VALIDATION OF GENEXPERT MACHINE USING EXTRAPULMONARY SAMPLES AT NATIONAL TUBERCULOSIS REFERRAL LABORATORY IN LESOTHO

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(_____)

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BLOEMFONTEIN
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DECLARATION OF INDEPENDENT WORK

DECLARATION WITH REGARD TO INDEPENDENT WORK

I, Nthabiseng Gladys Morabe, identity number _____ and student number _____, do hereby declare that this research project submitted to the Central University of Technology, Free State, for the Master of Health Science in Biomedical Technology degree, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules, and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

N. Morabe

07.08.2022

_____ _____

SIGNATURE OF STUDENT **DATE**

DEDICATION

I dedicate this dissertation to my lovely parents – Lekhooa Joseph Morabe and ‘Matankiso Pascalina Morabe – for their great support and tremendous understanding throughout this study period.

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LIST OF ABBREVIATIONS

µl	Microlitre
AFB	Acid-fast bacilli
AIDS	Acquired immunodeficiency syndrome
BCG	Bacillus Calmette-Guérin
CDC	Centers for Disease Control and Prevention
cfu/ml	Colony-forming unit(s) per millilitre
CSF	Cerebrospinal fluid
DISA	Descriptive and inferential statistical analysis
DNA	Deoxyribonucleic acid
DST	Drug susceptibility testing
EPTB	Extrapulmonary tuberculosis
FDA	Food and Drug Administration
FNA	Fine-needle aspirate
g/dL	Grams per decilitre
HIV	Human immunodeficiency virus
km	Kilometre
l	Litre(s)
LJ	Lowenstein-Jensen
LOD	Limit of detection
MDR	Multi-drug resistant
mg/dL	Milligrams per decilitre
MGIT	Mycobacteria Growth Indicator Tube
ml	Millilitre
mm	Millimetre
MTB/RIF	*Mycobacterium tuberculosis* / rifampicin
MTBC	*Mycobacterium tuberculosis* complex

NTB	Nontuberculous mycobacteria
NTRL	National Tuberculosis Reference Laboratory
OADC	Oleic albumin dextrose catalase
PCR	Polymerase chain reaction
PTB	Pulmonary tuberculosis
SPSS	Statistical Package for the Social Sciences
SR	Sarcoplasmic reticulum
TAT	Turn-around time
TB	Tuberculosis
TB/NTM	Tuberculosis / non-tuberculous mycobacterium
UNAIDS	Joint United Nations Programme on HIV and AIDS
WBC	White blood cell
WHO	World Health Organization
ZN	Ziehl-Neelsen [staining]

ABSTRACT

INTRODUCTION: The battle against tuberculosis (TB) is still a major public health scourge in Lesotho. Unfortunately, TB not only affects the lungs; it can also be found in other extrapulmonary areas such as the skin, brain, lymph nodes, genitourinary tract, and bones/joints. The GeneXpert is a sophisticated molecular assay that rapidly detects pulmonary TB and its resistance to rifampicin in sputum. The traditional culture TB-detection methods are still used for TB diagnosis of non-respiratory samples. The magnitude of delayed diagnosis of extrapulmonary tuberculosis (EPTB) can be addressed if rapid and effective methods are implemented. We sought to validate the GeneXpert System using two different cartridges; Xpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) and Xpert Ultra in the National Tuberculosis Reference Laboratory (NTRL) using extrapulmonary samples in this study.

METHODOLOGY: This study was mainly experimental, but existing data from the NTRL database were also included. The assessment of the Xpert MTB/RIF and Xpert Ultra was comparable to culture for identifying *Mycobacterium tuberculosis* in extrapulmonary clinical specimens. Extrapulmonary samples from various health facilities in Lesotho were tested using the culture method (gold standard) and the GeneXpert as method to be validated. Patients from all ages and genders were included. Statistical analysis was conducted on IBM's Statistical Package for Social Sciences (version 26).

RESULTS: A total of 352 samples were analysed from 2016 to 2019. Pleural fluid was the most collected sample type (39.2%), followed by ascitic fluid (14.2%) and cerebrospinal fluid (13.6%). Of the 352 samples tested, 34 EPTB cases were detected by the GeneXpert assays and culture methods. Twenty-one cases tested positive by the GeneXpert assays alone, while only one tested positive by the culture method, and the two methods were able to both detect *M. tuberculosis* in 12 specimens. The sensitivity and specificity of the GeneXpert assays were 92.3% and 87.1%, respectively. Pleural EPTB was predominant in males aged 21 to 40 years.

CONCLUSION: The findings from this study prove that pleural TB was the most prevalent EPTB. The outcomes of GeneXpert assays in this study do not replace culture as the gold method; however, the GeneXpert assays showed increased

sensitivity and a better turn-around time of less than two hours, which can assist clinicians in making a prompt diagnosis.

KEYWORDS: extrapulmonary tuberculosis, GeneXpert, *Mycobacterium tuberculosis*

CHAPTER 1:
BACKGROUND

1.1 INTRODUCTION

Tuberculosis (TB) is a life-threatening infectious disease mainly seen in people with a compromised immune system. These include people such as those living with human immunodeficiency virus (HIV), those suffering from malnutrition, diabetics, smokers, and alcohol abusers (World Health Organization [WHO], 2017). Several bacterial species from the genus Mycobacterium cause TB. These organisms include *M. tuberculosis*, *M. bovis, M. africanum, M. microti*, *and M. canettii*, among others. All these bacterial species are capable of causing TB infection in humans; however, *M. tuberculosis* is the common causative agent that leads to pulmonary tuberculosis (PTB) (Kock *et al.*, 2021; Tabong *et al*., 2021).

Mycobacterium bovis is more prevalent in cows and causes bovine TB but it can also be found in humans and can lead to a fatal form of TB called extrapulmonary tuberculosis (EPTB). Humans can be infected with *M. bovis* by consuming dairy products such as milk and the meat of animals infected with *M. bovis* (Jain, 2011; Khan *et al*., 2019; Todar, 2020). Tuberculosis can be classified into PTB or EPTB based on clinical presentation. The former is the most common type that attacks the lungs, and is more easily diagnosed. At the same time, the latter is found in other parts or organs of the body such as the brain, lymph nodes, genitourinary tract, and bones/joints, and it is not easily diagnosed (Purohit and Mustafa, 2015; Ravikumar and Bai, 2017).

Samples brought to the laboratory for diagnosis of EPTB may be tissues or body fluids. Samples such as bone marrow aspirates, ascetic fluids, cerebrospinal fluid (CSF), bone marrow aspirates, and pericardial fluids are usually sterile specimens that need aseptic collection, careful handling, and processing to minimise the risk of infections, as well as to obtain optimal results (National Tuberculosis Reference Laboratory [NTRL], 2015).

The magnitude of deaths resulting from EPTB can be decreased if rapid and effective methods are implemented for early diagnosis of this fatal disease (Yonge, 2015; Ravikumar and Bai, 2017). Various molecular methods can be used to

diagnose TB in general, and the GeneXpert is one of them. The GeneXpert is a sophisticated, in vitro, and a partially quantitative molecular test that uses real-time polymerase chain reaction (PCR) to detect the presence of the deoxyribonucleic acid (DNA) material of the *Mycobacterium tuberculosis* complex (MTBC) in sputum samples of clinically suspected TB patients. The assay is also used to confirm sputum samples that are either positive or negative for acid-fast bacilli (AFB) when smear microscopy is used. Moreover, the GeneXpert can determine mutations linked to rifampicin resistance in the *Rpo* B gene (Cepheid, 2012).

