AFRICA UNIVERSITY

(A United Methodist Related Institution)

AN ASSESSMENT OF THE EFFICACY OF TUBERCULOSIS SCREENING TOOLS AMONG ADULTS VISITING OPPORTUNISTIC INFECTIONS CLINICS IN HWANGE DISTRICT 2022-2023

BY

MREWA PATIENCE TENDAI

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER IN PUBLIC HEALTH IN THE COLLEGE OF HEALTH SCIENCES

2024

Abstract

Tuberculosis is a leading infectious disease causing a high burden of morbidity and mortality worldwide, the greatest debilitating effects are felt among the high risk group of people living with HIV. The disease is the number one opportunistic infection in this group of people and over 200 000 people living with HIV succumb to the disease each year. Over 85% of PLHIV live in Sub Saharan Africa, where Zimbabwe is located. Early TB detection through screening is central to intensified case finding and infection control therefore reducing the morbidity and mortality associated with the disease. Despite the availability of various TB screening tools, TB case detection remains poor. The TB case detection gap of about 44% reported in Zimbabwe in the year 2021 raises concern over the efficacy of the available screening tools and the methods being used for TB case finding. The WHO END TB strategy requires more than 90% case detection to combat Tuberculosis. Increased TB case detection needs screening tools that are highly sensitive and specific, meeting the targeted 90% sensitivity and 70% specificity. The performance of the screening tools in use in Zimbabwe needs evaluation to find the root cause of the case detection gap. The purpose of the study was to evaluate the efficacy of tuberculosis screening tools used among people living with HIV visiting opportunistic infections clinics in Hwange district, one of the remote districts with a high TB burden in the country. The study was a cross sectional study conducted for the period January 2022 to December 2023. Secondary data on TB screening was collected from the TB registers, O.I clinic attendance registers and presumptive TB registers to determine the number of people screened for TB, their screen outcomes and TB diagnostic outcomes on the gene xpert test for those who screened positive. All the data was captured and stored in Microsoft excel. Data presentation, analysis and processing was done using Stata software version 13. A total of 8 162 participants were screened for TB during the mentioned period. 2 256 participants with positive screen results were included in the study. 2119 participants screened positive on the symptom screening only, 45 screened positive on the CXR only and 92 participants screened positive on both CXR and symptom screening. Among these, 80 people were diagnosed with TB. The calculated sensitivity for symptom screening was 76.0% whilst specificity was 31.0%. The CXR had a sensitivity of 93.0% and specificity of 83.0%. Combining the screening tools had a sensitivity of 100.0% and specificity of 29.0%. Overall, the symptom screening tool had sub-optimal performance when compared to the WHO target profile for an efficacious screening tool. CXR performed better and met the required target profile for a screening tool. Combining the tools when screening for TB can increase case detection but there is need to resuscitate x-ray services and possibly upscale to computer aided detection of TB in Hwange if the set targets for the END TB strategy are to be achieved.

Key Words: Tuberculosis, sensitivity, specificity, People living with HIV.

Declaration Page

I declare that this dissertation proposal is my original work except where sources have been cited and acknowledged. The work has never been submitted, nor will it ever be submitted to another university for the award of a degree.

Mrewa Patience Tendai	pmrewa (30 **March 2024)**
_____	_____
Student's Full Name	Student's Signature (Date)
Dr E Mugomeri	 (30 March 2024)
_____	_____
Main Supervisor's Full Name (Date)	Supervisor's Signature

Copyright page

Acknowledgement

I would like to acknowledge the unwavering support and guidance I received from my academic and field supervisors, Dr E Mugomeri and Dr A. Kuretu in writing this dissertation proposal. I also want to acknowledge the continued support from the Opportunistic Infections and Family Child Health clinic staff at St Patricks Hospital in Hwange.

Dedication

I dedicate this dissertation to my husband, Nairos, who motivated me to take up the MPH degree and continuously supported me throughout the course of the degree.

LIST OF ABBREVIATIONS

Tas4TB	Targeted active screening for Tuberculosis
ACF	Active case findings
HRGs	High Risk Groups
CAD4TB	Computer -aided diagnosis for Tuberculosis
HIV	Human Immunodeficiency Virus.
UNAIDS	United Nations Joint Programme on HIV/AIDS
CXR	Chest Xray
LTBI	Latent Tuberculosis Infection
TPT	Tuberculosis Preventive Therapy
ICF	Intensified Case Finding
PLHIV	People Living with HIV
W4SS	WHO 4Symptom Screen
ART	Anti-Retroviral Therapy.
MDR-TB	Multi Drug Resistant TB
MTB	Mycobacterium Tuberculosis
O. I	Opportunistic Infections
O.I.C	Opportunistic Infections Clinic
NTP	National TB Program.
PPV	Positive predictive value
NPV	Negative predictive value

TABLE OF CONTENTS

List of Tables

List of Figures

List of Appendices

CHAPTER 1: INTRODUCTION

1.1 Introduction

Tuberculosis (TB) is among the top infectious causes of morbidity and mortality in the world, it is an enormous global public health problem especially in low and middle income countries (LMIC) Zimbabwe included (World Health Organisation [W.H.O], 2018). TB results in significant socioeconomic losses worldwide and particularly in highly burdened countries.

For people living with the immune-suppressing Human Immunodeficiency Virus (HIV), Tuberculosis continues to be the number one cause of morbidity and mortality. This makes improved prevention and treatment of HIV associated TB critical to ensuring long term survival of people living with HIV (PLHIV).

Screening for TB involves systematic identification of individuals at risk for the disease by assessing symptoms or using tests or other procedures that can be applied rapidly. Efficacious screening tests should efficiently distinguish people with a high probability of having the disease from those who are unlikely to have the disease (W.H.O, 2021). Consequently, only those with a positive screen test undergo diagnostic testing, whilst those who screen negative can be started on TB preventive therapy.

TB screening among PLHIV is central to implementation of World Health Organization's 3 I’s interventions (Isoniazide preventive treatment, Intensified case finding, and Infection control for TB) for reducing the impact of the TB and HIV syndemics.

The World Health Organisation (WHO) recommends TB screening at Opportunistic Infections Clinics (OICs) for PLHIV at every clinical encounter using the WHO recommended 4 symptom screen (W4SS) which comprises of any one of current cough, weight loss, fever or night sweats, use of C-Reactive protein with a cut off of >5mg/L and/or use of Chest Xrays (CXR) followed by confirmatory testing using a WHO recommended molecular rapid diagnostic test such as gene Xpert MTB/RIF or Xpert MTB/RIF ultra for those with a positive screen test.

The WHO target product profile for a TB screening test requires that its sensitivity be 90% and specificity 70% (WHO, 2014). This study aimed to find out the available and most commonly used pulmonary TB screening tools among PLHIV in Hwange district, the sensitivities and specificities of the screening tools were measured (against GeneXpert diagnostic test) and their usefulness in aiding diagnosis evaluated against the WHO set screening tool profile characteristics. The study was a cross sectional study making use of existing medical registers and records to extract the diagnostic outcomes of each individual who was screened for TB between the period January 2022 to December 2023. Findings from this study can help set the ground for further local research on the effectiveness of TB screening and diagnostic tools in use and thereby help inform policy in order to device methods to increase diagnostic yields of TB especially among the high risk group of PLHIV.

1.2 Background to the Study

Tuberculosis (TB) is an infectious disease dating back to ancient times, it is predominantly airborne spread, the primary infection site are the lungs but may also disseminate to other organs of the body. This study will focus on pulmonary TB. Transmission occurs when an individual with active disease coughs or sneezes,

releasing contaminated droplets into the surrounding air which are then inhaled by others (Centre for Disease prevention and Control CDC, 2016). The pathophysiology of TB starts with exposure to the infection (inhaling contaminated droplets), followed by the first stage known as latent tuberculosis infection (LTBI). In the latent stage the causal bacterium (mycobacterium tuberculosis) is sleeping and the host is asymptomatic and non-infectious. Progression to the second stage, known as active TB disease is subject to the immune status of the infected individual and is marked by symptoms, and an infectious host (World Health Organisation WHO, 2018).

According to Global TB report (2014) HIV infection increases the risk of TB disease at least 20 fold, of which the risk remains substantially high even after immune reconstitution with antiretroviral therapy (Golub, Durovni, & King, 2008). TB is also the leading opportunistic infection contributing to about one-third of deaths in this population (Owiti, Onyango, Momany & Harries, 2019). If not adequately addressed, TB has the potential to undermine the great strides made globally in revolutionizing HIV treatment and care. Early detection and treatment of TB disease in this high risk group are critical components of comprehensive HIV care, and this includes TB screening at every encounter as recommended by the World Health Organization. Those identified to have any TB-related symptoms are investigated and treated if diagnosed with TB.

In the year 2016 there were an estimated 10.4 million incident tuberculosis (TB) cases worldwide, of these cases, 10% were people living with HIV (PLHIV) (WHO, 2019). Nearly 1.7 million deaths were attributed to TB, with more than 374,000 of the deaths occurring among PLHIV. About one-third of deaths among PLHIV are attributed to TB (Gettahun, Gunnerburg, Grannich & Nunn 2012). Global TB incidence rates have

been falling steadily since year 2000. However, the decline in incidence has been slow, estimated at 1–2% per annum. Continuing at this rate of decline in incidence rate, it will not be possible to reach the 80% reduction in TB incidence that is necessary for meeting the End TB Strategy target and the Sustainable Development Goal of Ending the TB epidemic by 2030 (WHO, 2021).

Zimbabwe is among the highly TB burdened countries worldwide, also burdened by multidrug-resistant TB (MDR-TB) and TB/HIV coinfections (WHO, 2017). With 1.2 million PLHIV, Zimbabwe is also among the top countries globally with the highest numbers of HIV-infected individuals (UNAIDS, 2017). A recent nationwide prevalence survey showed that there were an estimated 29,000 incident TB cases in the country in 2020 (WHO, 2021), 17% of which were among PLHIV. However, the country notified only 16,000 cases to the NTP (WHO, 2022), representing a gap of about 44% who were either undiagnosed or unreported to the national TB programme. This has necessitated a strategic shift in focus to 'finding the missing cases', which entails active intensified screening for the disease.

Treating TB comes at a very high cost considering the health expenditure and the loss of income due to disability associated with disease complications (Gupta, Lucas, Fielding & Lawn, 2015). In Zimbabwe there has been progress in reducing the TB burden as the country was delisted among the top 30 countries with the highest TB burden in the world in 2021 (WHO, 2021). Although the incidence of TB in Zimbabwe is insidiously going down, the disease remains a nuisance among people living with the immunosuppressing Human Immunodeficiency Virus (HIV).

Detecting tuberculosis in people with the disease is often a significant challenge since screening algorithms are typically non-specific and the currently available rapid diagnostic tools may be inadequately sensitive (Nalunjogi, Mugabe, Kirenga, Komuhangi, Muhumuza, Mwesigwa & Siedner, 2021). These challenges are compounded in people living with HIV, who present with more highly variable clinical manifestations of TB than people without HIV (Kranzer, Houben Glynn, Bekker, Wood, & Lawn, 2010). As a result, the mortality rate among people with HIV and TB co-infection is high, and many of these people die without ever being diagnosed with TB (Gupta, Lucas, Fielding, & Lawn, 2015).

To improve TB case finding among people living with HIV, in 2011 the World Health Organization (WHO) recommended regular screening using a clinical algorithm (WHO, 2011). In a high TB prevalence setting, a person living with HIV presenting with current cough, fever, night sweats and/or weight loss is to be evaluated further to either identify TB or make another diagnosis. In addition to being the critical first step to intensified TB case finding, TB screening is the gateway to successful implementation of other crucial TB/HIV interventions including TB infection prevention and control, TB preventive therapy and anti-retro-viral therapy (ART)

The 4 symptom screening tool recommended by WHO was derived from a meta analysis of 12 studies that included about 10 000 people living with HIV, among whom 5.8% of them had TB disease (Anand & Surbhi, 2015). The meta-analysis showed that the 4 symptom screen had a sensitivity of 78.9%, thus identifying the majority of PLHIV with presumptive TB needing further diagnostic evaluation (Getahun, Kittikraisak, & Heilig, 2020). Several other studies have also confirmed that the 4

symptom screening tool had a sensitivity of above 65% (Nalunjogi, et al. 2021) (Santos, Batista, Braga, Silva, Maruza, & Souza, 2020).

The Chest Xray is a rapid radiological investigation that can be used to screen for TB and is an important diagnostic aid for the disease. CXR has a high sensitivity for pulmonary TB which is the commonest form of the disease. The tool however, has a poor specificity as some abnormalities present on xrays suggestive of TB are also present in other disease processes (WHO, 2017).

The C-Reactive Protein (CRP) is one of the biomarkers of tuberculosis, it is also a marker of several other diseases associated with acute or chronic inflammation (Wilson, Badri & Maartens, 2011). Among PLHIV, CRP was discovered to have a high sensitivity but the specificity being variable depending on the CD4 count and ART status (Shapiro, Hong & Govere, 2018). The CRP can be readily deployed at primary care level where the first presentations to a health facility occur as patients seek care for their symptoms, unlike other screening tools like CXR and molecular diagnostic tests which may be costly and use at primary care level may not be cost effective (Hanson, Osberg, & Brown, 2017).

Despite the gathered evidence in other settings and recommendations made by the WHO for TB screening, there are very few studies done locally to ascertain the efficacy of the screening tools among the Zimbabwean population. There is therefore need for evaluating the performance of these screening tools locally and explore alternative approaches.

1.3 Statement of the Problem

During a prevalence survey done in 2020 nationwide, it became apparent that there were an estimated 29,000 incident TB cases in Zimbabwe during that year (WHO, 2021). 17% of cases whom were among PLHIV. Only 16,000 cases had been notified to the National TB Program (WHO, 2022), leaving about 44% of cases being undiagnosed and some proportion unreported to the national TB programme. The efficacy of screening and diagnostic tests is therefore, a critical concern.

Despite the availability of various TB screening and diagnostic tools, TB case detection remains poor. The lack of effective screening and testing methods especially in low income countries like Zimbabwe has led to misdiagnosis, delayed diagnosis, poor treatment outcomes, and increased morbidity and mortality rates. A lot of TB cases go undetected because of inadequate and inaccurate screening and diagnostic tests. There is need to upscale screening and employ highly sensitive tools to increase case detection especially among the high risk groups like PLHIV. The efficacy of TB screening tools among the Zimbabwean population is not well documented, this study therefore, aims to evaluate the efficacy of available TB screening tools as applied among PLHIV focusing on a population in Hwange district.

1.4 Research Objectives

1.4.1 Broad Objective

To evaluate the efficacy of pulmonary Tuberculosis screening tools used among people living with HIV visiting opportunistic infections clinics in Hwange district during the period January 2022 to December 2023.

1.4.2 Specific Objectives

- To determine the available and most commonly used TB screening tools for adults living with HIV visiting Opportunistic Infections clinics in Hwange district during the period January 2022 to December 2023.
- To track TB diagnostic outcomes for HIV positive individuals who screen positive on the WHO 4 Symptom Screen (W4SS) and/or Chest Xray screening tools in Hwange during the period 2022-2023.
- To measure the sensitivity, specificity, positive and negative predictive values of the W4SS and CXR screening tools as applied among PLHIV during the period 2022-2023 in Hwange district.

1.5 Research Questions

- Which TB screening tools are used to screen for the disease among people living with HIV in Hwange?
- What are the Sensitivities and Specificities of the WHO 4 Sympton Screen and Chest Xray for TB screening as applied in Hwange district?
- What are the most effective screening methods for TB in people living with HIV in Hwange?

1.6 Significance of the Study

TB disease is a major public health problem worldwide. Despite the availability of various screening and testing methods, the diagnosis and treatment of TB remains a challenge, particularly in low and middle income countries. Many people with TB remain undiagnosed or untreated, especially in resource limited settings. One of the challenges in TB control is the low coverage and quality of TB screening and testing

services, which can result in delayed diagnosis, missed cases and poor outcomes. According to WHO in 2020, only 71% of the estimated TB cases were detected and notified, and the case detection gap was at 2.9 million. In Zimbabwe the case detection rate was at 68% and the case detection gap was 38000. There is a need to understand the factors that influence the access, utilization, and performance of TB screening and testing services, and to try and identify the best practices and strategies to improve the outcomes.

