIF and sputum culture
- Clean containers free from paraffin and other waxes should be used. The container should have an opening of at least 2cm and a capacity of at least 50ml.
- Sputum collection should be collected in open air away from other people and direct sunlight should be avoided.
- Give the patient confidence by explaining to him/her the importance of sputum collection
- Instruct the patient to rinse his/her mouth with water before producing the specimen. This will help to remove food particles and any debris in the mouth
- Instruct the patient to take two deep breaths, holding the breath for a few seconds after each inhalation and then exhaling slowly. Ask him or her to breathe in a third time and forcefully blow the air out. S/he should breathe again and then cough. This should produce a specimen from deep in the lungs. Ask the patient to hold the sputum container close to the lips and to spit it gently after a productive cough. Sputum is frequently thick and mucoid, but may be fluid, with chunks of dead tissue from a lesion in the lung. The colour may be a dull white or a dull light green. Bloody specimen will be red or brown. **Thin, clear saliva or nasopharyngeal discharge is not sputum** and is of little diagnostic value for tuberculosis.
- If the sputum in insufficient, encourage the patient to cough again until a satisfactory specimen is obtained. Remember that many patients cannot produce sputum from deep in the respiratory track in a few minutes. Give him/her sufficient time to produce an expectoration which she / he feels is produced by a deep cough.
- If there is there no expectoration, consider the container used and dispose of it in the appropriate manner.
- Check that the container is securely closed and labelled. Do not label the lid.
- Wash hands with soap and water
- Give the patient a new sputum container and make sure that she/he understands that a specimen must be produced as soon as she/he wakes up in the morning.
- Demonstrate to the patient how the container should be securely closed
- Instruct the patient to bring the specimen back the following day to the laboratory.

Sputum induction

Only HCW who have been trained in proper, safe technique should perform sputum induction. Patients must be observed at all times should the sputum induction procedure. Induction should be done in well ventilated areas and downwind if outside. HCW must wear N95 mask to safeguard against infectious droplets. Sputum induction can be used to obtain sputum from children or adults suspected with pulmonary TB, who are unable to produce sputum.

Do not perform sputum induction in patients with:
- Asthma
- Respiratory distress
- Pneumothorax
- Recent eye surgery
- Abnormal vital signs
- Chest trauma. Possible adverse events are mild coughing spells and broncho-constriction.

The procedure involves the insertion of a sterile suctioning tube through the nasopharynx after nebulisation with a 5% NaCl solution in order to help produce sputum and coughing. Sputum is then aspirated with a suctioning pump. Sputum induction can be done as an outpatient procedure and requires a fasting period of at least 3 hours prior to

procedure .Sputum induction is an aerosol generating procedure, and must thus be performed in an isolated room with adequate ventilation. If available, an ultraviolet light must be switched on when the room is not in use.

1. Materials

- Nebulizer kit
- Sterile oxygen mask (disposable)
- Sterile suction tube (disposable)attached to mucus trap (size 7 to 8 French)
- Sterile sputum container
- Sterile gloves
- Adult mask (N95)- for HCW
- Pulse oximeter
- 5% NaCl solution ('hypertonic saline')
- 0.9% NaCl solution ('normal saline')
- Salbutamol
- Disposable gloves
- Labels
- Permanent marker
- Transport box with cool packs
- Disposable waste container
- Oxygen cylinder

Explain to the participant the reason for sputum induction for sputum collection and how the procedure is performed. It is helpful to warn the patient that the inhalation might tickle a bit and can cause the urge to cough, but that it is not going to hurt. The patient's cooperation makes the procedure easier. Obtain an oral consent for the procedure.

2. Procedure
Preparation
- Setup area ahead of time to minimize anxiety of the patient
  - o Prepare the nebulizer and fit the aspiration pump with a new sterile suctioning tube
  - o Connect the air tube, one end to the compressor unit (nebuliser machine) and the other end to the bottom of the medication tank
  - o Ensure the pulse oximeter is working
  - o Fit the mask to the top of the medication tank
- Preload equipment with salbutamol & normal saline
- Fill the laboratory request forms
- Position patient in inclined/sitting position

Induction procedure
- Fit the mask to the patients face to ensure a close fit
- Run correct dosage of salbutamol nebulisation in normal saline (0.9% NaCl) for 3-5 minutes
- Add hypertonic saline (5% NaCl) to the solution and continue nebulization for 10-15 minutes
  - Record PR, RR, oxygen saturation throughout the procedure according to the control sheet for sputum induction
  - A sputum collection container should be available at all times. If the patient feels to urge to cough and expectorate spontaneously, even during inhalation, it should be done into the sputum collection container

Discontinue if:
- Respiratory distress including wheezing, increase in respiratory rate (more than 30% of baseline)
- Oxygen saturation of less than 90%
- Nausea and vomiting
- Light-headedness or dizziness

After procedure, all equipment is to be handled in sterile manner, and all equipment to be re-used will need to be disinfected and sterilized before use on an additional patient.

After induction
- Monitor the patient oxygen saturation and any signs of respiratory distress
- Ensure all samples are labelled
- Protect sputum from direct sunlight

CONDITIONS FOR SAMPLE REJECTION

A. Blood
- A specimen collected in a tube that is in appropriate for the test requested
- If the request form and the container do not contain the proper specified information, samples will be immediately discarded
- Any specimen that is obviously contaminated or rancid.
- Clotted specimens lead to erroneous result sand must be discarded.
- The correct volume of specimen must be collected. Over or under filling affects the blood: anticoagulant ratio leading to false results. Under filled or overfilled tubes will be rejected
- Specimens for which fasting is required that are known to have been collected in a non-fasting state.
- Specimens for which timed collection is critical that are not collected at the proper time. These include glucose tolerance, lactose tolerance, drug levels, and Troponin I.
- Specimens of insufficient quantity (however sample should not be discarded even though quantity is not sufficient)
- Haemolized specimens will invalidate many chemistry tests

B. Urine
- Any specimen received which is not properly labeled
- Any specimen collected in a non-sterile container.
- Urine unrefrigerated for more than 2 hours will be rejected.
- Any specimen that is obviously cloudy and characterized by extremely rancid smell, indicating bacteria multiplication in vitro.
- Urines known not to be collected at the proper time for those procedures requiring special timed voiding.
- Leaking containers

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