The WHO advocated the use of the fast and automated GeneXpert instrument using Xpert MTB/RIF cartridges and approved its use in 2010 to diagnose PTB in adults through testing of sputum samples. In 2013, the WHO also recommended that the Xpert MTB/RIF be used to test certain specimens for diagnosis of EPTB, especially tuberculosis meningitis (TBM), using a CSF specimen and that the assay may even be used to test children for EPTB (WHO, 2018). Currently, in Lesotho, the GeneXpert machines have not been validated and verified for extrapulmonary samples. Thus, it is necessary to validate and verify this instrument for extrapulmonary samples before its use for EPTB can be advocated. This research, therefore, aimed to evaluate the performance efficiency and reliability of the GeneXpert assay using non-respiratory samples for rapid EPTB diagnosis at the NTRL in Lesotho.

1.2 PROBLEM STATEMENT

Since EPTB is not as infectious as PTB, most TB strategies and control plans focus more on PTB patients, even internationally (Ayed *et al.*, 2018; Mekonnen *et al.*, 2019). EPTB diagnosis is a frustrating health problem in Lesotho. This is due to a lack of faster-advanced technologies and more effective methods than Ziehl-Neelsen (ZN) staining with an unsatisfactory turn-around time (TAT), sensitivity (microscopy), and culture with a longer TAT. There have been major successes with the use of the GeneXpert for the early diagnosis and treatment of various forms of EPTB globally (Kim *et al.*, 2015; Nataraj *et al.*, 2016; Kohli *et al.*, 2018; Habous *et al.*, 2019; Seo *et al.*, 2020; Hoel *et al.*, 2020; Binjomah *et al.*, 2021; Kohli *et al.*, 2021; Mokaddas *et al.*, 2021).

In Lesotho, GeneXpert instruments are used only to diagnose PTB from sputum samples. Extrapulmonary samples are brought to the referral laboratory for testing using the traditional culture method, which takes longer to provide results. To reduce the TAT for diagnosing TB in EPTB samples, the NTRL has embarked on validating the GeneXpert instrument as a diagnostic tool for EPTB samples. The GeneXpert instrument needs to be validated for extrapulmonary samples before supporting the use of the assay on EPTB samples to provide reliable and accurate results for patient care, as several studies have shown promising results of diagnosing EPTB with the GeneXpert assay compared to microscopy and culture. There is, thus, a need to fill the gap in the early diagnosis of EPTB using the rapid molecular method with a shorter TAT in Lesotho.

In Lesotho, this is the first study of this nature, and it also seeks to address other national problems, such as highlighting estimates of the prevalence of EPTB. The study aims to fill the gaps in rapid detection of EPTB in Lesotho and determine the prevalence thereof, as well as to add new knowledge regarding TB strategic plans in Lesotho.

1.3 AIM AND OBJECTIVES OF THIS STUDY

1.3.1 Aim

The aim of this study was to validate the GeneXpert for the diagnosis of TB from extrapulmonary samples (CSF, pleural fluid, ascetic fluid, and lymph) in Lesotho.

1.3.2 Objectives

The objectives of the study are as follows:

1) To examine various non-respiratory specimens using the GeneXpert;
2) To evaluate the performance efficiency and reliability of the GeneXpert in the rapid diagnosis of EPTB;
3) To determine the prevalence of EPTB; and
4) To compare results obtained from the GeneXpert with those obtained from culture, which is still considered the gold standard method.

CHAPTER 2:
LITERATURE REVIEW

2.1 INTRODUCTION

The battle against Tuberculosis (TB) is still a major public health scourge around the world. The WHO declared the TB pandemic a global emergency around three decades ago (WHO, 1994). Managing the disease has become challenging due to HIV-co-infection and the acquired drug resistance of *M. tuberculosis* to current TB drugs. Some TB strains are multi-drug resistant (MDR), while others are classified as extensively drug-resistant (WHO, 2014). Across the globe, approximately 10 million people are infected by TB every year (WHO, 2020).

Extrapulmonary tuberculosis (EPTB) is still a major public health concern in developing countries, with the prominent type being tuberculous lymphadenitis (Purohit and Mustafa, 2015; Ghariani *et al*., 2015; Manyepa, 2016; Seok *et al*., 2019). The diagnosis of EPTB mainly relies on histopathology, ZN staining, and *M. tuberculosis* culture results (Purohit and Mustafa, 2015; Mokaddas *et al*., 2021). However, the conventional method, ZN staining, is known to have limitations, such as low sensitivity that ranges from 20% to 43%. Even though it is a rapid and easy technique for detecting the acid-fast bacilli (AFB), multiple *M. tuberculosis* samples may be required for a diagnosis (Manyepa, 2016; Bayot *et al*., 2022).

Culture is still the reference standard for MTBC detection. Still, it has the major disadvantage of a prolonged TAT of approximately eight weeks, and there is a need for trained personnel and specialised biosafety laboratory protocols (Habous *et al*., 2019). Culture is also reported to have low sensitivity for various EPTB samples, such as CSF and pleural fluid, since these specimens usually have a low bacterial load (Hoel *et al*., 2020; Mokaddas *et al*., 2021). In addition, cultured biopsy samples usually come out negative even when histopathological findings indications of an active form of TB disease.

Possible reasons for the low positivity of biopsy samples could be the paucibacillary nature of the disease leading to some bacteriologically mute lesions (Kashyap *et al*., 2013; Li *et al*., 2020; Kohli *et al*., 2018; Mokaddas *et al*., 2021). Serological tests also have shortcomings of low sensitivity and specificity, whereas the PCR

tests (AdvanSure tuberculosis / non-tuberculous mycobacterium [TB/NTM] PCR and Cobas TaqMan Mycobacterium tuberculosis [MTB] PCR) are said to be examples of more sensitive PCR assays for TB diagnosis, despite their high cost, which makes them unsuitable for routine use (Cho *et al*., 2015; Manyepa, 2016).

Many attempts have been made to develop a suitable method that is fast, relatively inexpensive, and accurate for the diagnosis of TB. The GeneXpert assay has proven to meet the above attributes of a convenient diagnostic test and has been a game changer in the diagnosis of PTB, and its use is recommended by the WHO (Manyepa, 2016; Tiamiyu *et al*., 2020).

2.2 LESOTHO AND ITS TUBERCULOSIS/ HUMAN IMMUNODEFICIENCY VIRUS STATUS

Lesotho is a small mountainous country in the southern region of Africa with a population of about 2.2 million people. The mountain kingdom of Lesotho is surrounded by South Africa. Lesotho has 10 districts, namely Maseru (the capital city), Berea, Mafeteng, Mohale's Hoek, Quthing, Qacha's Nek, Thaba-Tseka, Leribe, Butha-Buthe, and Mokhotlong. Most parts of the country are in the highlands, where many villages are located in rural areas and are not easily accessed due to poor resources and infrastructure. These places are only accessible by foot or by using horses and small aircraft. Lesotho was categorised under the lower-middle-income countries in the year 2016 by the World Bank and is ranked high among the countries that are severely hit by poverty worldwide (Ministry of Health, Lesotho. 2019; World Bank, 2022).