Provision of highly sensitive screening and diagnostic tests that are easily accessible and cost effective will go a long way in reducing the burden of TB. Results of this study can help determine the usefulness of the available tools in order to recommend appropriate screening and diagnostic algorithms or continue with current procedures if they prove to be very effective in detecting active TB cases. Results from this study can also help set the ground for further research in efficacy of TB screening tools and hence on ways for improving TB case detection.

1.7 Delimitation of the Study

The study was carried out at one hospital of the 4 in Hwange district due to resource limitations and the short time that was available for the student to complete the study. The data was only collected from records of persons visiting the public hospital in Hwange town but there are individuals who are diagnosed and followed up at private and other clinics and these will be missed on analysis. Only outcomes of screening were analyzed in the study regardless of the algorithms used by the attending health care worker.

1.8 Limitation of the Study

Screening and testing for tuberculosis was not universal for every patient owing to the fact that some screening tests or imaging like Chest X-rays (CXR) were not done on every patient due to the high cost of the imaging which is out of reach for many and sometimes during the period under study the machines were down. .

CHAPTER 2: REVIEW OF RELATED LITERATURE

2.1 Introduction

In this chapter, the researcher will try to find related literature with regards to the research objectives. The aim of this chapter is to review previously conducted studies on the subject of TB screening tools and their respective sensitivities and specificities, identifying how arguments developed among different researchers; discussing how these researchers' findings complement or disagree with one another. This will then demonstrate similarities and differences of findings and recommendations among authors thus aiding the researcher to identify research gaps. The chapter will also include a conceptual framework on which the study will be based on.

TB screening and testing algorithm for Zimbabwe

Screening for TB is important for TB disease control as it allows early identification of patients with the disease in order to curb transmission and treat those with TB early to avoid the TB related morbidity and mortality. The diagram below outlines the basic steps followed by clinicians in screening and testing for TB and steps applicable for this study.

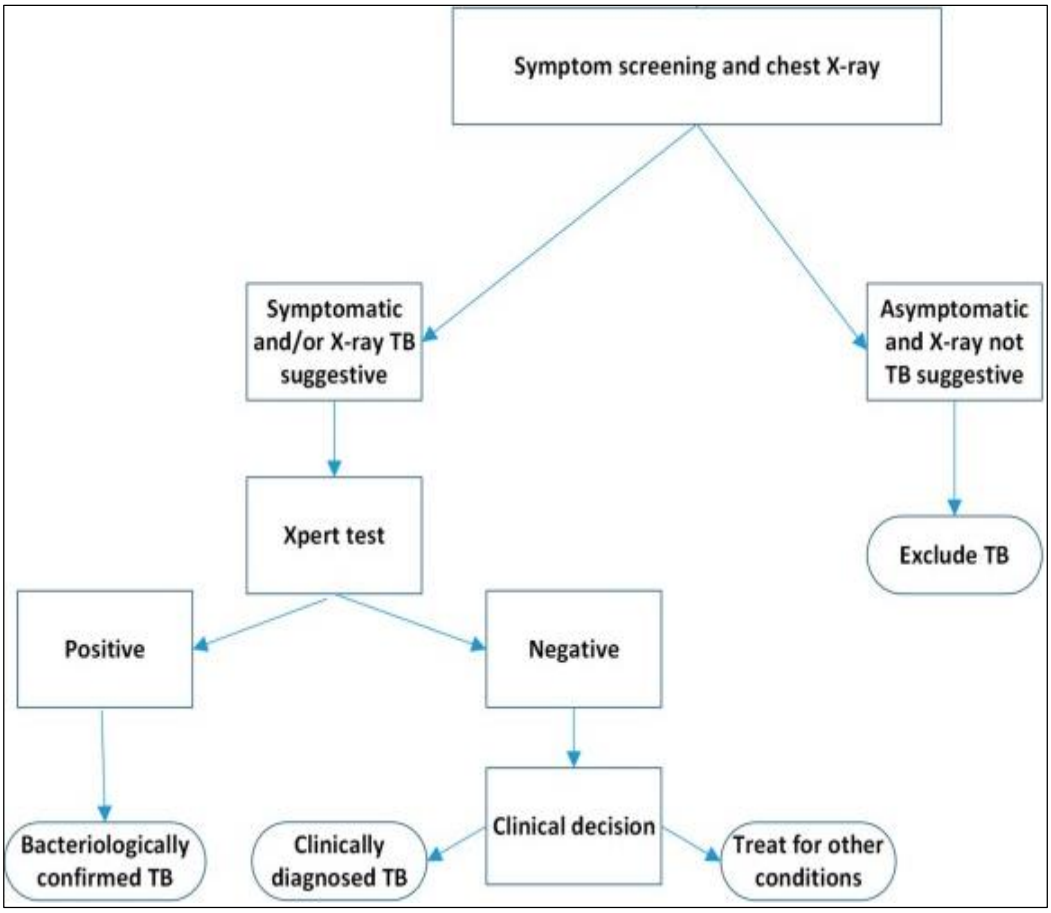


Fig 1. TB screening and testing algorithm

Adapted from Jerene, Muleta & Dressie (2022).

2.2 Conceptual Framework

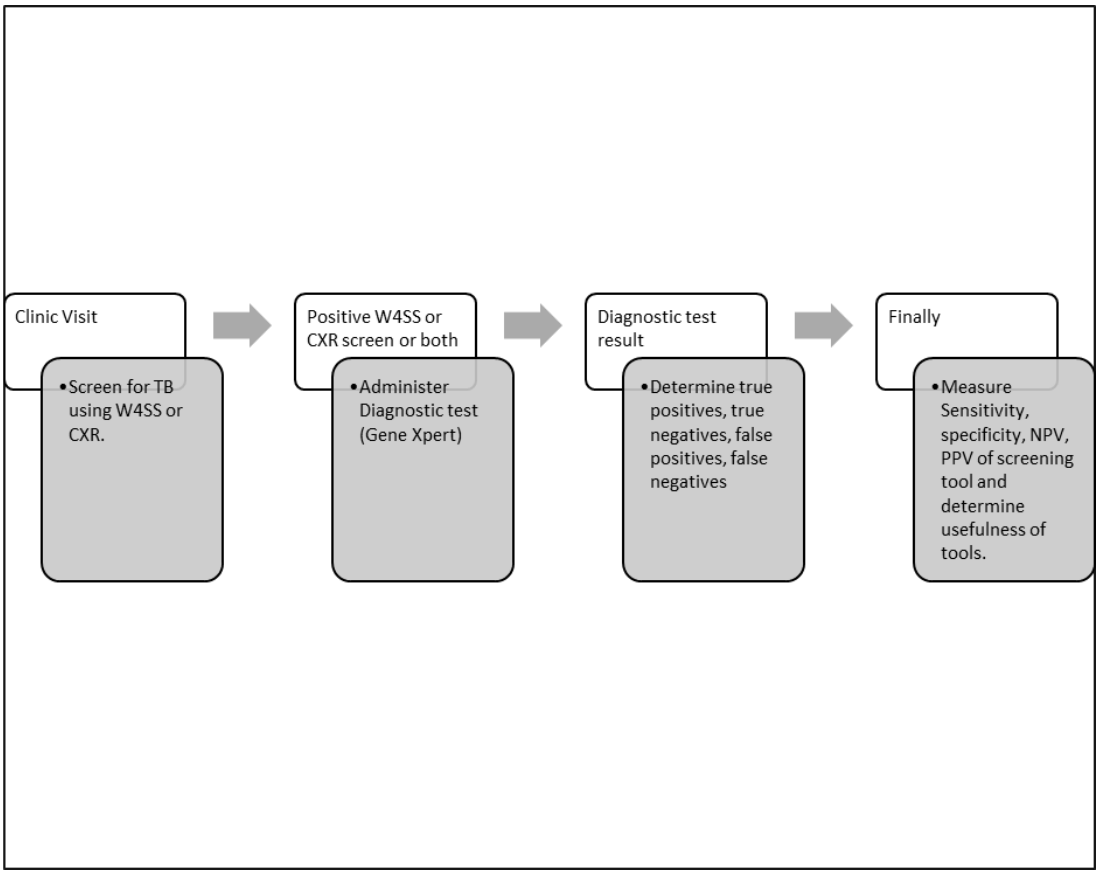


Figure 2. Conceptual Framework

2.3 Relevance of the Conceptual Framework to the Study

A conceptual framework is a structure which the researcher believes can best explain the natural progression of the phenomenon to be studied (Camp, 2001). It is the researcher's explanation of how the research problem will be explored, it presents an integrated way of looking at a problem under study (Liehr & Smith, 1999). The Conceptual Framework shown above tries to depict the sequence of activities that will characterize the research, At each clinic visit, a patient is screened for TB as per WHO guidelines, followed by administering a diagnostic test if the screening test is positive. TB preventive therapy (TPT) is given to individuals who have a negative screen test.

After a diagnostic test is administered, individuals who test positive will be started on TB treatment whilst those who test negative will be reevaluated either to start TPT or sometimes are started on TB treatment based on the physician's discretion. This research tried to determine the true positives (those with a positive screen test who also test positive on the diagnostic test Gene Xpert), true negatives, false positives and false negative results from the screening tests and subsequently measured the performance of the screening tools against the diagnostic test.

The sensitivities, specificities, negative and positive predictive values (NPV and PPV) of the screening tools (W4SS and CXR) were measured against the diagnostic test results on Gene xpert to estimate the performance of the screening tools as applied for the PLHIV in Hwange district. Secondary data from already existing TB, presumptive TB and other clinic registers were used for the study.

2.4 Tuberculosis screening

Screening can be defined as the systematic testing of individuals for pre-clinical disease and in individuals who are usually asymptomatic. The purpose of screening is to prevent or delay the development of advanced disease in the population with sub-clinical disease through early detection and treatment (Morrison, 2012). Screening for TB has greatly evolved over the years, In 1974, WHO recommended a shift away from indiscriminate TB case finding by mobile mass screening to more targeted and efficient approaches. Today, systematic screening for active TB involves risk based approaches, high risk groups such as healthcare workers, contacts of individuals with TB, PLHIV receive targeted screening.

Routine screening for TB among PLHIV is recommended in order to detect cases earlier, exclude active TB prior to initiating TB preventive therapy (TPT), initiate

treatment early and impact overall disease control. Screening is anticipated to result in a decrease in TB related morbidity and mortality (Telisinghe, Ruperez, Amofa-Sekyi, Mwenge, Mainga, Kumar, & Ayles, 2021). A major barrier to implementing systematic screening of TB for high risk groups is the lack of an adequate TB screening tool (Yoon, Semitala, & Atuhumuza, 2018). The WHO target product profile for a TB screening test requires that sensitivity be 90% and specificity 70% (WHO, 2014). The high sensitivity profile requirement ensures that individuals who screen negative have a low chance of active TB disease and can therefore initiate preventive therapy (TPT) safely. In addition, the test should be simple, low cost and available at the point of care so that the test can be done by front-line workers. Some researchers have noted that for use among people living with HIV, no current test satisfies the minimum requirements for a TB screening tool (Yoon, Semitala, & Atuhumuza, 2018).

2.5 Screening methods in use and their performance

2.5.1 WHO 4 symptom screening tool/questionnaire (W4SS)

The WHO recommends use of a **4 symptom screening tool** (W4SS) for PLHIV in resource constrained areas like Zimbabwe. The symptom screening tool is considered positive when an individual is found to have any one of a cough, night sweats, fever or weight loss. The screening tool categorizes people into asymptomatic individuals and TB suspects (symptomatic patients) who require further evaluation to exclude TB (WHO, 2020). The W4SS is simple to administer, low cost and readily available at the point of care, facilitating convenient categorization of patients into TB suspects who then need investigation with more definitive diagnostic tests like the Gene Xpert assays, sputum microscopy or TB culture when available.

There is however, debatable evidence on the effectiveness of the WHO 4 symptom screening tool with a study conducted in South Africa asserting that the symptom screening tool's performance is limited and varied by Anti-Retroviral Treatment (ART) status, the tool showed low sensitivity and was not useful for differentiating those with and without pulmonary TB among people on ART (Hanifa, Fielding & Charalambous, 2021). On the same study, the tool was highly sensitive and diagnostically useful among participants who were not on ART. The symptom screening questionnaire sensitivity was also low (23.8%) in the study by Rangaka, Wilkinson, Glynn, Boulle, Van Cutsem, Goliath & Maartens, (2014). although some confounding factors were not accounted for. On the contrary, a lot of other studies from Sub-Saharan Africa agree that the tool has over 90% sensitivity but the specificity is low, ranging from 5-33% (Lawn, Brooks & Kranzer, 2011) (Kufa, Mngomezulu & Charalambous, 2012) (Swindells, Komarow & Tripathy, 2013). In a local study by Corbett & Zezai (2010), the duo found out that symptom screening for TB was highly sensitive when used to identify TB suspects among people who present themselves at health facilities for ill health (passive case finding) than for active case finding among patients visiting the hospital for other services. In particular a chronic cough was found to have a high sensitivity and high positive predictive value for sputum positive TB. The same study by Corbett & Zezai also ascertained that in populations with easy access to TB diagnosis, all symptom combinations have very low sensitivity of about 20-45%. In a study by Khan, (2014) sensitivity of the W4SS among people on ART was 60% but combining symptom screening with CXR yielded more TB cases with sensitivity increasing to 95% and specificity 92%. In a randomized study in Brazil by Santos, Batista, Braga, Silva, Maruza, & Souza, (2020), the symptom screening among HIV patients on ART for more than 3 months had a

moderate sensitivity of 85.3%, a low specificity of 46.4% with a very low positive predictive value of 13.6% but a high negative predictive value of 97% but screening performance remained the same after chest xray inclusion with a 44% specificity, 13.1% positive predictive value and a negative predictive value of 96.8%.

More recently, provider initiated TB screening as part of active case finding, has become an integral part of HIV care especially in resource constrained settings and symptom screening is often the only practical approach (Lawn, Myer, Bekker & Wood, 2016). To facilitate intensive case finding among the high risk group of PLHIV, there is need for a screening method that has a higher specificity for active TB, but maintain the other attributes of the W4SS. In Zimbabwe the 4 symptom screening questionnaire is widely in use and at central hospitals every patient who comes in for whatever service is screened for TB using the tool which is often in a stamp form and the frontline worker at the entry point ticks any presence of the stated symptoms (Cough, night sweats, fever, weight loss).

2.5.2 C-Reactive protein

The emergence of drug resistant strains of TB has prompted the demand for more triage tests to identify active pulmonary TB and also evaluate their disease severity. Thus, acute phase reaction serum tests like C reactive protein are instrumental for monitoring TB patients.

C reactice protein (CRP) is an acute phase reactant, it is a non specific inflammatory biomarker that WHO has recently recommended for TB screening. Its concentration rises in response to inflammation induced by diseases like TB (Sage, Noursadeghi & Evans, 2014). and can be quantitatively analyzed through point of care cost effective assays. A meta-analysis by Meca, Turcu-Stiolica, Bogdan & Pisoschi, (2022) found

CRP to be highly sensitive and moderately specific for active TB in outpatients with confirmed HIV infection. For a CRP threshold of 10mg/L, sensitivity estimates ranged between 20% and 97%, and specificity between 26% and 95%, where twelve studies with 3 751 patients were included.

A systematic review conducted in China found CRP levels of >10mg/L among persons undergoing TB screening to have a pooled sensitivity of 93% and pooled specificity of 60% for culture positive pulmonary TB (Yoon, Chaisson, Patel, Allen, Drain, Wilson & Cattamanchi, 2017) almost meeting the WHO's minimum requirements for sensitivity and specificity for a triage test for active TB. In the same study, adding the symptom screening to the CRP screening algorithm had not much difference in the TB yield obtained and had similar sensitivity and specificity. Yoon, Chaisson, Patel, Allen, Drain, Wilson & Cattamanchi (2017) also ascertained in their study that the use of CRP identified all cases of Gene Xpert positive TB .

CRP has been consistently shown to have higher sensitivity for pulmonary TB compared to other markers of inflammation like erythrocyte sedimentation rate (ESR) and lactate dehydrogenase (LH) (Polzin, Pietz, & Erbes, 2013) (Schleicher, Herbert, & Brink, 2015). CRP studies have confirmed that the test is more sensitive when screening patients who present to a health facility with ill health (passive case finding) than among patients visiting health centers for other reasons

Although elevations in CRP (>10mg/L) are not specific for active TB, it has been found to have 2-6 fold higher specificity compared to the symptom screening (Yoon, Davies & Huang, 2014) (Lawn, Kerkhoff, Vogt & Wood, 2013). Theses studies highlighted that the CRP which is already available as a simple, rapid and low cost

point of care test (results are available in 3minutes and cost is about $2 per test) is a promising approach to TB screening.

2.5.3 Chest X-ray (CXR)

Is a rapid imaging technique that allows lung abnormalities to be identified, the chest x-ray has historically been one of the primary tools for detecting TB. The recognition that new strategies are needed to control TB has led to reconsidering the chest x-ray as a screening tool by WHO. Innovative, lower cost methods for performing CXR (digital radiography) and reading them (computer aided) have become available (Jaeger, Karargyris & Candemir, 2014). Different studies have tried to determine the sensitivity and specificity of the CXr in TB diagnosis. In a systematic review done among HIV negative and HIV positive individuals (Van't Hoog, Langendam, & Mitchell, 2013) the CXR was found to have 98% sensitivity and 75% specificity . A study by Cohen, Muzaffar, Capellan, Azar, & Chinikamwala (2006) found a sensitivity of the CXR for TB detection between 74% to 79% and a specificity of 60% to 63% in a high risk population. Similar results were obtained by Den Boon, Bateman & Enarson (2005) who compared the diagnostic value of TB symptoms versus CXR in a prevalence survey, he noted that peculiar findings like milliary TB had sensitivities of between 59% to 69% with specificity as high as 97% to 100%, the detection of enlarged lymph nodes had a sensitivity of 67% and specificity 59%. The use of more sensitive TB screening tools such as the CXR will increase the pool of presumptive TB cases hence increasing case yielding as more people will be subjected to the diagnostic tests (gene xpert or culture).

The prohibitive costs of a CXR are the number one deterring factor in widespread use of the tool. According to WHO (2016), CXR has a high sensitivity for pulmonary TB

when read to identify any abnormalities consistent with TB, they however note that it has a poor specificity. Many lung abnormalities that are seen for TB on CXR are also present on CXR for other lung diseases. The inter-observer differences in interpretation of CXRs and also the difficulties in interpreting prior and/or active infection makes it a difficult tool to rely on for TB diagnosis and can lead to over or under-diagnosis (WHO, 2016). WHO insists that rigorous efforts to base a TB diagnosis on bacteriological tests should therefore be made.

2.5.4 The GeneXpert MTB/RIF or MTB/RIF ultra assays

The gene xpert or gene xpert ultra assays are also used to screen for TB in some settings, these are new tests that are revolutionizing TB control by contributing to the rapid diagnosis of TB disease and drug resistance. The gene xpert is a nucleic acid amplification test of low complexity and is almost fully automated (WHO, 2016). The test simultaneously detects Mycobacterium TB (MTB) and resistance to Rifampicin (a drug used as the backbone for TB first line treatment) in less than 2 hours (Rimal, Shrestha, Pyakurel, Poudel, Shrestha, Rai, & Rai, 2022). Gene Xpert assays can be performed on respiratory specimens and also some extrapulmonary specimens.

Sensitivity of the MTB/RIF ultra assay is higher than that of MTB/RIF assay and is preferred for HIV infected patients, children and extra pulmonary specimens (Center for Disease Control and Prevention, CDC, 2018). According to a study by Shah (2016), the diagnostic accuracy of the Gene Xpert was 96.23%. Another study concluded that the Gene Xpert test is an excellent clinical tool for detection of MTB in early stages where the direct smear is negative (Prakash, Datta, Goyal, Chatterjee & Gopal, 2016).

The sensitivity and specificity of the Xpert test from a study in India was noted to be 95.7% and 99.3% respectively (Sanker, Kottuthodi, Ambika, Santhosh, Balakrishnan, Mrithunjayan, & Moosan, 2017). In higher prevalence settings, Gene Xpert has a pooled sensitivity of between 69.4% to 85.7% and specificity of 98.4% to 98.8% (Shapiro, Ross, Yao, Schiller, Kohli, Dendukuri, Steingart & Horne, 2021). Many other studies on the sensitivity and specificity of the Gene Xpert test have produced similar results. The study by Rimal, Shrestha, Pyakurel, Poudel, Shrestha, Rai, & Rai, (2022) found that there was not much difference in performance of the Gene Xpert as a diagnostic tool for TB compared with the TB culture which is the gold standard in diagnosing TB, the Xpert assay had a sensitivity of 80.2%, a specificity of 96.6%, positive predictive value was 89% whilst negative predictive value was 92%.

It has been noted however that for optimal laboratory diagnosis using the Gene Xpert tests, patients need to appropriately expectorate sputum from deep within the lungs in order to optimize the chances of having a high TB bacilli density (Zifodya, Kreniske, Schiller, Kohli, Dendukuri, Schumacher, & Pai, 2021).

Use of the Gene Xpert in resource constrained areas is challenging as it requires a constant supply of electricity, huge capital investments for equipment and consumables and persistent maintenance, these characteristics can make use of the test out of reach for many in poor economies but in Zimbabwe, a subsidiary program from the global fund against TB, malaria and HIV has made it possible for the test to be widely available in most district hospitals (Kuretu, 2023).

Case study use of CXR and Gene xpert testing in Zimbabwe (2017)

According to the tuberculosis (TB) intervention program in Zimbabwe, the Tas4TB algorithm encompasses a two-pronged approach involving symptom inquiry and

digital chest X-ray (CXR) as initial screening tools, with subsequent diagnostic confirmation through Xpert MTB/RIF testing from Cepheid, Sunnyvale, CA, USA (Timire, Sandy & Ngwenya, 2019).

Tas4TB program data from 2017 indicates that a substantial number of patients are initiated into treatment based on field CXR results and/or symptom screening. Of the 35,610 individuals screened, 11,213 (31%) were identified as presumptive TB cases, and 705 (2%) were clinically diagnosed with TB (all forms) and commenced treatment. Only 89 out of 705 cases (13%) were confirmed through bacteriological testing. All digital CXR images are retained by the Tas4TB program. In 2017, 230 images were available for presumptive TB patients who commenced treatment but yielded sputum-negative results on Xpert or on Xpert plus smear microscopy (Timire, Sandy, & Ngwenya, 2019). In cases where there was no bacteriological confirmation with smear microscopy or Xpert, clinical assessment alongside CXR yielded a sensitivity of just 24% (95% confidence interval [CI] 10–51) (WHO, 2015). Concerns have been raised that some patients may be receiving over-treatment during Tas4TB programs as a large proportion do not receive bacteriological confirmation. Over-treatment occurs when individuals without active TB are misdiagnosed and initiated on TB treatment.

It is worth noting that the benefits and risks of TB over-treatment vary for different high-risk groups (HRGs), depending on the potential advantages of early treatment versus the adverse consequences of over-treatment (Lönnroth, Corbett & Golub, 2014). The acceptability of false positives may differ among people living with HIV (PLHIV), where the potential benefits of early treatment are significant; however, the specific groups receiving over-treatment remain unknown.

Accurate identification of HRGs during Tas4TB is crucial in reducing false-positive results. As the identification of HRGs may be relatively straightforward, a reliable active case-finding (ACF) algorithm is essential. The study algorithm involved digital CXR and Xpert testing. While Xpert is automated and subject to minimal human error, the interpretation of CXR ratings may be susceptible to variability. Studies have shown that inconsistencies in CXR scores are common when read by medical officers. However, when assessed by radiologists, higher sensitivities and specificities have been reported compared to both medical officers and computer-aided diagnosis for TB (CAD4TB) from Delft Imaging Systems, Neenendaal, The Netherlands (Rahman, Codlin & Rahman, 2017). The reliability of field CXR ratings in Zimbabwe is uncertain, as medical officers do not routinely undergo assessments for CXR competence. Accurate interpretation of CXR is pivotal as it serves as a screening tool, identifying patients who then proceed to Xpert testing or TB treatment.

2.5.5 Sputum Smear microscopy

While the conventional method for diagnosing pulmonary TB involves culturing sputum in liquid media, the challenges posed by limited access to culture facilities and lengthy processing times have led many programs to rely on direct Ziehl-Neelsen (Z-N) microscopy as the primary diagnostic tool for detecting acid-fast bacilli (AFB) in sputum smears (Uddin, Chowdhury & Ahmed, 2014). This straightforward approach involves smearing sputum directly onto slides without any pre-processing and subjecting it to ZN staining. AFB microscopy is widely considered a practical and expedited technique for diagnosing pulmonary TB, particularly in regions with a high burden of TB cases, such as developing countries (Singh, Saket & Kachhi, 2019). Although direct smear microscopy is known for its specificity in areas with high TB

prevalence (CDC, 2017), it has limitations, including lower sensitivity and specificity compared to culture-based methods. Studies have shown that under optimal conditions, the sensitivity of AFB microscopy ranges from 22% to 60%, notably lower than that of culture methods, especially in cases involving children and individuals co-infected with HIV (Singhal & Myneedu, 2015; Fry, Barnabas & Cotton, 2019).

Enhancing the sensitivity of sputum smear microscopy has been a focus of researchers in recent years. Various techniques involving liquefaction of sputum using chemical reagents and subsequent concentration by centrifugation or sedimentation prior to staining have been proposed to significantly improve microscopy performance (Uddin, Chowdhury & Ahmed, 2014). Among these methods, the utilization of N-acetyl-L-cysteine (NALC) with 2% sodium hydroxide (NaOH) has emerged as one of the most effective approaches. NALC acts as a potent mucus digester, resulting in smears with reduced debris and higher AFB concentration, thereby enhancing sensitivity (Singhal & Myneedu, 2015). Despite its potential to significantly boost sensitivity, this technique requires specialized staff training, extended diagnostic time, and biosafety measures to safeguard laboratory personnel, hindering its widespread adoption, particularly in developing countries with limited resources (CDC, 2017).

Another noteworthy chemical, sodium hypochlorite, commonly known as household bleach, has been identified as an ideal processing agent in low-income settings. Its affordability and availability make it an attractive option, with its disinfectant properties enhancing infection control in laboratories lacking proper biosafety facilities. Reports suggest that bleach treatment enhances smear microscopy sensitivity by digesting mucus and debris in sputum, resulting in clearer microscopic fields (Singhal & Myneedu, 2015). However, a significant drawback of using bleach

for sedimentation is its impact on mycobacterial culture, as it can lead to the destruction of M. tuberculosis.

The emergence of fluorescence microscopy (FM) represents a recent development in smear microscopy that promises higher sensitivity and faster processing times compared to traditional Z-N microscopy (Ahmed, Shukla, Fatima, Varshney, Shameem & Tayyaba, 2019). FM has been shown to improve sensitivity by 10% compared to Z-N microscopy, offering faster examination times and potentially increasing diagnostic efficiency, particularly when employing LED-based FM systems that are cost-effective and suitable for resource-constrained settings (CDC, 2017).

Smear microscopy has been noted to have several limitations including a low sensitivity rate, being unable to differentiate between viable and non viable mycobacterium, lack of detection of susceptibility of bacilli to TB drugs (Tortoli, 2014). Smear microscopy according to W.H.O is no longer the recommended initial diagnostic test for TB but still plays a role in monitoring response to treatment and particularly useful in low resource settings where the more recent diagnostic tests are not readily available (WHO, 2020). In conclusion, variations in smearing techniques and the need for improved sensitivity underscore the importance of exploring techniques such as concentrated smearing to enhance diagnostic accuracy in TB diagnosis.

2.5.6 Sputum Culture - Lowenstein–Jensen Method (LJ)

Sputum culture is not used for screening TB but is the gold standard for the diagnosis of Tuberculosis and involves growing M.tuberculosis bacterium in solid media or specific liquid media. Compared to sputum smear microscopy, culture can increase

sensitivity by up to 30%, providing definitive confirmation of M. tuberculosis presence and enabling drug susceptibility testing (Rodrigues da Costa, Silva, Augusto & Gonçalves Leite, 2022). Sample decontamination is a crucial step in the preparation of biological samples before they are inoculated on Lowenstein–Jensen medium and liquid MGIT medium, distinct techniques used in the diagnosis of tuberculosis (Tortoli, 2014). Decontamination is performed by use of N-acetyl-L-cysteine (NALC) and sodium hydroxide (NaOH), Chlorhexidine, Ogawa-Kudoh among others.

There are different types of culture examinations that can be employed including using MGIT 960 Technology. The MGIT 960 technique (Mycobacteria Growth Indicator Tube 960) is an advanced and automated method for the detection and cultivation of the bacterium *M. tuberculosis* (Rodrigues da Costa, Silva, Augusto & Gonçalves Leite, 2022).

The Löwenstein–Jensen (LJ) culture technique involves spreading a biological sample, such as sputum, evenly over a solid medium and allowing the mycobacteria to grow under controlled temperature and humidity conditions, forming characteristic colonies (Tortoli, 2014).

Examining these colonies under a microscope can provide crucial information for accurate diagnosis. Despite its higher sensitivity compared to acid-fast bacilli smear examination (80–85%), the LJ method is time-consuming, with results available after 4–6 weeks of incubation (Moreira, Huf & Vieira, 2015). False positive MTB cultures are rare but still occur at rates of 2% to 4%. Therefore, strict adherence to laboratory techniques and awareness of the possibility of false positive MTB cultures, especially when inconsistent with clinical data, are crucial for preventing tuberculosis misdiagnosis(Mohammed Adam, Ebraheem & Bedri, 2022).

A study conducted in Indonesia over one year aimed to assess the performance of Thin-Layer Agar T (TLA) culture for tuberculosis diagnosis compared to the LJ culture method. The sensitivity of TLA was significantly higher than that of LJ, with a substantially shorter median time to detection. As a result, TLA has been suggested as an equally sensitive but faster alternative to the traditional LJ method (Battaglioli, Rintiswati & Martin, 2014).

Culture is also necessary to confirm treatment failure, assess treatment response in patients with drug resistant TB and evaluate treatment outcome in drug resistant TB. Culture is also useful to differentiate M.tuberculosis and non tuberculous forms of myco-bacterium, it may also help to diagnose TB when other bacteriological tests are negative or inconclusive (CDC, 2018). The test has its own limitations like requirement for specialized laboratories that implement systematic quality assurance procedures like the national reference laboratories in Zimbabwe, it is labour intensive and has a long turnaround time of between 2 to 4 weeks (WHO, 2014).

2.5.7 Tuberculin Skin Testing

The tuberculin (purified protein derivative - PPD) skin test (TST) was first described by Robert Koch in 1890, and subsequently developed by Felix Mandel in 1908. Charles Mantoux is attributed with formulating the technique of intradermal injection on the inner surface of the forearm. In 1934, Florence Seibert contributed significantly by devising a method for obtaining the purified-protein derivative, thereby leading to the establishment of the PPD test (Kestler & Tyler, 2022).

The tuberculin protein utilized in the test is derived from cultures of Mycobacterium tuberculosis and is employed as a purified-protein derivative. A standardized PPD-S involves the use of a tuberculous mycobacterium. Non-tuberculous mycobacteria are

designated by a letter other than S. The interpretation of the test results hinges on the measurement of the delayed-type hypersensitivity reaction, with the peak of the induration reaction appearing 24 hours after the test due to cell infiltration. It typically takes six to eight weeks after exposure to the bacteria for the PPD test to yield a positive result, which should be read between 48 and 72 hours following administration (Rehman, Bibi & Imran, 2018). Notably, the PPD TST is performed on the surface of the forearm, and no specimen collection is necessary (Almeida Santos, Duarte & Nunes, 2020).