In 2016, Lesotho's top five causes of death were HIV, which leads to acquired immunodeficiency syndrome (AIDS), TB, diarrhoea, cerebrovascular diseases, and lower respiratory infections (Ministry of Health, Lesotho, 2019). Lesotho's TB status was ranked number two globally, with 611 cases per 100 000 people (WHO, 2019). In 2020, the country still maintained its rank globally but with a markedly increased TB incidence of 724 cases per 100 000 people, which led to approximately 15 276 people who developed TB that year (Pacheco, 2020).

The prevalence of HIV in persons aged 15 to 59 years was 25.6% in 2016 to 2017 (Ministry of Health, Lesotho, 2018; 2019), 23.6% in 2018 (Joint United Nations

Programme on HIV and AIDS [UNAIDS], 2019), and 22.8% in 2019 (WHO, 2019). The prevalence of HIV among TB patients was 75% (WHO, 2019). Most notified TB cases are seen in young working adults aged 24 to 35 years. This reflects the age distribution pattern in HIV-positive people, which denotes ongoing transmission instead of infections that are reactivated. Approximately 80% of TB patients are co-infected with HIV. Other super-spreaders of TB include overcrowding, malnutrition, the mining community, alcohol abuse, and poor ventilation (WHO, 2014). Although the treatment success rate in Lesotho is around 77%, there is still a notable increase in multi drug resistant- TB (MDR-TB) cases (Pacheco, 2020).

2.3 LESOTHO HEALTHCARE AND LABORATORY SERVICES

The healthcare services in the country are divided into three categories: primary, secondary, and tertiary healthcare. As per the Master of Health list of 2017, there were 290 healthcare facilities, of which 261 fall under primary health centres/clinics, 21 are regarded as general hospitals, four are primary hospitals, and four are filter clinics. Unfortunately, among all these healthcare facilities, there is only one tertiary/referral hospital (Queen 'Mamohato Memorial Hospital). There are approximately 20 medical laboratories found in the 25 hospitals. For instance, there is a laboratory in the Berea, Mafeteng, Seboche, Maluti Adventist, Motebang, Paray Mission, Queen Elizabeth II, and Butha-Buthe hospitals, to mention a few. It is also worth noting that there is only one TB reference laboratory in the country, namely the NTRL (NTRL, 2015; Ministry of Health, Lesotho, 2019).

The latter is a Level 3 biosafety laboratory, with the mandate to serve as a reference laboratory for the diagnosis of TB for patient care and management countrywide. Tests include microscopy, GeneXpert, mycobacterial culture, drug susceptibility testing (DST), and line probe assay. The NTRL receives specimens from various hospital laboratories and health centres throughout Lesotho. These specimens must be collected without preservatives and reach the NTRL within three days. The most prioritised samples are CSF, which should be transported to the NTRL immediately and processed urgently or be kept at a temperature of 2 °C to 8 °C and then transported to the NTRL for processing within three days. Urine samples should also reach the NTRL within four hours or be refrigerated at 2 °C to

8 °C and then transported to the NTRL for processing within three days (NTRL, 2015).

2.4 TUBERCULOSIS AND ITS GLOBAL STATUS

Before the advent of the COVID-19 pandemic, TB was the leading cause of mortality from a single infectious pathogen (more than the deadly HIV/AIDS) worldwide. *Mycobacterium tuberculosis* is said to be the most predominant single agent that leads to TB (WHO, 2021a). The immune system also plays a vital role in TB infection; the probability of one contracting TB increases if the immune system of such a patient is already compromised/suppressed, as TB is an opportunistic infection. Tuberculosis is mainly predominant in HIV-positive patients as their immune system is very low due to their lowered CD4 count (Yonge, 2015; Centers for Disease Control and Prevention [CDC], 2016; WHO, 2020).

Ravikumar and Bai (2017) also support that TB/HIV-infected patients account for 50% to 70% of the population compared to the 10% to 34% of TB patients not infected by HIV. It was estimated in 2020 that approximately 9.9 million people had TB globally (WHO, 2021a). This number slightly decreased from 2019, estimated to be 10.0 million (WHO, 2020). The number of TB notification cases in 2019 was 7.1 million, slightly increasing from the 7 million cases detected and reported in 2018. A significant increase from the 6.4 million cases reported in 2017 (WHO, 2020). From 2009 to 2012, a total of 5.7 to 5.8 million new cases were reported yearly, according to the WHO (2020). Based on the findings in the Global TB Report (WHO, 2020), Lesotho was also in the top 20 listed countries seriously hit by TB infection and TB/HIV co-infection worldwide (see Figure 2.1).

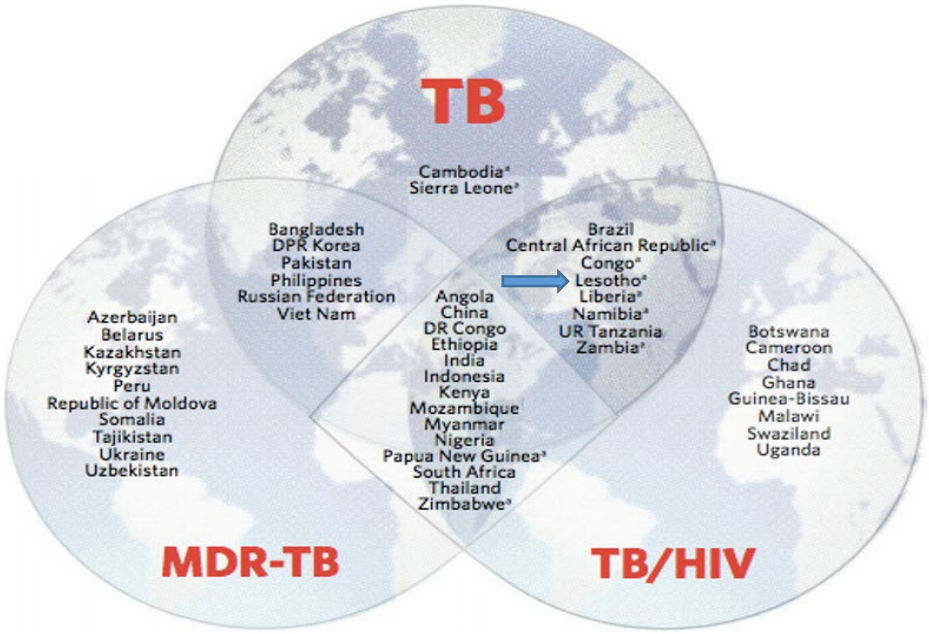

Figure 2.1: Countries with a high tuberculosis burden, tuberculosis/ human immunodeficiency virus and multi-drug-resistant tuberculosis; Lesotho being one of those with a high tuberculosis/ human immunodeficiency virus burden
Source: WHO (2020)

Although there was a notable increase in newly confirmed cases in 2019, there was an enormous gap (approximately 3 million) between the reported newly diagnosed cases and the approximately 10 million people who were ill due to TB. This was mainly a result of the under-reporting of confirmed cases and low testing capacity (WHO, 2020). Another significant factor was the advent of the COVID-19 pandemic, which diverted most countries' focus, resources, and funds to fight the new pandemic, thus sidelining other deadly diseases such as TB. For example, some countries used the GeneXpert instrument (which was previously used for TB testing) to test for COVID-19 using specific cartridges designed for COVID-19 diagnosis, thus decreasing TB testing (WHO, 2020).