In the United States, two FDA-approved PPD tuberculin antigens - Tubersol and Aplisol - are available for use. Although other tuberculin antigens, such as RT-23, are occasionally employed outside the United States, their dosages may differ from PPD-S formulations, with the dosage for PPD-S consisting of five units in 0.1 ml. No alterations to the dosage are warranted for patients with hepatic or renal failure. According to the guidelines from the Centre for Disease Control and Prevention (CDC), the test is conducted using the Mantoux technique, entailing the injection of 0.1 ml of a solution containing five units of tuberculin-purified protein derivative into the inner surface of the forearm via the intradermal route, typically two or more inches from the elbow, wrist, or other injection sites. The test is performed using a one ml tuberculin syringe and a needle shorter than 0.5 inches, with the needle bevel inserted slowly at an angle of five to fifteen degrees, ensuring the wheal or elevation of the skin measures six to ten mm in diameter upon intradermal injection of a small amount of the PPD solution. Documentation of the injection site, date and time of test administration, the administrator's name, product lot number, and manufacturer is essential, and the patient should be advised to refrain from touching the area to

maintain cleanliness. Given the potential for an allergic reaction to tuberculin, epinephrine should be made readily available (CDC, 2014).

The reading of the PPD skin test involves the identification and measurement of induration, which is palpable and should be assessed through inspection and palpation, yielding more reliable results than the measurement of erythema, and must take place between 48 and 72 hours post-test placement - as tests read after 72 hours tend to underestimate the size of the skin reaction, compromising the reliability of the test. Repeat testing is recommended if the initial reaction is not read within 72 hours, preferably within seven days to avoid a boosting effect by administering the second test at a different location, such as the other arm (Lardizabal & Reichman, 2021).

The indications for TB screening encompass the assessment of individuals with an increased risk of new TB infection and those at heightened risk of reactivation disease. These individuals include those with known exposure to M. tuberculosis, close and casual contacts with cases of untreated, active respiratory TB, individuals with latent TB infection and an elevated risk of disease progression, employees and residents of homeless shelters and correctional facilities, and certain healthcare workers necessitating serial testing due to high exposure risks (Tchakounte & Youngui, 2022). Moreover, those with heightened susceptibility to reactivation disease are individuals with severe immunocompromise, including chemotherapy or organ transplant recipients, individuals with HIV infection, lymphoma, leukemia, head or neck cancer, evidence of healed TB on chest radiograph, silicosis, renal failure necessitating hemodialysis, and recipients of treatment with tumor necrosis factor-alpha (TNF) inhibitors (Sharma, Vashishtha, Chauhan, Sreenivas & Seth, 2017).

It is important to base the selection of TB screening tests on CDC guidelines. Specifically, the PPD skin test is favoured for the serial testing of individuals who require regular testing, while an Interferon Gamma Release Assay (IGRA) is preferred for cases with a low to intermediate risk of progression from latent to active disease and the PPD test may serve as an alternative when IGRA is unavailable or costly. The decision to conduct dual testing with IGRA and PPD is contingent on the initial test results, which necessitates further diagnostic confirmation through IGRA testing when the PPD test is positive (CDC, 2014). Additionally, the prompt initiation of treatment for latent TB is crucial to forestall the progression to active disease, and shorter treatment regimens are recommended by the CDC for improved compliance with three months of weekly isoniazid and rifapentine, four months of daily rifampin, or three months of daily isoniazid and rifampin.

When interpreting a positive Tuberculin Skin Test (TST), it is essential to take into account more than just the size of the induration. Instead, the positive TST result should be evaluated based on three key aspects: the magnitude of induration (considering the current test and any prior tests), the likelihood of infection before testing, and the potential risk of disease if the individual is genuinely infected (Pai, Denkinger & Kik, 2014).

The value of the PPD skin test in identifying TB infection cannot be understated due to its simplicity, cost-effectiveness, and rapidity. Consequently, it is essential that proper administration and interpretation be based on standardized procedures and good staff training and supervision, particularly for such an internationally utilized test with its inherent interpretational complexities (Mulder, Erkens, Kouw & Huisman, 2019).

2.5.8 Interferon Gamma Release Assays (IGRA)

IGRAs serve as in vitro blood assessments of cellular immune activity, gauging the T-cell discharge of IFN-γ upon exposure to antigens exclusive to the M. tuberculosis complex, such as early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), encoded by genes within the region of difference 1 (RD1) locus of the M. tuberculosis genome (Jia, Yang & Dong, 2017). These tests exhibit heightened specificity towards M. tuberculosis in comparison to PPD, as they are absent in the genomes of most BCG vaccine strains and a range of non-tuberculous mycobacteria (NTM), with some exceptions like M. marinum, M. kansasii, M. szulgai, and M. flavescens (Gcebe, Michel, Gey van Pittius & Rutten, 2016). Notably, cross-reactivity between ESAT-6 and CFP-10 of M. tuberculosis and M. leprae has been observed (Krasilnikov, Lehnherr-Ilyina, Djonovic, Artamonova, Nikitin, & Kislichkin, 2024).

Internationally, two prominent IGRAs are widely accessible: the QuantiFERON-TB Gold In-Tube (QFT) assay and the T-SPOT.TB assay, both endorsed by the U.S. Food and Drug Administration (FDA), Health Canada, and CE marked for use in Europe. The QFT assay, an enzyme-linked immunosorbent assay (ELISA)-based test, evaluates IFN-γ levels in international units (IU) per milliliter, with positivity indicating a heightened response to TB antigens surpassing the test cutoff limit. In contrast, the T-SPOT.TB assay, an enzyme-linked immunosorbent spot (ELISPOT) test, quantifies the number of IFN-γ-producing T cells (spot-forming cells) after PBMCs are stimulated with ESAT-6 and CFP-10 peptides, enabling a positive diagnosis when spot counts exceed specified thresholds in TB antigen wells compared to negative-control wells. Additionally, inconclusive IGRA outcomes may arise from minimal IFN-γ response to the positive control or elevated background levels in the negative control.

Broadly, the sensitivity and specificity assessments for Latent Tuberculosis Infection (LTBI) are approximated using surrogate standards due to the absence of a definitive gold standard. Extensive meta-analyses (Chen, Nakagawa, Takamori, 2022; Cohen, Mathiasen, Schön, & Wejse, 2019; Ngozi, 2018) indicate that IGRAs demonstrate a specificity surpassing 95% for TB diagnosis in low TB incidence settings, unaffected by BCG vaccination status. Similarly, the TST exhibits high specificity in non-BCG-vaccinated populations (97%), but this specificity diminishes markedly (to around 60%) and fluctuates in BCG-vaccinated groups, contingent upon the frequency and timing of BCG inoculations. Notably, the T-SPOT.TB assay exhibits a higher sensitivity compared to the QFT assay and TST (approximately 90%, 80%, and 80%, respectively), albeit compromised by HIV infection and in pediatric populations (Starke & Byington, 2014).

Due to insusceptibility to BCG vaccination status, IGRAs prove invaluable for LTBI assessments in BCG-vaccinated individuals, especially in regions where BCG vaccinations occur post-infancy or involve multiple booster doses (Starke, 2014). Manufacturing anomalies can influence IGRA precision, with scrutiny from researchers like Slater and collaborators revealing fluctuations in QFT assay outcomes following a sudden rise in positive results at a U.S. academic establishment (Slater, Parsonnet & Banaei, 2012).

Within the clinical context, sources of variance in IGRA outcomes stem from both analytical and immunological factors. Analytical variances encompass measurement fluctuations induced by arbitrary factors in biological fluids, imprecision in handling procedures, and signal measurement errors, contrasting with systematically mappable

preanalytical variances (Oh, Ortiz-Brizuela, Bastos & Menzies, 2021). Several studies, including those of Metcalfe and Whitworth and their teams, have underscored sizeable fluctuations in quantitative results within and between test runs, often leading to conflicting outcomes nearing the assay threshold (Metcalfe, Cattamanchi, McCulloch, Lew, Ha & Graviss, 2013; Whitworth, Hamilton & Goodwin, 2012). While interreader discrepancies are more notable in the T-SPOT. TB assay compared to the QFT assay (Franken, Thijsen & Wolterbeek, 2009).

Immunological variations in IGRA outcomes, on the other hand, stem from immune boosting and immunomodulation. Studies by Van Zyl-Smit and colleagues expounded on augmented TB responses when IGRA tests are conducted post 3 days following PPD administration, attributable to the mobilization of preexisting memory T cells (Banaei, Gaur & Pai, 2016). Further research has echoed similar observations (Naseer, Naqvi & Kampmann, 2007; Vilaplana, Ruiz-Manzano, Gil et al., 2008 in Pai, et al., 2014) while substantiating the mechanism of TST immune augmentation via the recall of existing memory T cells to RD1 antigens present in PPD (Pai, Denkinger, & Kik, 2014). Conversely, prior IGRA test results do not influence subsequent outcomes, given the ex vivo nature of the tests.

Another facet of immunological variability stems from immunomodulation triggered by pathogen-associated molecular patterns (PAMPs), like lipopolysaccharide and peptidoglycan (Pai, Denkinger & Kik, 2014). Recognition of PAMPs by innate immune cells through pathogen recognition receptors, notably the Toll-like receptor (TLR) family, initiates cascades leading to the expression of inflammatory mediators, potentiating the maturation of antigen-presenting cells and consequent adaptive immune responses (Pai, Denkinger & Kik, 2014).

Overall, while a comprehensible means to evaluate Tuberculosis (TB) infection, IGRA results are subject to diverse influences, necessitating meticulous consideration of all variances. Elimination or minimization of systematic variances can be facilitated through standardization by test manufacturers and users, albeit accounting for inherent random variances is imperative for unbiased result interpretation. Once the total variances impacting IGRA responses are delineated, the establishment of suitable cutoff levels and borderline zones is essential in the interpretation of serial test outcomes relative to patient-specific TB risk factors and local lab practices (Pai, 2012).

2.6 Summary

This chapter outlined the conceptual framework of the study and reviewed the available literature on the screening and diagnostic tools available for TB in the high risk group of people living with HIV. Gaps available for TB screening were also discussed including areas for future research and innovation.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Introduction

This chapter describes the study setting, study population, study period together with the sample size and the sampling techniques that were used in the study. The data collection tools that were utilized and the methods of data analysis employed are also detailed in this chapter. Ethical considerations that were observed are also outlined.

3.2 The Research Design

A research design can be defined as a strategy for answering research questions using empirical data. It is a blueprint for conducting a study with control over factors that may interfere with the validity of the findings (Saunders, Lewis & Thornhill, 2019).

The study design that was used in this research was a cross-sectional study employing secondary analysis of the data collected during the period January 2022 to December 2023. The study looked into data that was recorded in Opportunistic Infections clinic attendance registers, presumptive TB registers and the TB register including other medical records for people living with HIV who visited Opportunistic Infections clinics and were screened for TB, then tested using GeneXpert assay during the period that was studied. According to the national HIV and TB guidelines People living with HIV should be screened for TB at every clinic visit, individuals who screen positive on the WHO 4 symptom screening (W4SS) tool then proceed to submit sputum for GeneXpert testing. Chest x-ray imaging is also a screening tool that is used and individuals with an x-ray suggestive of TB abnormalities also submit sputum for testing using the GeneXpert test .

The outcomes on the screening and diagnostic algorithms were analysed, true positives (those who had a positive screen test and positive GeneXpert test), true

negatives (those who had a negative screen test and negative GeneXpert but had tested positive on a different screening tool hence the submission of sputum), false positives (those who had a positive screen test and a negative Gene xpert assay) and false negative (those who had a negative screen test and a positive Gene xpert but had tested positive on a different screening tool) results for the screening tools (for both CXR and W4SS) were determined by comparing screen results to Gene Xpert results and consequently the sensitivity, specificity, negative predictive and positive predictive values of the screening tools/tests were estimated.

The cross sectional study was chosen as it is the appropriate study design for comparing screening tests with a reference standard (Rothman & Greenland, 2008) (Shreffler & Huecker, 2023). This particular study provided for review of already screened and tested patients with documented outcomes, this saved time and financial resources for the researcher as there was limited time to conduct the study.

3.3 Study population

The study included data from people living with HIV who were already on anti-retroviral therapy and were screened for TB at every clinic visit during the period 2022-2023. There is a high prevalence of tuberculosis among PLHIV and the need to reduce disease burden in this population makes it critical to research into the efficacy of screening tools for the disease for purposes of improvement of diagnosis and care in this high risk group.

3.4 Inclusion Criteria

The data and records for PLHIV above the age of 18 who visited O.I clinics during the period January 2022-December 2023 and were screened for tuberculosis, subsequently submitting sputa for gene xpert were included.

3.5 Exclusion Criteria

Data for individuals below the age of 18 were not included in the study, data for patients already on TB treatment at the beginning of 2022 were also excluded.

3.6 Sampling and sample size

The aim of the study was to evaluate the efficacy of TB screening methods/tools as applied to PLHIV for the period January 2022 to December 2023. Data was collected from all entries for PLHIV in the registers that had screened for TB and screened positive on either the symptom screening or CXR or both and had their sputa examined for presence of TB bacilli during the specified period. The sampling method used was stratified random sampling, then simple random sampling for each strata during each month . The sample size was calculated using Epi Info version 7.2.5.0, using 95% confidence interval, and a study power of 80% . The minimum sample size calculated was 1200. The number of individuals whose complete data was recorded during the period under study was the sample size included and amounted to 2 256.

3.7 Study Site

The study was conducted at St Patrick's hospital. The site was chosen for convenience. It is a public hospital with high numbers of people utilizing O.I clinic services. The facility is used by the low income population, among whom HIV and TB are more

prevalent. St Patrick's hospital is in Hwange district, a district in Matebeleland North Province Northwest of Bulawayo city. Hwange is a coal mining community, with most miners living in clustered homes and carry substantial risk for TB infections.

Figure 3. Zimbabwe Map.

3.8 Data Collection Procedure

Authority to collect data from the O.I.C registers was sought from the Ministry of Health in order to utilize the data for purposes of this research. Data was collected from medical records and O.I registers for the period under study, with documentation on checklists and tallying of outcomes using variables on a data collection instrument attached in the appendix. The data was collected for individuals who screened positive on the symptom screen and those who had chest xray findings suggestive of TB that proceeded to get a Gene Xpert test done on their sputum, subsequently the abilities of the screening tools to detect true TB cases was calculated (sensitivity). Individuals who had negative screen tests on one tool and positive on another and also proceeded

to do GeneXpert testing also had their data collected and analysed to come up with specificity of the tool (CXR or W4SS) under study.

3.9 Data Collection instruments

Medical Records (TB registers, presumptive TB registers and O.I attendance registers) were the data sources used to collect information. Instruments used included a data extraction tool and tally sheets. The instruments helped extract information for screen results on CXR and W4SS and also the outcomes obtained on diagnostic testing using the Gene Xpert assay. The instruments were chosen in the retrospective study because of the lack of sufficient time to conduct the study, A prospective study or randomised trial would take a long time to yield results but in this retrospective study, the information is already recorded and the researcher will just extract from the records as secondary data.

Validity and reliability

Validity is the degree to which a test or an instrument measures what it purports to measure (Nachmias & Nachmias, 1996), it is about the accuracy of a measure. The data collection instrument is meant to measure the number of people who screened positive on the screening tools and in turn determine the true positive, true negative, false positive and false negative cases. The instrument can accurately assist in determining this information by collecting the right variables needed for the study to yield the desired results, this was observed on the pilot study and the researcher was satisfied with the results from the pilot study. **Reliability** is the ability of the test instrument to consistently give the same results even when used in a different setting (Huck, 2007). The measuring instrument in this study, being quantitative, has the ability to consistently give the same results.

3.10 Pretesting tools

Pretesting of data extraction instrument and data extraction and collection procedures was done using the data from the registers entered between the period January to April 2022 which was an extract from the data used for the study.

3.11 Analysis and Organization of Data

The data collected was entered into Microsoft excel then cleaned for errors, duplications, missing data fields, inconsistent data and out of range data. Descriptive analysis for the participants' variables was done using Stata version 13 to generate frequencies, tables and graphs. Stata was also used to calculate the sensitivities, specificities, Negative Predictive Value (NPV) and Positive Predictive Values (PPV) for the screening tools in reference to the Gene Xpert assay results. Some of the participants' variables that were analysed included age, sex, symptoms of TB if any, viral load, CD4 count, time-frame on ART, screening tools used and the screen outcomes then diagnostic outcomes on the Gene Xpert assay.

3.12 Ethical Considerations

During the study, there was no collection or documentation of any personal identification information like names, identification numbers, addresses, contact numbers from study participants. The student sought authority to conduct the study from the Provincial Medical Director, District Medical Officer and Medical Superintendent for the institution that was under study. Permission was granted and approval letter is attached in the appendix of this document. Ethical approval to conduct the study was also sought from AUREC and approval was granted as

evidenced by the approval letter also in the appendix section of this dissertation write up.

3.13 Plan for dissemination of results

Findings from this study are important to policy makers and also to the custodians of the data who are responsible for documenting outcomes of patients in medical registers and relevant clinic books. The results will be presented first at provincial level in Matebeleland North and also to the district and the institution that was involved in the study.

3.14 Summary

This chapter detailed the study methodology by describing the study design, study setting, population under study, sampling technique that was used, data collection tools and procedures and also data analysis together with ethical considerations that guided the study.

CHAPTER 4: DATA PRESENTATION, ANALYSIS AND INTERPRETATION

4.1 Introduction

This chapter focuses on the results of the study. Descriptive statistics and analytical statistics are shown as charts and tables. Variables are summarised using percentages. The STARD 2015 guidelines (Cohen, Korevaar & Altman, 2015) were used as a guide to report the results of the study. Data was analysed using STATA version 13. Records with any one of cough, fever, night sweats or weight loss were analysed as positive screen for symptoms whilst those with xrays indicative of active TB abnormalities were analysed as positive CXR screen. The GeneXpert results (whether positive or negative) following a positive CXR or symptom screen was used to determine the performance of the screening tool.

4.2 Data Presentation and Analysis

4.2.1 Study profile

Over the period January 2022- December 2023, 8 162 PLHIV were screened for TB. 2 571 (31%) people screened positive for TB using either the W4SS or the Chest Xray. 2 256 records of participants were included in the study as shown in the flow diagram below.

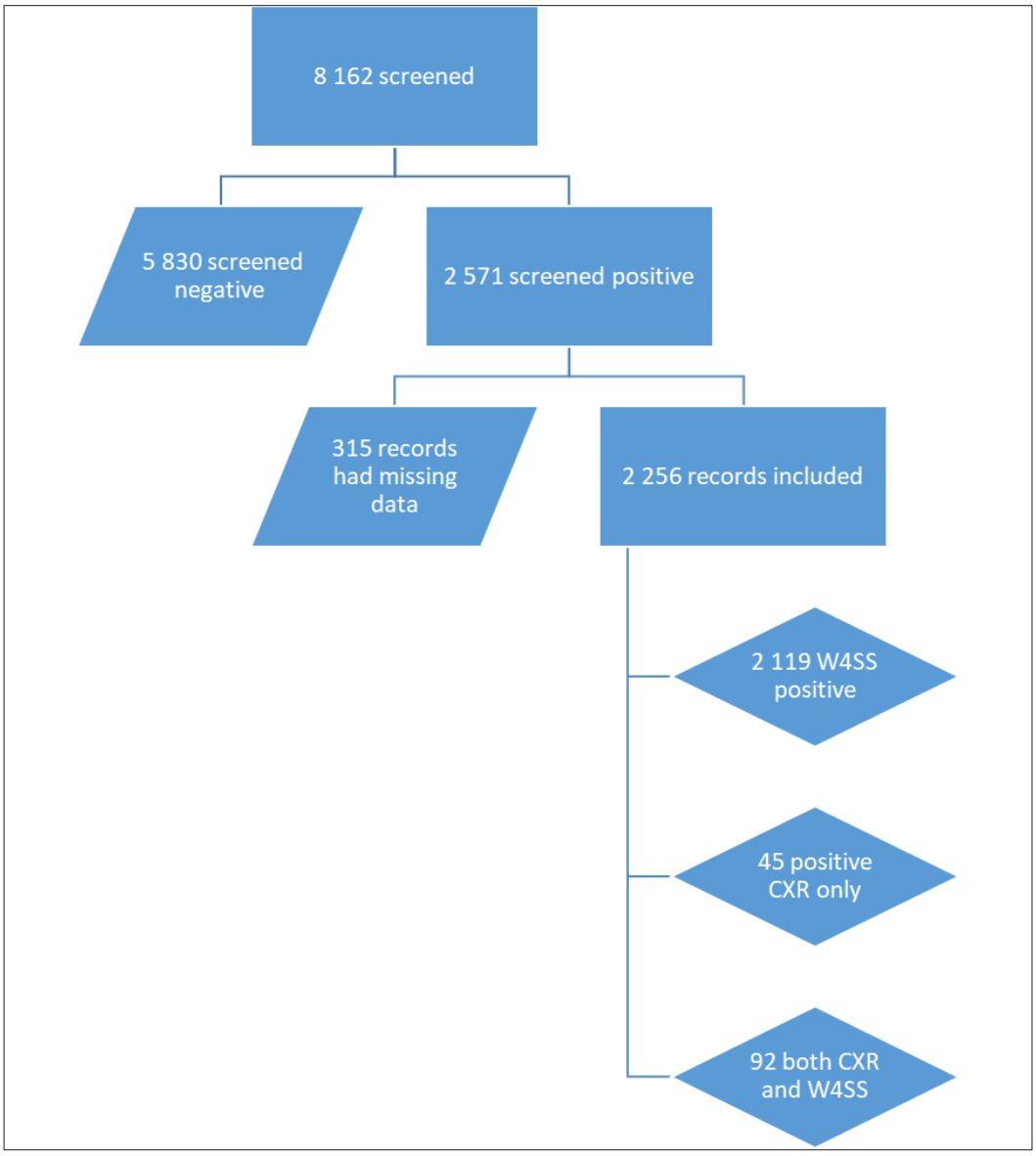


Figure 4. Research flow chart.

4.2.2 Demographic characteristics of patients screening for TB

A total of 2 256 records of adults who screened positive during the period 2022-2023 at a clinical encounter at the O.I clinic were used for the study. 52% of the participants included were female whilst 48% were male. Participants screening positive for each age group are shown in the table below. Participants were also categorized as having mining exposure or not as one of the high risk groups indicated in the presumptive TB registers also Hwange being a mining town.

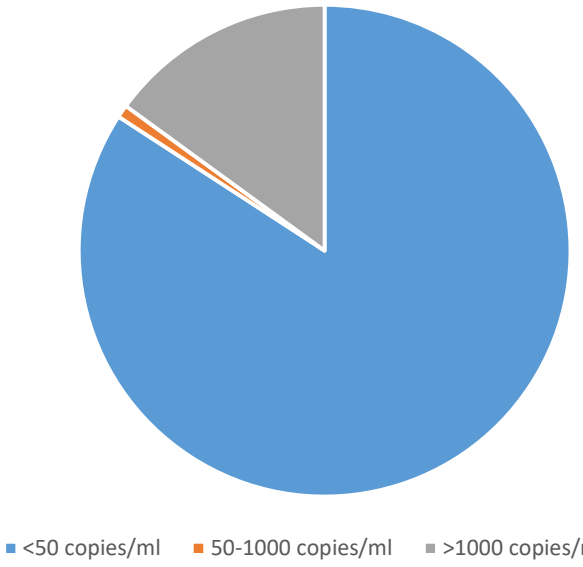
Table 1: Demographic characteristics of participants who were included in the study

Variable	Frequency	Proportion (%)
Sex		
Male	1083	48
Female	1173	52
Age (years)		
15-24	384	17
25-34	429	19
35-44	428	19
45-54	361	16
55-64	406	18
65+	248	11
Mining exposure	857	38
No mining exposure	1399	62

4.2.3 Positive screen test against Viral load

The majority (2 076, about 92%) of the participants in the study had been on antiretroviral therapy for more than 3 months. 1 918 (85%) of participants who screened positive had HIV viral loads less than 1 000 copies per mililitre (ml) of which 1 899 of these had viral loads less than 50 copies/ml. These results are a bit worrying as we move towards UNAIDS target of 95% of people on ART being virally suppressed although this population may not be representative of the institutional statistics. 15% had viral loads of greater than 1 000 copies per ml.

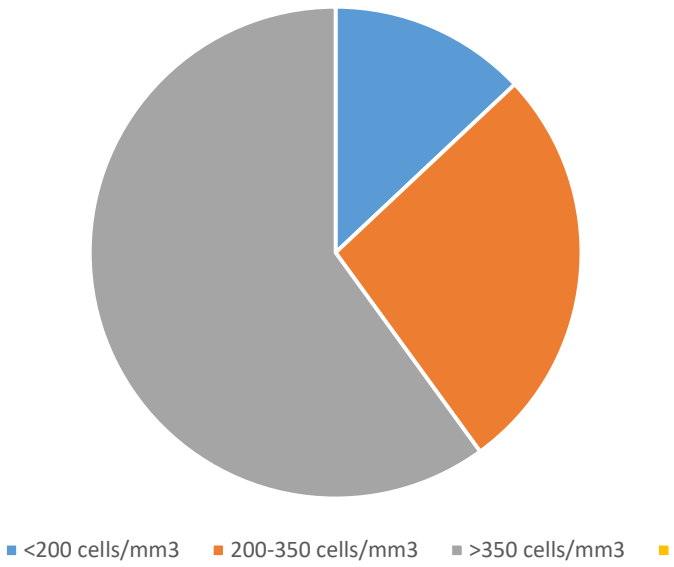
Fig. 5 Participants viral load distribution



4.2.4 Positive screen test against CD4+ count

13% (293) of the participants had CD4+ counts of less than 200 cells/cubic millimetre (mm3) whist 87% had counts greater than 200 cells/mm3.

Fig. 6 Participants CD4 counts



4.2.5 Commonly used screening tools

Screening tools widely in use in Hwange district for TB screening during the period under study were the Chest Xray (CXR) and the Symptom screening (W4SS). No individuals were screened for TB using the C reactive protein. 26 results for individuals who had urine Lipoarabinomannan (LAM) done were documented in the TB presumptive register with 3 having screened positive and the test was used as an aid for TB diagnosis.

The results of screening using the W4SS and the CXR for individuals who tested positive are shown below. Including cough of any duration increased the number of individuals screening positive on the symptom screening. On analysis of the results, a cough of more than 2 weeks was used to calculate performance of the symptom screening tool. 439 chest xray (5% of screened individuals) results were documented in the O.I clinic registers as below, with 137 (31%) of the xrays done for TB screening reported to have signs of active TB.

Symptom screening	
Cough	1413 (63%)
Fever	334 (15%)
Night sweats	394 (17%)
Weight loss	304 (13%)
CXR characteristics (N=439)	
Normal	188 (43%)
Abnormal healed TB	36 (8%)
Abnormal active TB	137 (31%)
Abnormal not TB	78 (18%)

Table 2. Screening tools used and results obtained

4.2.6 TB diagnostic outcomes for individuals who screened positive for the disease

Over the 2 year period under study, 80 PLHIV (3.5%) who were screened and tested for TB using the CXR, W4SS and Gene Xpert algorithm to conform to the study were diagnosed and treated for TB. Of the 137 people who screened positive on CXR, 74 (54%) were diagnosed with TB using the Gene Xpert test whilst 61 who screened positive on the symptom screening tested positive on the Gene Xpert test. 6 people were missed by the CXR screen and picked by symptom screening subsequently testing positive on the Gene Xpert assay. Symptom screening missed 19 who then tested positive on Gene Xpert assay.

4.2.7 Performance of TB screening tools

Performance of the symptom screening and chest xray are reported as sensitivity, specificity, and negative and positive predictive values. The proportion of cases confirmed to be positive on Gene Xpert that had screened positive on CXR is reported as the yield due to the CXR in percentages. The proportion of cases confirmed on Gene Xpert that screened positive on symptoms screening is reported as the yield due to symptoms screening. Below are the basic mathematical formulas for calculating performance/accuracy of the screening tools from the 2x2 table:

Status of person according to Gene xpert

		Has the condition	Does not have the condition
Screen test result	Positive	**True positive**	**False positive**
	Negative	**False negative**	**True negative**

Table 3. The basis for deriving sensitivity, specificity, PPV and NPV

Sensitivity = True positives/[True positives + False negatives]

Specificity = True negatives/[True negatives + False positives]

Positive predictive value = True positives/[True positives + False positives]

Negative predictive value = True negatives/[True negatives + False negatives]

4.2.7.1 Performance of W4SS in screening Gene Xpert confirmed pulmonary TB

61 cases of patients who screened positive on the symptom screen tested positive on the Gene Xpert diagnostic test. There was a high rate of false positive among the symptom screened patients. Use of the WHO recommended 4 Symptom screen (W4SS) in this study had a sensitivity of 76% with a specificity of 31% as shown below. The positive predictive value of symptom screening was 4%, which is critically low and shows that for every symptom screen positive case, there is just a 4% chance that the patient has TB, and a 96% chance of false positive results. A positive screen result for the W4SS should therefore be interpreted with caution, the low prevalence of the TB disease among the studied population also contributes to the low positive predictive value which is a product of or influenced by disease prevalence, sensitivity and specificity of the screening tool. The negative predictive value of the screening tool was 97%, interpreted as a 97% chance that a case screened as negative is healthy

or does not have the disease. The screening tool can therefore be trusted when a negative result is obtained.

Tool	Confirmed TB	Total number	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Prevalence	80	2 256				
Symptoms	61	2 256	76 (69, 83)	31 (30, 32)	4 (3, 5)	97 (96,98)

Table 4. Screening performance of symptom screening

4.2.7.2 Performance of Chest x-ray in screening Gene Xpert confirmed pulmonary TB

74 cases of patients who screened positive on the CXR were bacteriologically confirmed to have TB by the Gene Xpert test. 6 TB cases were missed by the CXR screening. The sensitivity of the CXR was calculated to be 93% with a specificity of 83%. A positive predictive value of 54% indicates that there is a 54% chance that a case detected as positive screen using chest x ray actually has the disease, low prevalence of TB among the population also contributes to the relatively low PPV. Then there's a 98% chance that a case detected to be negative on CXR truly does not have TB, the CXR has an excellent negative predictive value and negative results can be treated as such with confidence.

Tool	Confirmed TB	Total Number	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Prevalence	80	2256				
CXR	74	439	93 (87, 96)	83 (80, 86)	54(48,60)	98(98,100)

Table 5. Screening performance of chest x-ray

4.2.7.3 Performance of CXR plus symptoms in screening Gene xpert confirmed TB

Combining the CXR and symptom screening picked all cases of Gene Xpert positive TB. The sensitivity of the screening tools combined was 100% whilst the specificity was 29%. The positive predictive value of 5% was low as it indicates there's a 95% chance of false positives when using the screening tools combined, the low disease prevalence also affects the result.

Tool	Confirmed TB	Total Number	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Prevalence	80	2 256				
CXR + Symptoms	80	2 256	100(98,100)	29 (28, 31)	5 (4, 6)	100(99,100)

Table 6. Screening performance of symptom screening plus CXR

4.2.7.4 Incremental yield of adding the CXR in a TB screening algorithm

Using the W4SS alone, 61 cases out of 80 would have been diagnosed. The addition of CXR as a screening tool increased the cases of TB diagnosed by 13 (about 16%). Use of both the CXR and symptom screening yielded all cases of TB hence increasing

yield by 24%. Combining both CXR and symptom screening was therefore critical under this study in order to increase yield of TB diagnosis.