Towards the end of 2019, many countries across the globe failed to attain the 2020 milestone towards ending the TB pandemic. The milestone strategies are now extended to 2035 and are as follows, as stated by the WHO (2021a):

- The number of deaths due to TB is to be reduced by 95% by 2035;
- The number of new cases is to be reduced by 90% by 2035; and

- Catastrophic cost due to TB to be at 0% in households with people infected by TB by 2035.

Although the TB incidence rate seemed to be declining, there is still a long way to go, as the drop statistically was not significant enough to reach the 2020 milestone of a 20% decrease in new TB cases and deaths between 2015 and 2020.

2.5 EXTRAPULMONARY TUBERCULOSIS

In most cases, TB affects the lungs (PTB) but can also disseminate to other body organs/parts either via the hematogenous (blood) or lymphatic (lymph) system and is then called EPTB. The latter is often a consequence of a primary lung infection, as demonstrated in Figure 2.2. Samples can be taken from various body parts to detect *M. tuberculosis*, including urine, lymph node aspirates, pleural fluids, blood, CSF, and ascitic fluids (Purohit and Mustafa, 2015; WHO, 2017).

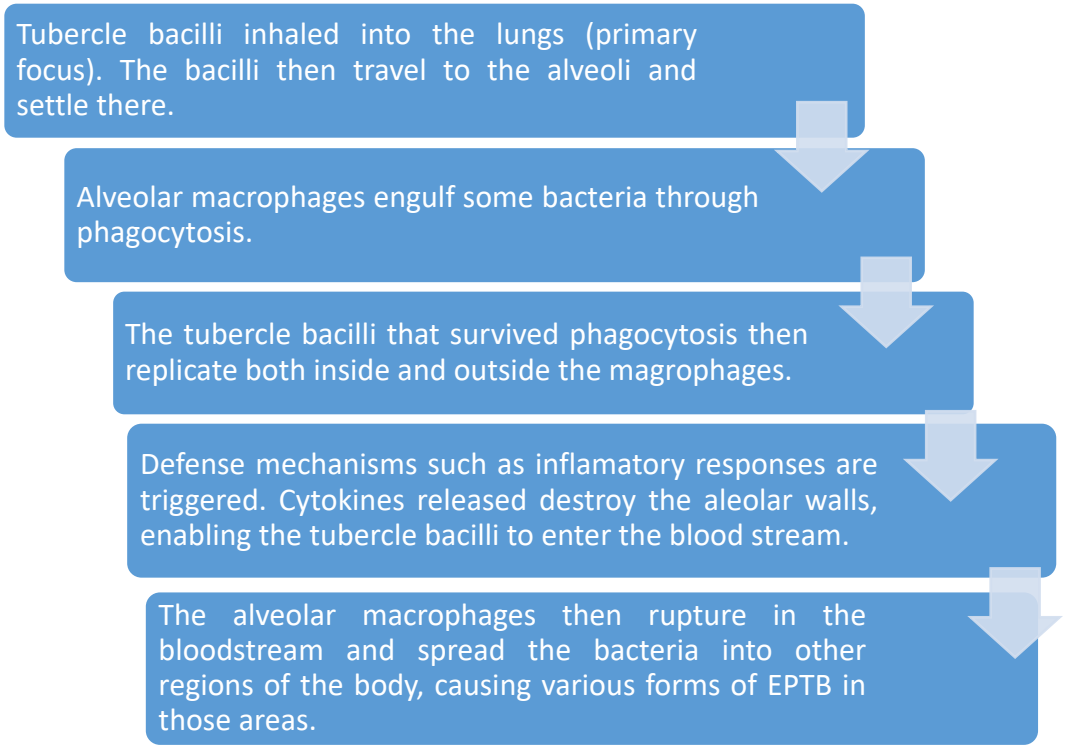

Figure 2.2: The pathogenesis of tuberculosis

Source: European Centre for Disease Prevention and Control (2013); Lekhooa (2015)

As stated by the Minister of Health and Social Welfare, Lesotho (2010) and Heemskerk *et al*. (2015), the clinical manifestations of EPTB depend on the site of

infection, as shown in Figure 2.3. These may include pleural chest pain, abdominal pain, lymphadenopathy, severe headaches, urinary problems, hepatomegaly, weight loss, anorexia, fever, erythema nodosum, skeletal pains, malaise, altered mental status, and dyspnoea.

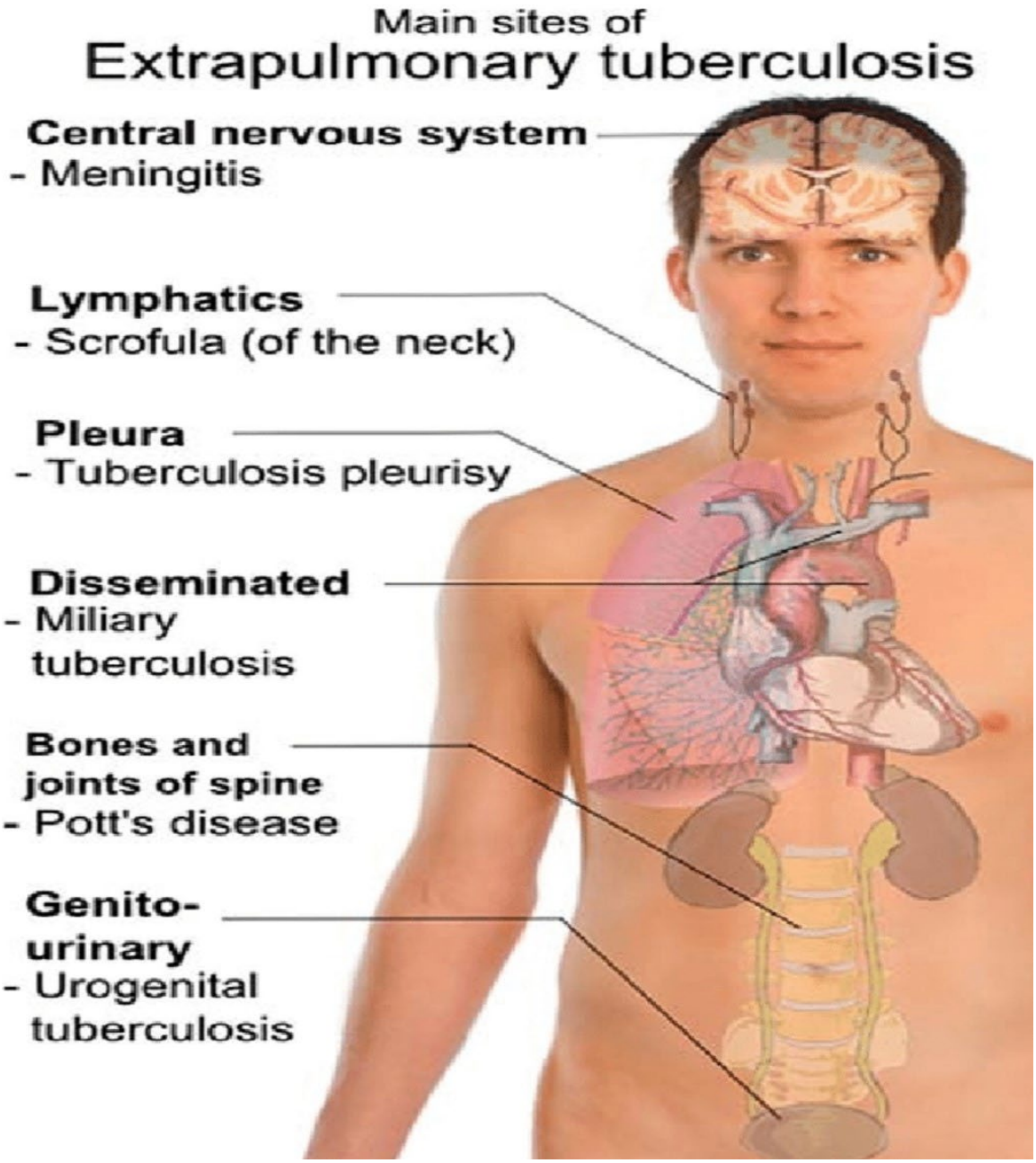

Figure 2.3: Common sites where extrapulmonary tuberculosis can develop
Source: Stewart (2017)

According to the WHO (2017), an approximate prevalence of 16% of EPTB was reported in Africa. This ranked Africa second after a 24% prevalence was observed

in the Eastern Mediterranean (Mohammed *et al*., 2018). In 2019, there were 7.1 million new TB cases, of which 16% were EPTB globally (WHO, 2020). Extrapulmonary TB is not easily diagnosed compared to PTB. Reasons include the low yield of the bacteria found in extrapulmonary sites. The disease can present many (non-specific) signs and symptoms, which hinders correct and early diagnosis. Moreover, invasive procedures to extract samples and inadequate facilities used in laboratories with insufficient resources also contribute to EPTB diagnosis challenges (Ramírez-Lapausa *et al*., 2015).

A high index of clinical suspicion is required for the correct diagnosis of EPTB and, ultimately, timely treatment. Unfortunately, for results suspected EPTB patients usually having to wait for a lengthy period before their final diagnosis of EPTB is confirmed (Marouane *et al*., 2016; Tadesse *et al*., 2019; Uppe *et al*., 2020; Li *et al*., 2020). Once EPTB is confirmed in such individuals, they start a six- to nine-month treatment. However, in some cases, medicine must be taken longer, such as in the case of tuberculosis meningitis. Surgery may also be needed in some types of EPTB, such as urinary system TB and spinal TB (Lee, 2015; Cantres-Fonseca *et al*., 2018).

Both direct and indirect methods are used to detect the presence of causative agents of EPTB, as presented in Table 2.1. Direct methods detect the presence or viability of strains of the TB bacilli and their products. Still, the limiting factor for most of these methods is their low sensitivity to detect the microorganism *M. tuberculosis* in extrapulmonary sites where the organism is found in low quantities (inadequate for detection). Indirect methods, on the other hand, rely on the degree of one's adaptive immune responses towards the pathogen (*M. tuberculosis*) that has invaded the body tissues and organs. The indirect method of diagnosing EPTB is reported to have high sensitivity but lower specificity than the direct method (Purohit and Mustafa, 2015; Zellweger *et al*., 2020), as indicated in Table 2.1.

Table 2.1: The sensitivity and specificity of various direct and indirect methods for the detection of *Mycobacterium tuberculosis* in non-respiratory samples

Method	Direct	Indirect	Sensitivity	Specificity
BACTEC culture system	✓	x	↑↑↑	↑↑
Skin test	x	✓	↑↑	↓↓↓
ZN staining	✓	x	↓↓↓	↑↑↑
Cytology	x	✓	↑↑	↓↓

GeneXpert	✓	x	↑↑	↑↑
LJ culture	✓	x	↓↓	↑↑↑
Interferon-y release assay	x	✓	↑↑	↑↑
IHC and ICC	✓	X	↑↑	↑↑

NOTE: ZN = Ziehl-Neelsen, LJ = Lowenstein-Jensen, IHC and ICC = immunohistochemistry and immunocytochemistry

KEY: ↑↑↑ (very high), ↑↑ (high)

↓↓↓ (very low), ↓↓ (low)

Source: Purohit and Mustafa (2015)

According to Haas (2018), in the TB diagnostic laboratory, EPTB can be diagnosed by the following methods:

- Positive AFB as observed from the smear microscopy;
- Culture;
- Nuclei acid amplification tests;
- Increased protein levels:
  - Pleural/peritoneal protein ranging from 4-5 g/dL; and
  - CSF proteins range from 100-500 mg/dL.
- Slightly reduced CSF glucose levels;
- Necrotising granulomata (Ghon complex); and
- Elevated white blood cell (WBC) count in:
    - CSF (100-500/µl);
  - Blood (blood film presenting lymphocytosis); and
    - Pleural fluid (1000-5000 WBC/µl).

However, despite numerous EPTB markers (Denkinger, 2014), culture and histology are regarded as the cornerstones of EPTB diagnosis.

2.5.1 Types of extrapulmonary tuberculosis

In most cases, EPTB is spread to other body organs and sites by blood (hematogenous dissemination). There are many types of EPTB, including tuberculosis meningitis, tuberculous lymphadenitis, pleural TB, genitourinary tuberculosis (GTB), cutaneous TB (scrofuloderma), gastrointestinal TB, TB peritonitis, and TB pericarditis, which are discussed in the following subsections (Sevgi *et al*., 2013; Ramírez-Lapausa *et al*., 2015).

2.5.1.1 Tuberculosis meningitis

Roughly 5% of all EPTB cases are due to tuberculosis meningitis (TBM). This is one of the deadliest forms of EPTB that leads to a high death rate (50%) among HIV-infected people. The disease can be found in all age groups but seems to be predominant in birth and children under the age of five years (Tierney and Nardell, 2018; Thakur *et al*., 2018). Like most forms of EPTB, TBM develops as a respiratory infection through inhalation of the aerosolised droplet nuclei of *M. tuberculosis* that lodge in the lungs. This stimulates the alveolar macrophages, neutrophils, and dendritic cell to liberate cytokines, chemokines, and antimicrobial peptides in response to the localised infection. Infected dendritic cells are triggered to move towards the local draining lymph node by cytokines and chemokines which also trigger T helper 1 cells to differentiate and liberate interferon gamma and tumour necrosis factor alpha at the primary focus. The infection is contained by the activated alveolar macrophages together with the dendritic cells, which release cytokines and antimicrobial peptides. Eventually there will be a development of a granuloma that is composed of tubercle bacilli in a dormant state (Davis *et al*., 2019; Manyelo *et al*., 2021).