Tool/Algorithm	screened	Tested	Number of TB cases	% of confirmed TB	Incremental yield (%)
W4SS only	2571	2256	61	76	ref
CXR only	2571	439	74	93	16
W4SS and Xray	2571	439	80	100	24

Table 7. Incremental yield of adding chest x-ray in screening

4.3 Summary

A total of 2 256 records of participants were included in the study, 94% of the individuals who screened positive were for symptom screening whilst 2% screened positive on CXR and 4% screened positive on both the CXR and W4SS. 80 participants who were screened using the CXR and W4SS followed by Gene Xpert testing were diagnosed with TB over the study period. The sensitivity of the W4SS was calculated to be 76% whilst the specificity was 31%. The sensitivity of the CXR was 93% and specificity was 83%. Combining CXR and symptom screening had a sensitivity of 100% and specificity of 29%. From the study, it can be concluded that combining both screening tools has the ability to detect all TB positive cases but a low rate of picking negative cases as being truly negative. The low positive predictive values were obtained because of the low prevalence of TB among the tested population and also the high rate of false positives by the symptom screening tool.

CHAPTER 5: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

In this chapter, the researcher gives a summary of the study findings in relation to the research objectives. It also gives conclusions based on the findings in this study. The researcher also gave recommendations to the policy makers and suggest areas of further study to improve TB program implementation for PLHIV.

5.2 Discussion

TB is a leading global health problem and its diagnosis is crucial in order to achieve the global End TB Strategy and should be scaled up to increase diagnostic yield and move towards incidence reduction through infection control and also reduction in morbidity and mortality. This study looked into efficacy of the available screening tools for tuberculosis among PLHIV who are a high risk group for TB disease.

Available TB screening tools in Hwange were mainly the WHO recommended four symptom screen (W4SS) and the chest xray (CXR). The CXR being a costly radiological investigation, not too many people can afford to have an xray done for screening as evidenced by only 439 (5.4%) PLHIV having done chest x-rays over the 2 year period out of the 8 162 who were screened. X-ray machine down time is also a contributor to the low numbers of x-rays done for TB screening as during the time of the study, there were several occasions when x-rays could not be done owing to lack of chemicals for developing the films and also due to electricity load shedding. A single x-ray costs about $25 united states dollars which is out of reach for many people who visit St Patrick's mission hospital who are mainly from low socioeconomic settings. Need for chest x-ray for TB screening and investigation is a huge driver for

TB catastrophic costs in people of low socioeconomic status. Of concern is also the fact that the institution is still using an analogue x-ray machine when other institutions have upgraded to digital x-rays with no need for x-ray films. One of the other TB screening tools recommended by WHO, the C reactive protein was not offered by the institution's laboratory and only had to be done at a next hospital which is a private hospital about 7km away but having higher costs for laboratory tests, the CRP was tagged at $20 per tests which is way too high for a TB screening test which according to WHO should cost $2 or less.

In this study, the W4SS was the main screening tool in use in Hwange district among people living with HIV, the tool was estimated to have a sensitivity of 76% and a specificity of 31%. Although the W4SS has a higher sensitivity than specificity as preferred by the World Health Organization, it does not have the target characteristics for a WHO screening tool target profile which requires that a screening tool have a sensitivity of 90% and a specificity of 70% (WHO, 2014). in this study, the W4SS had an estimated positive predictive value of 4% which is very low and indicates that the probability of having TB disease among those screening positive is only 4%, consequently meaning the test can not be relied on. The negative predictive value of 97% is satisfactorily high for clinicians to have confidence in a negative screen result.

The sensitivity of 76% and specificity of 31% obtained in this study are similar to the estimates obtained in a systematic review conducted by Van't Hoog, Langendam, & Mitchell (2013) of TB screening algorithms where sensitivities of the W4SS ranged between 67% to 79% whilst specificity ranged between 29% to 37% in the various studies that were reviewed. The results obtained in this study are again, comparable to those obtained in Uganda during a retrospective cross sectional study by Nalunjogi et

al (2021) in which the symptom screening had a sensitivity of 73% and a specificity of 37% among outpatients living with HIV during a national TB prevalence survey. In contrast, during a survey done in South Africa, the symptom screening was observed to have a sensitivity of 40.7% and a specificity of 81.3% considering the presence of any cough, fever, night sweats or weight loss (Claassens, van Schalkwyk, Floyd, Ayles & Beyers, 2017). Another study conducted in China to assess the diagnostic accuracy of symptom screening for TB showed the symptom screening to have a sensitivity of around 51% and a specificity of 93% (Cheng, Wang, Zhang & Xia, 2015) with very low positive predictive value of 2.2% but their symptom profile included only cough and haemoptysis and they noted the sensitivities of the symptom screening could have been improved by adding other symptoms like fever, night sweats and weight loss.

The World Health Organization End TB strategy states that it requires greater than 90% case detection in order to combat TB (WHO, 2015). It is estimated that up to 44% of individuals with TB are asymptomatic (Nalunjogi, et al., 2021) this makes symptom screening a sub optimal screening tool as it runs a risk of missing TB patients because of lack of symptoms especially in those with early TB disease who then continue to spread the infection in the community, and likely have poor treatment outcomes due to late presentation to health facilities with more advanced disease. These factors require that a more sensitive and specific tool be applied especially when conducting intensified or active case finding.

The End TB Strategy has led to the reconsideration of CXR as a screening tool for TB as innovative and lower costs strategies for taking xrays (digital xrays) and interpreting them (computer aided) are now available (Den Boon, Bateman, & Enarson, 2014).

Xray image interpretation requires trained human readers of which these are in short supply in many high TB burden countries. In our study, xrays were interpreted by medical officers who have not received additional training in radiology and this could potentially lead to inter observer bias and inaccurate interpretations, a lot of other pathological xray findings could easily be mistaken for TB hence a low specificity. The technique used for taking xrays was still the archaic analogue technique. In the study, CXR has been estimated to have a sensitivity of 93% and a specificity of 83%. The results are comparable to the results obtained during a cross sectional study done in Uganda by Nalunjogi et al. (2021) where the sensitivity of the CXR screening for TB was estimated to be 92% with a specificity of 97% and the xrays were being read by radiologists. A systematic review by Piccazzo, Paparo & Garlaschi (2014) yielded similar results, concluding that the presence of any abnormalities on CXR for TB screening among PLHIV had a sensitivity of 97% while the specificity of any detectable abnormalities was 67%. Cohen (2006) found the sensitivity of the CXR to be between 73-79% with a specificity of between 60-63% in a TB high risk population with xrays read by medical officers. In our study, xrays were done only for some of the people presenting with chest symptoms and sometimes those screening positive on the symptom screening and could afford the radiological investigation which was used as a diagnostic aid. The fact that xrays were only done for sick individuals could have improved the measured accuracy of the CXR as a TB screening tool, having obtained a high sensitivity and specificity.

In this study, the CXR was found to perform better than the W4SS in correlation with positive gene xpert results. Gene xpert was used as the diagnostic test to compare with because of the low accessibility (only Harare and Bulawayo national reference

laboratories process TB culture specimens) to the gold standard TB culture and low results turnover for the few samples sent as evidenced by only 7 sputum samples sent for TB culture had results documented in the TB register over the two year period. The gene xpert has become the gold standard for TB diagnosis in settings like Hwange.

The screening algorithm using both symptom screening and CXR increased the sensitivity to 100% and decreased specificity to 29%. These results are comparable to those of a study in Kenya that conducted intensified case finding among PLHIV, they found that adding the CXR to symptom screening increased sensitivity of the screening algorithm from 74% to 90.2% but decreased the specificity from 49.5% to 32% (Modi, Cavanaugh, Shiraishi, Alexander, McCarthy & Burmen, 2016). The results however, differ from those obtained by Van't Hoog, Langendam, & Mitchell (2013) where the sensitivity of a screening algorithm involving both the CXR and symptom screening had a sensitivity of 87% and a specificity of 90%. The differences obtained in our study could be due to the fact that only individuals screening positive on CXR or W4SS had their sputa collected and examined hence overestimating sensitivity at the expense of the specificity. Overall, the CXR increased the TB diagnostic yield and should be added to TB screening algorithms for PLHIV to aid case detection.

This study however, had some limitations, the TB culture diagnostic test which should be used as the gold standard to compare performance of screening tools was not readily accessible and the study had to compare against the gene xpert assay. Although documented to have high sensitivity and specificity, the gene xpert test largely relies on the type of sputum expectorated and false negative results can also be obtained using the gene xpert test, thereby affecting the accuracy of the study results.

5.3 Conclusion

This study made use of routine data collected in the Opportunistic infections clinic (O.I.C) during the period January 2022 to December 2023 to determine the performance of the available screening tools in Hwange district (CXR and Symptom screening) in correlation with the gene xpert assay which is used for bacteriological confirmation of TB disease. The results indicated that symptom screening had a sensitivity of 76% and a specificity of 31%, with a positive predictive value of 4%. There is a high false positive rate for symptom screening due to the coexistence of other illnesses with similar symptoms among PLHIV. These results do not meet the requisite characteristics of a screening tool recommended by WHO to have a target profile of 90% sensitivity and 70% specificity. The missing of 19 TB cases by the symptom screening during this study is a reflection of how inadequate the screening tool is when used in isolation for TB screening. The symptom screen does however meet the other technical requirements of a screening tool like being cost effective, readily available and easy to apply. The CXR is another screening tool employed by Hwange district and was found to have a sensitivity of 93% and a specificity of 83% with a positive predictive value of 54%, this tool has better screening performance than symptom screening but requires complicated equipment and interpretation, is also costly and can be a major driver of TB catastrophic costs among the community which is of poor socioeconomic status. Adding the CXR to the symptom screening algorithm increased case detection by 19 (24%) detecting 100% of the cases. The tools combined yielded a sensitivity of 100% and a specificity of 29%. It is of paramount importance to screen TB using an additional screening tool when employing the symptom screening strategy. In the current setting, clinical evaluation of signs and symptoms together with application of CXR can go a long way in detecting TB cases although

early TB disease is likely to be missed. Symptom screening is vital and can not be discarded out rightly before introduction of other newer and advanced screening tools for TB.

5.5 Recommendations

Several gaps for TB screening need to be filled in Zimbabwe. Provision of additional screening tools like the WHO recommended C-reactive protein which may be more objective than symptom screening will go a long way in aiding TB diagnosis especially among PLHIV. Tests like the CRP can be intergrated into community HIV groups and be administered by village health workers who can then refer those screening positive to the nearest health center.

The high costs of doing x-ray examination among TB patients should be subsidized like other TB investigations such as sputum examination to encourage screening and increase likelihood of case detection among PLHIV. The introduction of digital x-rays and artificial intelligence soft-wares like computer aided detection for TB (CAD4TB) in these remote communities where radiologists can not be readily accessed will go a long way in 'finding the missing millions' in TB case detection.

Community education on the importance of TB screening should be scaled up in order to have a high turn-up of HIV patients for screening, some people were never screened or only screened once during the two year period, such people sent their relatives for ART pill refill rather than presenting to hospital themselves, these missed opportunities for screening are a major driver to 'missing thousands' who may have TB. Awareness campaigns on TB will also go a long way in stigma reduction which will increase turn up for TB screening.

Further education and training for O.I clinic health workers should be prioritized to scale up accurate screening for HIV patients as this study indicated a high rate of false positive which may have been because of inaccurate identification of significant symptoms which may point to TB. Medical officers also need additional training courses on x-ray reading to be fully equiped in identifying x-ray changes suggestive of TB.

The TB case detection gap in Zimbabwe is very disturbing, further research in screening tools that can be applied easily to communities like Hwange but with better accuracy profiles for TB detection should be expedited in order to avert the effects of the disease especially among people living with HIV.

References

Acharya, B., Acharya, A., Gautam, S., Ghimire, S. P., Mishra, G., Parajuli, N., & Sapkota, B. (2020). Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. Molecular biology reports, 47, 4065-4075.

Ahmed, S., Shukla, I., Fatima, N., Varshney, S. K., Shameem, M., & Tayyaba, U. (2019). Light-emitting diode-fluorescent microscopy: Determining its sensitivity and specificity in diagnosis of pulmonary tuberculosis in a high-burden tuberculosis region and resource-limited country like India. *CHRISMED Journal of Health and Research*, *6*(1), 44-51.

Ahmad Khan, F.,Verkuijl, S., Parrish, A., Chikwava, F., Ntumy, R., El-Sadr, W., & Howard, A. (2014). Performance of symptom based tuberculosis screening among people living with HIV: not as great as hoped. AIDS (London, England), 28(10), 1463-1472. https://doi.org/10.1097/QAD.0000000000.

Almeida Santos J, Duarte R, Nunes C. (2020). Tuberculin skin test and predictive host factors for false-negative results in patients with pulmonary and extrapulmonary tuberculosis. *Clin Respir J*. Jun;14(6):541-548.

Anand, D., & Surbhi, M., (2015). TB Screening Among People Living With HIV/AIDS in Resource-Limited Settings.

Banaei, N., Gaur, R. L., & Pai, M. (2016). Interferon Gamma Release Assays for Latent Tuberculosis: What Are the Sources of Variability?. *Journal of clinical microbiology*, *54*(4), 845–850. https://doi.org/10.1128/JCM.02803-15.

Battaglioli, T., Rintiswati, N., Martin, A., Palupi, K. R., Bernaerts, G., Dwihardiani, B., ... & Van Der Stuyft, P. (2014). Comparative performance of thin layer agar and Löwenstein–Jensen culture for diagnosis of tuberculosis. *Clinical Microbiology and Infection*, *19*(11), E502-E508.

Camp, W. (2001). Formulating and evaluating theoretical frameworks for career and technical education research. *Journal of Vocational Educational Research, 26(1), 27-39.*

Centre for Disease Control and Prevention, CDC, (2018). Diagnosis of Tuberculosis in high burden countries.

Centre for Disease Control and Prevention (2019). Updated Recommendations for TB Screening, Testing, and Treatment of U.S. Health Care Personnel. https://www.cdc.gov/nchhstp/newsroom/2019/recommendations-for-tb-screening.html.

Chaisson, R. & Golub J. (2017). Preventing tuberculosis in people with HIV—no more excuses. Lancet Glob Health; 5(11):e1048–9.

Chappity, P., Mamidi, D., & Pradhan, S. (2021). Diagnostic high grade tuberculin sensitivity. *IDCases*, *24*.

Cheng, J., Wang, L., Zhang, H., & Xia, Y. (2015). "Diagnostic value of symptom screening for pulmonary tuberculosis in China,"PLoS One, 10(5), e0127725. https://doi.org/10.1371/journal.pone.0127725.

Chen, H., Nakagawa, A., Takamori, M., Abe, S., Ueno, D., Horita, N., ... & Seki, N. (2022). Diagnostic accuracy of the interferon-gamma release assay in acquired

immunodeficiency syndrome patients with suspected tuberculosis infection: a meta-analysis. *Infection, 50*(3), 597-606.

Claassens, M., van Schalkwyk, C., Floyd, S., Ayles, H., & Beyers, N. (2017) "Symptom screening rules to identify active pulmonary tuberculosis: findings from the Zambian South African Tuberculosis and HIV/AIDS Reduction (ZAMSTAR) trial prevalence surveys," *PLoS One,* 12(3), e0172881.

Cohen, A., Mathiasen, V. D., Schön, T., & Wejse, C. (2019). The global prevalence of latent tuberculosis: a systematic review and meta-analysis. *European Respiratory Journal, 54*(3).

Cohen, J., Korevaar, D., Altman, D., Bruns, D.,Gatsonis, C., Hooft, L.,…..& Bossuyt, P. (2016) 'STARD 2015 guidelines for reporting diagnostic accuracy studies: Explanation and elaboration,' *BMJ Open*, 6(11), e012799.