Mycobacterium tuberculosis bacilli can spread hematogenously to the central nervous system in two ways:

1) The tubercle bacilli disseminate into the local draining lymph nodes in the early-stage infection of PTB and develop bacteraemia.
2) Reactivating the latent infection may result in lung tissue damage (typically in older people, immunosuppressed individuals, and children).

The tubercle bacilli can cross other membranes in the body, such as the blood-brain barrier and blood-CSF barrier, by the Trojan horse mechanism. This phenomenon is when engulfed bacteria use the infected cells, namely the neutrophils and macrophages, as their carriers or when *M. tuberculosis* enters the brain endothelium in large numbers through the help of *M. tuberculosis* pknD (*Rv0931c*). Once the bacilli settle in the brain, they form lesions called Rich foci. These lesions may rupture into the subarachnoid space or ventricles in the brain. It may then lead to the formation of other granulomas and, ultimately, inflammation of the meninges (Manyelo *et al*., 2021).

Patients with TBM may present the following clinical manifestations: severe headache, drowsiness, hydrocephalus (as shown in Figure 2.4), blindness, mild fever, nausea, neurological disorders, stupor, and, even worse, coma (National Organization for Rare Disorders, 2009; Tierney and Nardell, 2018).

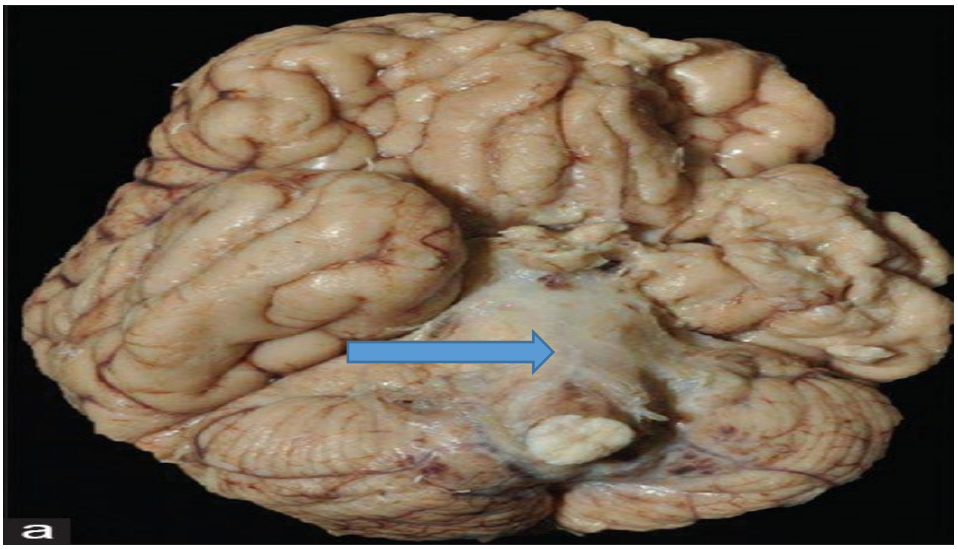

Figure 2.4: The brain of a patient with tuberculosis meningitis
Source: Chatterjee *et al*. (2015)

The most prevalent diagnosis of TBM is based on the typical clinical manifestation, neuroimaging findings, CSF examination, and a comprehensive evaluation of the response to anti-TB medication. The presence of *M. tuberculosis* in CSF smears or bacterial culture is sufficient to make a definitive diagnosis. Both techniques (smear microscopy and culture) have a poor positivity rate for *M. tuberculosis* detection, which is usually due to the paucibacillary nature of CSF samples. However, AFB microscopy seems to be the most effective diagnostic method compared to the conventional culture method (Luo, 2018; Mokaddas *et al*., 2021). Several studies have been conducted that show good performance of the GeneXpert in the diagnosis of TBM (Bahr *et al*., 2015; Metcalf *et al*., 2018; Wu *et al*., 2019; Cresswell *et al*., 2020; Kohli *et al*., 2021; Mokaddas *et al*., 2021; Binjomah *et al*., 2021).

2.5.1.2 Tuberculous lymphadenitis

The lymph nodes are the most commonly affected sites of EPTB infection (Sevgi *et al*., 2013; Guler *et al*., 2015; Tadesse *et al*., 2019; Seo *et al*., 2020). It is a scrofula that is seen frequently in adults and young children. The typical clinical presentation is the enlargement of the lymph nodes (generalised lymphadenopathy) due to the infection by *M. tuberculosis* or *M. bovis*. However, chronic lymphadenitis can also be caused by infection with non-tuberculous mycobacteria including *M. scrofulaceum*, *M. avium*, and *M. haemophilum*. Other causes can be organisms like *Toxoplasma* species, *Bartonella* species, and fungi. Neoplasms, sarcoidosis, Castleman disease, adverse drug effects, and non-specific reactive hyperplasia are examples of non-infectious causes (Reuter and Wood, 2009; Fontanilla *et al*., 2011; Ramírez-Lapausa *et al*., 2015).

Mycobacterium tuberculosis typically disseminates in the body through hematogenous and lymphatic spread. The foremost lymph nodes to be affected are the hilar and mediastinal lymph nodes. The oropharyngeal mucosa serves as the route of entry for the tubercle bacilli in children. *Mycobacterium tuberculosis* may also gain its entry at the tonsils and then disseminate to the lymphatics and cervical lymph nodes. The formation of necrotic lymph nodes also significantly enhances the likelihood of TB in various extrapulmonary regions. The bacilli can spread via the blood to the lymph node and then settle in the sinuses, where the inactive macrophages engulf the foreign particles (bacilli) during phagocytosis. The phagocytosed bacteria replicate inside the macrophages and release their contents together with the replicated bacilli (Ramírez-Lapausa et al., 2015; Rodriguez-Takeuchi *et al*., 2019).

Other immune cells, such as monocytes and activated macrophages, are stimulated, and a granuloma is formed to combat the infection and restrict the bacilli replication rate. Eventually, the granuloma changes into a lesion, which shows features of a necrotic centre, together with peripheral granulation tissue. The most predominant manifestation of the lymphatic system is bilateral cervical lymphadenitis, which is painless. Symptoms of cervical tuberculous lymphadenitis include swollen, very hard lymph nodes that, when inflamed, lead to draining

fistulas (Ferry, 2018; Tierney and Nardell, 2018; Rodriguez-Takeuchi *et al*., 2019; Mathiasen *et al*., 2020).

Culture or PCR results indicating the presence of *M. tuberculosis* in an afflicted lymph node can be used to provide a definitive diagnosis of tuberculous lymphadenitis. The gold standard for diagnosis is still culture, which allows differentiation of *M. tuberculosis* from other mycobacteria that can cause lymphadenitis, although results can take up to four weeks. New molecular approaches, such as PCR nucleic acid amplification tests, are increasingly applied in the rapid diagnosis of tuberculous lymphadenitis. These methods are sensitive and specific, producing findings within 24 to 48 hours (Salvador *et al*., 2015).

The presence of AFB and granulomatous inflammation may be suggestive of mycobacterial aetiology. AFB microscopy has high specificity for *M. tuberculosis* in adults; however, its sensitivity is lower than that of culture. When both the AFB and the culture results are negative, histological findings such as non-specific lymphoid infiltrates, non-caseating granulomas or Langerhans' giant cells in areas of severe caseous necrosis might help diagnose presumptive TB. Excisional biopsies have the best sensitivity (80%). However, fine-needle aspirate (FNA) is usually suitable for diagnosing this disease as it is less invasive, more practical, and safer than excisional biopsy. Fine-needle aspirates could therefore be helpful for diagnosing tuberculous lymphadenitis, particularly for immunosuppressed patients and in resource-limited settings (Fontanilla *et al*., 2011; Salvador *et al.*, 2015).

2.5.1.3 Pleural tuberculosis

Pleural tuberculosis is the second most common EPTB presentation after tuberculous lymphadenitis. Pleural tuberculosis can occur due to PTB or as a long-term implication of the disseminated tubercle bacilli several years after the initial pulmonary infection. It is said to be a complication in the pleural space due to hypersensitivity responses that occur in battling TB antigens. Ruptured or leaking pleural space poses a great threat as the tubercle bacilli can use such loopholes as entry points to the blood or lymphatic systems (Ramírez-Lapausa *et al*., 2015; Casalini *et al*., 2018).

The invasion of the bacilli from the primary focus to the pleural space stimulates a cascade of immune events mediated by T cells. This results in the production of cytokines and stimulation of the macrophages, leading to granuloma formation. These reactions also lead to inflammatory responses such as increased vascular permeability and a high number of WBCs that enter the pleural space, which ultimately culminates in increased fluid and cell concentration, which are features of pleural exudates. Other mediators that are stimulated include interlukin-8, transforming growth factor-β1, and tumour necrosis factor-α (Antonangelo *et al*., 2012). *Mycobacterium tuberculosis* in pleural fluids and pleural biopsies is mainly detected using AFB smear microscopy and/or culture or histopathology (Vorster *et al*., 2015).

2.5.1.4 Genitourinary tuberculosis

Genitourinary tuberculosis (GTB) is another prevalent type of EPTB, representing 20% to 40% of all EPTB cases (Zajaczkowski, 2012; Raina *et al*., 2021). Tubercle bacilli spread to the genitourinary tract commences in the lungs or during the reactivation of latent primary infection in the lungs. The reactivated infection is prominent in HIV-positive people and steroid users. The medullary region of the kidney, the prostate, and seminal vesicles are often the initial site of this form of TB (Amaya- Tapia and Aguirre-Avalos, 2018; Jha and Budh, 2021; Raina *et al*., 2021).

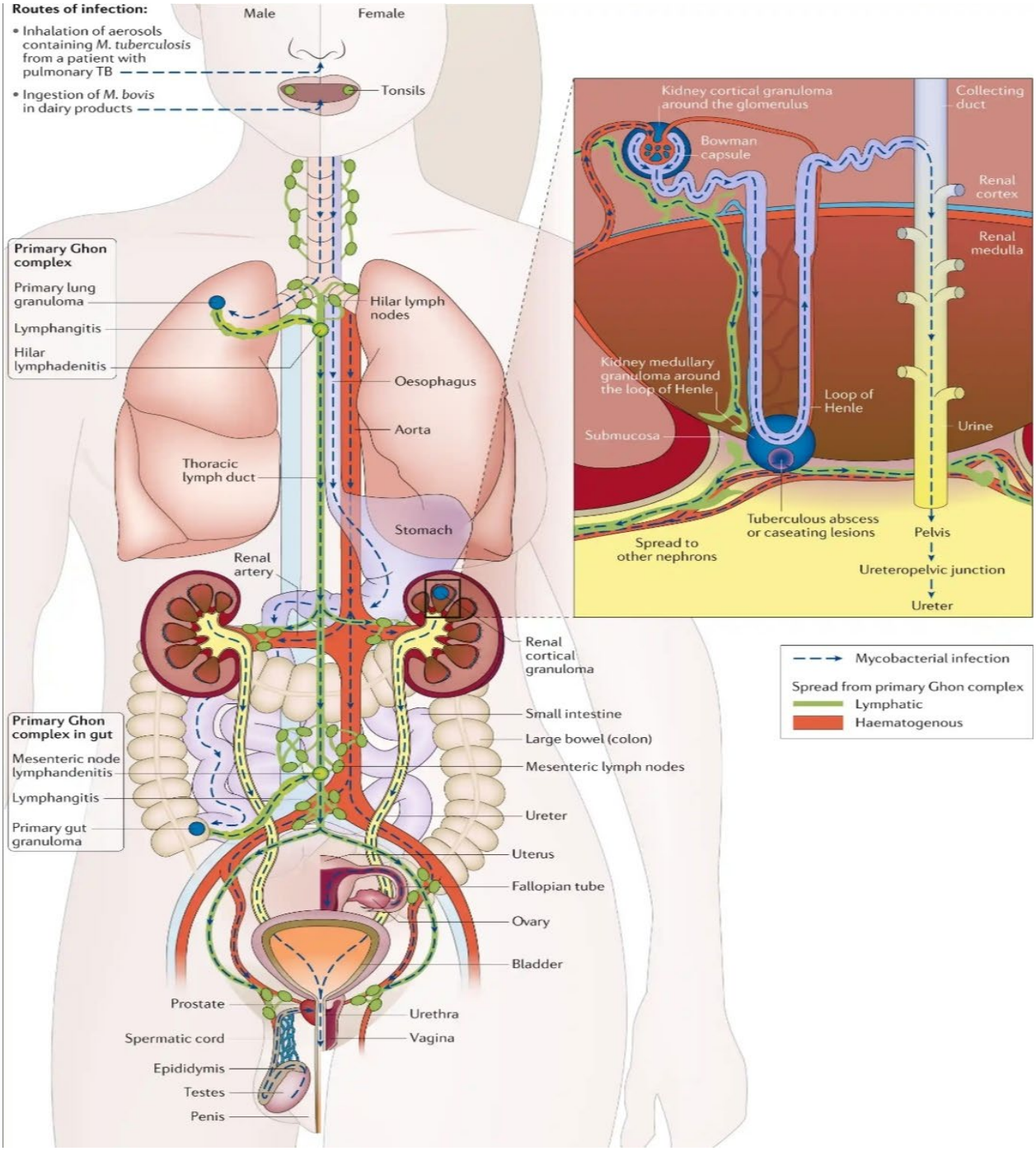

Figure 2.5: The hematogenous and lymphatic spread of *Mycobacterium tuberculosis* infection in various parts and organs of the body, particularly in the kidney, as illustrated in the cross-section

Source: Muneer *et al*. (2019)

Mycobacterium tuberculosis reaches the kidneys through the hematogenous spread, as illustrated in Figure 2.5. These form a granuloma, which can either heal and develop into fibrous tissue, remain dormant for years, or break down and

spread into the tubular lumen. The tubercle bacilli are then excreted via urine into the urinary tract. Further bacterial dissemination can reach the ureter and bladder and form a stricture and fibrous tissue, which consequentially leads to the obstruction of the urinary tract and hydronephrosis (Amaya- Tapia and Aguirre-Avalos, 2018; Jha and Budh, 2021).