Cohen, R., Muzaffar, S., Capellan, J., Azar, H., & Chinikamwala, M. (2006). The validity of classic symptoms and chest radiographic configuration in predicting pulmonary tuberculosis. Chest 2006;109:420-3.

Corbett, E., & Zezai, A. (2010). The impact of HIV on Tuberculosis in Zimbabwe: a study of the diagnostic accuracy of a urine based test for tuberculosi in an HIV positive population. *AIDS (London, England)*, 24(7), 1039-1045.

Coulibaly, G., Togo, G., Somboro, M., Kone, M., Traore, G., Diallo, F., ... & Diarra, B. (2023). Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum as proficient alternative tool in the diagnosis of pulmonary tuberculosis in countries with limited resource settings. *The International Journal of Mycobacteriology*, *12*(2), 144-150.

Den Boon, S., Bateman, E., & Enarson, D. (2005). “Development and evaluation of a new chest radiograph reading and recording system for epidemiological surveys of tuberculosis and lung disease,” The International Journal of Tuberculosis and Lung Disease, 9(10), 1088–1096.

Franken, W. P., Thijsen, S., Wolterbeek, R., Bouwman, J. J., el Bannoudi, H., Kik, S. V., ... & Arend, S. M. (2009). Variation in T-SPOT. TB spot interpretation between independent observers from different laboratories. *Clinical and Vaccine Immunology*, *16*(10), 1439-1442.

Fry, S. H. L., Barnabas, S. L., & Cotton, M. F. (2019). Tuberculosis and HIV—An Update on the “cursed duet” in children. *Frontiers in pediatrics*, *7*, 159.

Gaur, R. L., Suhosk, M. M., & Banaei, N. (2012). In vitro immunomodulation of a whole blood IFN-γ release assay enhances T cell responses in subjects with latent tuberculosis infection. *PLoS One*, *7*(10), e48027.

Gcebe, N., Michel, A., Gey van Pittius, N. C., & Rutten, V. (2016). Comparative genomics and proteomic analysis of four non-tuberculous Mycobacterium species and Mycobacterium tuberculosis complex: occurrence of shared immunogenic proteins. *Frontiers in microbiology*, *7*, 160883.

Getahun, H., Harrington, M., Brien, R., & Nunn P (2007). Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. Lancet. 2007; 369: 2042–2049. https://doi.org/10.1016/S0140-6736(07)60284-0 PMID: 17574096 3.

Getahun, H., Gunneberg, C., Granich, R., & Nunn P (2012). HIV infection-associated tuberculosis: the epidemiology and the response. Clin Infect Dis. 2012; 50 Suppl 3: S201–7. https://doi.org/10.1086/651492 PMID: 20397949 4.

Getahun H, Kittikraisak J, & Heilig C. (2020). Development of a standardized screening rule for tuberculosis in people living with HIV in resource limited settings: individual data meta-analysis of observational studies. *PLoS Med. 8:e1000391. doi:10.1371/journal.pmed.1000391*.

Golub, J., Durovni, B., & King, B. (2008). Recurrent tuberculosis in HIVinfected patients in Rio de Janeiro, Brazil. AIDS. 22:2527–2533.

Gupta, R., Lucas, S., Fielding, K., & Lawn S (2015). Prevalence of tuberculosis in post-mortem studies of HIVinfected adults and children in resource-limited settings: a systematic review and meta-analysis. AIDS 2015; 29: 1987–2002. https://doi.org/10.1097/QAD.0000000000000802 PMID: 26266773

Hanifa, Y., Fielding, K., Charalambous, S., Variava, E., Luke, B., & Churchyard, G. (2012). Tuberculosis among adults starting antiretroviral therapy in South Africa: the need for routine case finding. *Int J Tuberc Lung Dis. 16(9):1252–1259.* [PubMed: 22794030]

Hanson, C., Osberg, M., & Brown, J. (2017). Finding the missing patients with tuberculosis: lessons learnt from patient pathway analyses in 5 countries. *J Infect Dis* 216:S686-95.

Holani, A. G., Ganvir, S. M., Shah, N. N., Bansode, S. C., Shende, I., Jawade, R., & Bijjargi, S. C. (2014). Demonstration of mycobacterium tuberculosis in

sputum and saliva smears of tuberculosis patients using ziehl neelsen and flurochrome staining-a comparative study. *Journal of clinical and diagnostic research: JCDR, 8*(7), ZC42.

Huck, S. (2007). Reading statistics and research, United States of America, Allyn & Bacon.

Jaeger, C., Karargyris, A., Candemir, S., Folio, L., Siegelman, J., Callaghan, J……& McDonald, C. (2014) "Automatic Tuberculosis screening using chest radio graphs," IEEE transactions on medical imaging, 33(2), 233-245.

Jerene, D., Muleta, C., & Dressie, S. (2022). The yield of chest Xray based versus symptom based screening among patients with diabetes mellitus in public health facilities in Addis Ababa, Ethiopia. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases, 29, 1000333.*

Jia, X., Yang, L., Dong, M., Chen, S., Lv, L., Cao, D., ... & Chen, F. (2017). The bioinformatics analysis of comparative genomics of Mycobacterium tuberculosis complex (MTBC) provides insight into dissimilarities between intraspecific groups differing in host association, virulence, and epitope diversity. *Frontiers in cellular and infection microbiology*, *7*, 88.

Jo, J., Fox G., & Marais B, (2016). Passive case finding for tuberculosis is not enough. Int J Mycobacteriol 2016; 5: 374–378.

Kestler, B., & Tyler, S. K. (2022). Latent tuberculosis testing through the ages: the search for a sleeping killer. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *322*(3), L412-L419.

Khan, M., Dar, O., Tahseen, S., & GodfreyFausset, P. (2009). Judging respiratory specimen acceptability for AFB microscopy: visual vs microscopic screening. *Trop Med Int Health;14:571-5.*

Khan, F., Verkuiji, S., Parrish, A., Chikwava, F., Ntumy, R., El-Sadr, W., & Howard, A. (2014). Performance of symptom based tuberculosis screening among people living with HIV: not as great as hoped. *AIDS 28(10): 1463-1472.*

Kranzer, K., Houben, R., Glynn, J., Bekker L, Wood, R., & Lawn S (2010). Yield of HIV-associated tuberculosis during intensified case finding in resource-limited settings: a systematic review and meta-analysis. *Lancet Infect Dis. 2010;* 10: 93–102. https://doi.org/10.1016/S1473-3099(09)70326-3 PMID: 20113978 2.

Krasilnikov, I., Lehnherr-Ilyina, T., Djonovic, M., Artamonova, I., Nikitin, M., & Kislichkin, N. (2024). Cracking the antigenic code of mycobacteria: CFP-10/ESAT-6 tuberculosis skin test and misleading results. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, 100436.

Kufa, T., Mngomezulu, V., Charalambous, S., et al. (2012). Undiagnosed tuberculosis among HIV clinic attendees: association with antiretroviral therapy and implications for intensified case finding, isoniazid preventive therapy, and infection control. *J Acquir Immune Defic Syndr. 60:e22–e28.* [PubMed: 22627184].

Kuretu, A. (2023). Ministry of Health and Child Care; Provincial Medical Director, Matebeleland North Province. (Local attachment supervisor).

Lardizabal, A., & Reichman, B. (2021). Diagnosis of latent tuberculosis infection. *Tuberculosis and Nontuberculous Mycobacterial Infections,* 75-82.

Lawn, S., Myer, L., Bekker, L., & Wood, R. (2006). Burden of tuberculosis in an antiretroviral treatment programme in sub-saharan Africa: impact on treatment outcomes and implications for tuberculosis control. *AIDS 20:1605-12 PMID: 16868441.*

Lawn, S., Brooks, S., & Kranzer, K. (2011). Screening for HIV- associated tuberculosis and rifampin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: A prospective study. *PLoS Med. 8(7):e1001067*. [PubMed: 21818180]

Lawn, S., Kerkhoff, A., Vogt, M., & Wood R (2013). HIV-associated tuberculosis: relationship between disease severity and the sensitivity of new sputum-based and urine-based diagnostic assays. BMC Med. BMC Medicine; 2013;11: https://doi.org/10.1186/1741-7015-11-1.

Liehr, P. & Smith, M. (1999). Middle range theory: Spinning research and practice to create knowledge for the new millennium. *Advances in Nursing Science, 21(4): 81-91.*

Lönnroth, K., Corbett, E., & Golub, D. (2014). Systematic screening for active tuberculosis: rationale, definitions and key considerations. *Int J Tuberc Lung Dis. 17(3):289–298.*

Meca, A., Turcu-Stiolica, A., Bogdan, M., Subtirelu, M., Cocos, R., Ungureanu, B., Mahler, B., & Pisoschi, C. (2022). Screening performance of C-reactive protein for active pulmonary tuberculosis in HIV-positive patients: A

systematic review with meta- analysis. *Journal of Infection and Public Health,* 14(10), 1380-1386. DOI: 10.1016.

Metcalfe, Z., Cattamanchi, A., McCulloch, C. E., Lew, J. D., Ha, N. P., & Graviss, E. A. (2013). Test variability of the QuantiFERON-TB gold in-tube assay in clinical practice. *American journal of respiratory and critical care medicine*, *187*(2), 206-211.

Modi, S., Cavanaugh, J., Shiraishi, R., Alexander, H., McCarthy, K., & Burmen, B. (2016). Performance of clinical screening algorithms for tuberculosis intensified case finding among people living with HIV in Western Kenya. *PLoS ONE 11(12): e0167685. doi:10.1371/journal.pone.0167685.*

Mohammed Adam, A., Ebraheem, M., & Bedri, A. (2022). Statistical Investigation of High Culture Contamination Rates in Mycobacteriology Laboratory. *Frontiers in Microbiology*, *13*, 789725.

Moreira, R., Huf, G., Vieira, S., Costa, D., Aguiar, F., Marsico, A. G., ... & Kritski, L. (2015). Liquid vs solid culture medium to evaluate proportion and time to change in management of suspects of tuberculosis—a pragmatic randomized trial in secondary and tertiary health care units in Brazil. *PLoS One*, *10*(6), e0127588.

Morrison, A.(2012). Introduction in: Screening in chronic disease. 2nd ed. New York: *Oxford University Press. 2012:3-20.*

Mulder, C., Erkens, C., Kouw, P., Huisman, E., Toumanian, S., & van den Hof, S. (2019). Tuberculin skin test reaction depends on type of purified protein

derivative: implications for cut-off values. *The International Journal of Tuberculosis and Lung Disease, 23*(12), 1327-1334.

Nachmias, D., & Nachmias, C. (1996). Research Methods in the Social Sciences (5th Ed.,) New York: St. Martins Press.

Nalunjogi, J., Mugabe, F., Najjingo, I., Lusiba, P., Olweny, F., Mubiru, J…….& Kirenga, B. (2021). Accuracy of the chest xray in screening for tuberculosis in Uganda: A cross sectional study. *Tuberculosis Research and Treatment*, 6622809.

Ngozi, U. J. (2018). The Prevalence of Latent Tuberculosis Infection and Associated Risk Factors Among Young People in South Africa: A Systematic Review and Meta-analysis

Orina, F., Mwangi, M., Githui, W., Ogaro, T., Kiptoo, M., Sang, W., Kariuki, J., & Wanzala, P. (2019). Effects of Sputum quality on Xpert MTB/RIF Results in The Detection of Mycobacterium from Persons Presumed to have TB in EAPHLN Project Operational Research Study Sites in Kenya. *African Journal of Health Sciences 32:6.*

Owiti, P., Onyango, D., Momanyi, R., & Harries, A. (2019). Screening and testing for tuberculosis among the HIV infected: Outcomes from a large HIV programme in Western Kenya. *BMC Public Health*, 19, 29.

Pai, M. (2012). Serial testing with TB interferon-γ release assays: toward a nuanced understanding. *Chest*, *142*(6), 1366-1368.

Pai, M., Denkinger, M., Kik, S. V., Rangaka, M. X., Zwerling, A., Oxlade, O., Metcalfe, J. Z., Cattamanchi, A., Dowdy, D. W., Dheda, K., & Banaei, N.

(2014). Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. *Clinical microbiology reviews*, *27*(1), 3–20. https://doi.org/10.1128/CMR.00034-13.

Piccazzo, R., Paparo, F., & Garlaschi, G. (2014). Diagnostic accuracy of chest radiography for the diagnosis of tuberculosis (TB) and its role in the detection of latent TB infection: a systematic review. *J Rheumatol Suppl, 91, 32-40.*

Polzin, A., Pletz, M., & Erbes, R. (2003). Procalcitonin as a diagnostic tool in lower respiratory tract infections and tuberculosis. *Eur Respir J. 2003; 21:939–943*. [PubMed: 12797485].

Prakash, A., Datta, B., Goyal, P., Chatterjee, A., & Gopal, J. (2016). Implementing upfront mobile digital chest x-ray for tuberculosis diagnosis in India feasibility and benefits. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 110(4), 215-222.

Rahman, M. T., Codlin, A. J., Rahman, M. M., Nahar, A., Reja, M., Islam, T., ... & Creswell, J. (2017). An evaluation of automated chest radiography reading software for tuberculosis screening among public-and private-sector patients. *European Respiratory Journal*, *49*(5).

Rangaka, M., Wilkinson, R., Glynn, J., Boulle, A., Van Cutsem, G., Goliath, R……& Maartens, G. (2014). Effect of antiretroviaral therapy no the diagnostic accuracy of symptom screening for intensified tuberculosis case finding in South African HIV clinic. *Clinical Infectious Diseases*, 59(4), 527-534.

Rehman, M., Bibi, S., Imran Khan, Z., Khan, A., Hussain Gilani, S., & Baig M. (2018). Role Of Tuberculin Test As A Diagnostic Tool For Tuberculosis. J Ayub Med Coll Abbottabad. Oct-Dec;30(4):529-533.

Rimal, R., Shrestha, D., Pyakurel, S., Poudel, R., Shrestha, P., Rai, K…..& Rai, S. (2022). Diagnostic performance of GeneXpert MTB/RIF in detecting MTB in smear-negative presumptive TB patients. *BMC Infectious Diseases, 22, 321.*

Rodrigues da Costa, R., Silva, M. R., Augusto, C. J., & Gonçalves Leite, I. C. (2022). Fast, simple and cheap: method modified from conventional cultivation for tuberculosis diagnosis allows seeding on Löwenstein–Jensen of any swab-embedded pulmonary samples decontaminated with sodium hydroxide. *Transactions of The Royal Society of Tropical Medicine and Hygiene, 116*(6), 523-530.

Rothman, K., & Greenland, S. (2008). Modern Epidemiology (2nd edition). Philadelphia: Lippincott Williams and Wilkins.

Sage, E., Noursadeghi, M., & Evans, H. (2014). Prognostic value of C-reactive protein in HIV-infected patients with Pneumocystis jirovecii pneumonia. *Int J STD AIDS.21(4):288–292.* [PubMed: 20378904]

Sanker, P., Kottuthodi, R., Ambika, A., Santhosh, V., Balakrishnan, R.,Mrithunjayan, S., & Moosan, H. (2017). Predictable repeatability issue with Gene xpert-xpert MTB/RIF derived Rifampicin Resistant Tuberculosis Results from South India: Appreciating the limits of a technological Marvel.

Santos, M., Batista, J., Braga, C., Silva, A., Maruza, M., & Souza, W. (2020). Effectiveness of screening for tuberculosis in HIV: a pragmatic clinical trial. *Rev Saude Publica. 2021; 55:45.*

Saunders, M., Lewis, P., & Thornhill, A. (2019). Research Methods for Business Students (8th ed). New York: Pearson.

Satia I, Bashagha S, Bibi A, Ahmed R, Mellor S, Zaman F. (2014). Assessing the accuracy and certainty in interpreting chest X-rays in the medical division. *Clin Med (Lond)* 13(4):349–352.

Schleicher, G., Herbert, V., & Brink, A. (2005). Procalcitonin and C-reactive protein levels in HIV positive subjects with tuberculosis and pneumonia. *Eur Respir J. 25(4):688–692.* [PubMed: 15802344].

Shapiro, A., Hong, T., & Govere, S. (2018). C-Reactive protein as a screening test for HIV associated pulmonary tuberculosis prior to antiretroviral therapy in South Africa. *AIDS* 32: 1811-20

Shapiro, A., Ross, J., Yao, M., Schiller, I., Kohli, M., Dendukuri, N., Steingart, K., & Horne, D. (2021). Xpert MTB/RIF and Xpert Ultra assays for screening for pulmonary tuberculosis and rifampicin resistance in adults, irrespective of signs or symptoms. Cochrane Database of Systematic Reviews, 2021(3). https://doi.org/10.1002/14651858.CD013694.pub2.

Shar, W. (2016). To determine diagnostic accuracy of GeneXpert and sputum Ziehl-Neelsen staining taking sputum culture as gold standard. *European Respiratory Journal*, 48, PA2779.

Sharma, S. K., Vashishtha, R., Chauhan, L. S., Sreenivas, V., & Seth, D. (2017). Comparison of TST and IGRA in diagnosis of latent tuberculosis infection in a high TB-burden setting. *PloS one*, *12*(1), e0169539.

Shen, F., & Sergi, C. (2020). Sputum analysis.

Shreffler, J., & Huecker, M. (2023). Diagnostic Testing Accuracy: Sensitivity, Specificity, Predictive Values and Likelihood Ratios. University of Louisville, United States of America.

Singhal, R., & Myneedu, V. P. (2015). Microscopy as a diagnostic tool in pulmonary tuberculosis. *The International Journal of Mycobacteriology*, *4*(1), 1-6.

Singh, P., Saket, V. K., & Kachhi, R. (2019). Diagnosis of TB: From conventional to modern molecular protocols. *Front. Biosci*, *11*, 38-60.

Starke, J. R., Committee On Infectious Diseases, Byington, C. L., Maldonado, Y. A., Barnett, E. D., Davies, H. D., ... & Zaoutis, T. E. (2014). Interferon-γ release assays for diagnosis of tuberculosis infection and disease in children. *Pediatrics*, *134*(6), e1763-e1773.

Steiner, A., Mangu, C., van den Hombergh, J., van Deutekom, H., van Ginneken, B., Clowes, P., ... & Hoelscher, M. (2015). Screening for pulmonary tuberculosis in a Tanzanian prison and computer-aided interpretation of chest X-rays. *Public health action*, *5*(4), 249-254.

Swindells, S., Komarow, L., Tripathy, S., Cain, K., MacGregor, R., Achkar, J……& Katzenstein, D. (2013). Screening for pulmonary tuberculosis in HIV infected individuals: Aids Clinical Trials Group Protocol A5253. *International Journal of Tuberculosis and Lung disease,* 17(4), 532-539.

Tchakounte Youngui, B., Tchounga, B. K., Graham, S. M., & Bonnet, M. (2022). Tuberculosis infection in children and adolescents. *Pathogens, 11*(12), 1512.

Telisinghe, L., Ruperez, M., Amofa-Sekyi, M., Mwenge, L., Mainga, T., Kumar, R…….& Ayles, H. (2021). Does Tuberculosis screening improve individual outcomes? A systematic review. *EClinicalMedicine, 40, 101127.*

Timire, C., Sandy, C., Ngwenya, M., Woznitza, N., Kumar, A. M. V., Takarinda, K. C., Sengai, T., & Harries, A. D. (2019). Targeted active screening for tuberculosis in Zimbabwe: are field digital chest X-ray ratings reliable?. *Public health action*, *9*(3), 96–101. https://doi.org/10.5588/pha.19.0003.

Tortoli, M. (2014). Smear microscopy for Tuberculosis diagnosis: Relevance for clinical practice. *Clinical Microbiology reviews*, 27(2), 389-405.

Uddin, M. K., Chowdhury, M. R., Ahmed, S., Rahman, M. T., Khatun, R., van Leth, F., & Banu, S. (2014). Comparison of direct versus concentrated smear microscopy in detection of pulmonary tuberculosis. *BMC research notes*, *6*, 291. https://doi.org/10.1186/1756-0500-6-291.

Van't Hoog, A., Langendam, M & Mitchell, E. (2013). "A systematic review of the sensitivity and specificity of symptom and chest radiography screening for active pulmonary tuberculosis in HIV negative persons and persons with unknown HIV status," *REPORT-Version March 2013,* World Health Organization, Geneva, Switzerland.

Whitworth, C., Hamilton, R., Goodwin, J., Barrera, C., West, B., Racster, L., ... & Mazurek, G. (2012). Within-subject interlaboratory variability of QuantiFERON-TB Gold In-Tube tests.

United Nations Joint Programme on HIV/AIDS [UNAIDS], (2017). Fast Track commitments to end AIDS by 2030 Joint United Nations Programme on HIV/AIDS.

Wilson, D., Badri, M. & Maartens, G. (2011). Performance of serum C reactive protein as a screening test for smear negative tuberculosis in an ambulatory high HIV prevalence population. PLoS one 6:e15248.

World Health Organization (2011). Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource- constrained settings. World Health. Geneva; 2011; 1: 142. doi:ISBN 978 92 4 150070 8

World Health Organization (2012). WHO policy on collaborative TB/HIV activities. In: guidelines for national programs and other stakeholders. Geneva, Switzerland; WHO/HIV/2012.1

World Health Organization (2012). Recommendations for investigating contacts of persons with infectious tuberculosis in low- and middle-income countries. WHO/HTM/TB/2012.9. Geneva, Switzerland.

World Health Organization (2013). Systematic screening for active tuberculosis: principles and recommendations, WHO, Geneva (Switzerland).

World Health Organization, (2013). Systematic screening for active tuberculosis. Principles and recommendations. WHO/HTM/TB/2013.04. Geneva, Switzerland: WHO.

World Health Organization (2013). Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and

rifampicin resistance: Xpert MTB / RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update. W [Internet]. Geneva; Available: http://www.who.int/tb/publications/xpert-mtb-rif-assay-diagnosis-policy-update/en/

World Health Organization. Global TB Report (2014). WHO/HTM/TB/2014.08.Geneva, Switzerland.

World Health Organization (2014). High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva, Switzerland.

World Health Organization (2015). Systematic screening for active tuberculosis: an operational guide. Geneva, Switzerland; WHO/HTM/TB/2015.16

World Health Organization (2015). The end TB strategy. Geneva, Switzerland.

World Health Organization (2015). The End TB Strategy: global strategy and targets for tuberculosis prevention, care and control after. WHO, Geneva, Swirtzerland.

World Health Organization (2015). *Systematic screening for active tuberculosis: principles and recommendations.* WHO, Geneva, Switzerland.

World Health Organization (2018). Global tuberculosis report, 2018. WHO/CDS/ TB/2018.20. Geneva, Switzerland: WHO.

World Health Organization (2020). Global tuberculosis report 2020, World Health Organization, Geneva (Switzerland).

Yoon, C., Davis, J., & Huang, L. (2014). Point-of-care C-reactive protein testing to facilitate implementation of isoniazid preventive therapy for people living

with HIV. *J Acquir Immune Defic Syndr. 65(5):551–556.* [PubMed: 24346636]

Yoon, C., Semitala, F., Atuhumuza, E., Katende, J., Mwebe, S., Asege, L., ……& Cattamanchi, A. (2017). Point of care C-reactive protein based tuberculosis screening for people living with HIV; a diagnostic accuracy study. *Lancet Infectious Diseases 17(12):1285-1292.*

Yoon, C., Chaisson, L, Patel, S., Allen, I., Drain, P., Wilson, D., & Cattamanchi, A. (2017). Diagnostic accuracy of C-reactive protein for active pulmonary tuberculosis: a meta- analysis. *Int J Tuber Lung Dis 21:1013-1019.* Crossref. PubMed. ISI.

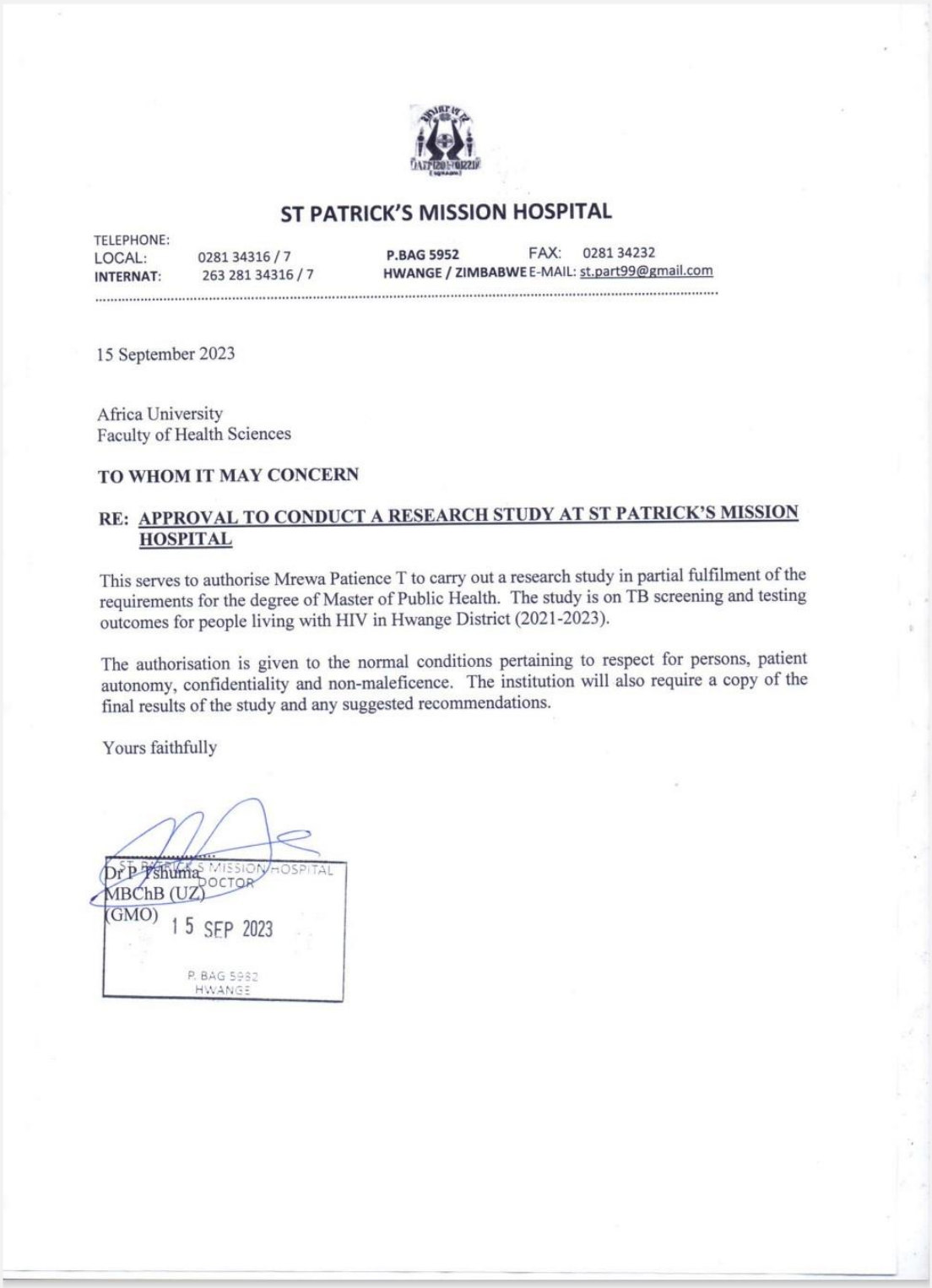
Zifodya, J., Kreniske, J., Schiller, I., Kohli, M., Dendukuri, N., Schumacher, S…,….& Pai M. (2021). Xpert Ultra versus Xpert MTB/RIF for pulmonary tuberculosis and rifampicin resistance in adults with presumptive pulmonary tuberculosis. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD009593.pub5.

Appendix 1: Study financial budget in usd$

ITEM	UNIT COST (USD$)	QUANTITY	TOTAL (USD$)

Airtime & Bundles	80	-	80
Stationery Printing services	5 [bond paper] 30	2 1	10 30
Fuel	1.68	100	168
TOTAL			288

Appendix 2: Institutional approval letter



ST PATRICK'S MISSION HOSPITAL

TELEPHONE:
LOCAL: 0281 34316 / 7
INTERNAT: 263 281 34316 / 7
P.BAG 5952
HWANGE / ZIMBABWE
FAX: 0281 34232
E-MAIL: st.part99@gmail.com

15 September 2023

Africa University
Faculty of Health Sciences

TO WHOM IT MAY CONCERN

RE: APPROVAL TO CONDUCT A RESEARCH STUDY AT ST PATRICK'S MISSION HOSPITAL

This serves to authorise Mrewa Patience T to carry out a research study in partial fulfilment of the requirements for the degree of Master of Public Health. The study is on TB screening and testing outcomes for people living with HIV in Hwange District (2021-2023).

The authorisation is given to the normal conditions pertaining to respect for persons, patient autonomy, confidentiality and non-maleficence. The institution will also require a copy of the final results of the study and any suggested recommendations.

Yours faithfully

Dr P Tshuma
MBChB (UZ)
(GMO)

ST PATRICK'S MISSION HOSPITAL
DOCTOR
15 SEP 2023
P. BAG 5952
HWANGE

Appendix 3: AUREC approval letter

AFRICA UNIVERSITY
A United Methodist-Related Institution
Investing in Africa's future

AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE (AUREC)

P.O. Box 1320 Mutare, Zimbabwe, Off Nyanga Road, Old Mutare-Tel (+263-20) 60075/60026/61611 Fax (+263 20) 61785 website: www.africau.edu

Ref: AU3159/23 7 November, 2023

MREWA PATIENCE T
C/O Africa University
Box 1320
MUTARE

RE: **AN ASSESSMENT OF THE EFFICACY OF TUBERCULOSIS SCREENING AND TESTING TOOLS AMONG ADULTS VISITING OPPORTUNISTIC INFECTIONS CLINICS IN HWANGE DISTRICT 2022-2023**

Thank you for the above-titled proposal that you submitted to the Africa University Research Ethics Committee for review. Please be advised that AUREC has reviewed and approved your application to conduct the above research.

The approval is based on the following.

a) Research proposal

- **APPROVAL NUMBER** AUREC 3028/23
  This number should be used on all correspondences, consent forms, and appropriate documents.
- **AUREC MEETING DATE** NA
- **APPROVAL DATE** November 7, 2023
- **EXPIRATION DATE** November 7, 2024
- **TYPE OF MEETING** Expedited
  After the expiration date, this research may only continue upon renewal. A progress report on a standard AUREC form should be submitted a month before the expiration date for renewal purposes.
- **SERIOUS ADVERSE EVENTS** All serious problems having to do with subject safety must be reported to AUREC within 3 working days on standard AUREC form.
- **MODIFICATIONS** Prior AUREC approval is required before implementing any changes in the proposal (including changes in the consent documents)
- **TERMINATION OF STUDY** Upon termination of the study a report has to be submitted to AUREC.

AFRICA UNIVERSITY
RESEARCH ETHICS COMMITTEE (AUREC)
APPROVED
P.O. BOX 1320, MUTARE, ZIMBABWE

Yours Faithfully

MARY CHINZOU
ASSISTANT RESEARCH OFFICER: FOR CHAIRPERSON
AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE

Appendix 4: Data extraction instrument

Age…….

Sex. Male…….Female…….

Mining Exposure as an occupation Yes……No…….

Previous TB treatment Yes……..No…….

CD4+ count……..

Viral load……

Number of months on ART…….

Screened for TB Yes……..No…….

Symptom screen results Positive…….Negative…….

Symptoms present Cough…….Fever…….Night sweats…….Weight loss……

CXR done Yes……..No…….

CXR results Suggest TB……..Not suggestive of TB……

Sputum for Gene Xpert results……Positive…… Negative……