Pyelonephritis frequently presents in patients with GTB (Verma *et al*., 2016). An enlarged scrotal mass may be observed in cases where TB has spread and affected men's bladder, prostate gland, or epididymis (Herchline, 2018). In women, the tubercle bacilli can affect the fallopian tubes, which leads to salpingo-oophoritis. These women may present with severe pelvis pain, infertility, or ectopic pregnancy (Tierney and Nardell, 2018).

The diagnosis of GTB is commonly made by LJ culture and smear microscopy. The latter has significant disadvantages of low sensitivity (due to low bacterial load in clinical specimens such as urine) and false positives. False-positive results can be due to urine contaminated by the generally non-pathological *M. smegmatis*, a species similar to *M. tuberculosis*. Despite the better sensitivity of LJ culture, definite diagnostic results may take approximately eight weeks (Amaya- Tapia and Aguirre-Avalos, 2018; Muneer *et al*., 2019).

Nevertheless, like any EPTB, a high index of clinical suspicion and multiple tests are required to diagnose GTB. Deoxyribonucleic acid amplification tests also play a vital role in GTB diagnosis, for which improved sensitivity and specificity are reported, with the added advantage of reduced TAT to obtain results (Amaya-Tapia and Aguirre-Avalos, 2018). Moreover, genital ulcer biopsies and bladder tubercles from infected areas may be examined by histopathological examination to aid in the diagnosis. Epithelioid granuloma and caseous necrosis will be identified in the presence of GTB disease (Amaya- Tapia and Aguirre-Avalos, 2018; Jha and Budh, 2021).

2.5.1.5 Cutaneous Tuberculosis (scrofuloderma)

Tuberculosis that affects skin and soft tissues is known as cutaneous TB. Most cutaneous TB cases are reported to be caused by *M. tuberculosis* and rarely due to *M. bovis* or the TB vaccine Bacillus Calmette-Guérin (BCG). Cutaneous TB

represents around 1% to 2% of all cases of EPTB (Charifa *et al*., 2022). This type occurs secondarily from TB infection that has intensified from other body regions such as bones or joints, which ultimately affects the cutaneous part covering that area, which may develop sinus tracts or even ulcers, as shown in Figure 2.6 (Gunawan *et al*., 2018).

The invasion of *M. tuberculosis* in the skin can be classified as endogenous or exogenous. The former is a result of hematogenous or lymphatic spread from the pulmonary parenchyma, while the latter is a result of direct inoculation of the tubercle bacilli. Subsequently, a cascade of immune reactions occurs where a tuberculous granuloma forms. Skin conditions such as tuberculous chancres and TB verrucosa cutis are examples of exogenous cutaneous TB. In contrast, lupus vulgaris, orificial TB, and tuberculous gumma fall under endogenous cutaneous TB (Santos, 2013; Ramírez-Lapausa *et al*., 2015; Charifa *et al*., 2022).

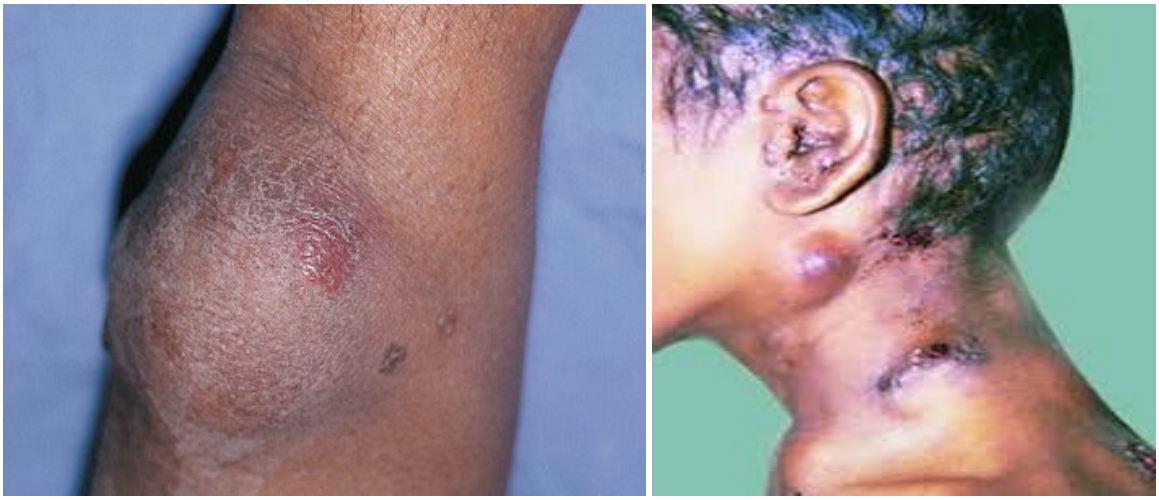

Figure 2.6: Tuberculosis affecting the knees and joints and nodules on the surface of the skin, forming lesions

Source: Tierney and Nardell (2018)

The presence of the bacillus, usually *M. tuberculosis*, confirms the diagnosis of TB, which can, unfortunately, be problematic in cases of cutaneous TB because traditional diagnostic procedures have lower sensitivity and specificity for skin manifestations compared to the pulmonary form (Costa and Veasey, 2021). If there are adequate clinical suspicions, microscopic observation of AFB in tissue or secretions allows for empiric treatment. Other microorganisms such as *Nocardia*, *Corynebacterium*, non-tuberculous mycobacteria, and even artefacts can show

acid-fast properties; this, therefore, does not indicate cutaneous TB (Khadka *et al*., 2018).

In this kind of EPTB, culture is still the gold standard approach to detect *M. tuberculosis*. The protracted growth period of the tubercle bacilli and the poor sensitivity of culture on skin lesions and tissue samples add to the difficulty of diagnosing cutaneous TB quickly and accurately. Identifying the *Mycobacterium* genus with PCR tests employing bacterial 16S ribosomal DNA is a watershed moment in diagnosing PTB and some forms of cutaneous TB. Deoxyribonucleic acid from fresh tissues, blood, or even formalin-fixed paraffin-embedded sections is multiplied and identified, suggesting the presence of TB (Costa and Veasey, 2021). Tuberculin skin testing has a specificity of 63% and a sensitivity ranging from 33% to 96% for cutaneous TB, based on BCG vaccination status. In people suffering from cutaneous TB, interferon-gamma release assays have a sensitivity of 92% and a specificity of 76% (Franco-Paredes *et al*., 2018). Costa and Veasey (2021) reported a case of scrofuloderma in which the GeneXpert was used to examine the discharge of a cervical lesion and provided a rapid diagnosis and early treatment.

2.5.1.6 Gastrointestinal tuberculosis

Gastrointestinal tuberculosis affects different organs that make up the gastrointestinal tract. The ileocecal is most frequently affected by tuberculous enteritis. Various mechanisms in which one can be infected by tuberculous enteritis include consumption of meat and milk products with *M. bovis*, dissemination of the active disease from the primary focus (lungs) or other infected regions of the body, and if people swallow their phlegm that is already infected (Ramírez-Lapausa *et al*., 2015).

Gastrointestinal TB is notoriously difficult to diagnose as it is a paucibacillary disease, with only a tiny percentage of cases able to be proven microbiologically. This increases the risk of under- and overdiagnosis, as well as uncertainty regarding the appropriate treatment approaches and under-reporting of cases. These diagnostic challenges are exacerbated by the existence of key differential diagnoses that might resemble the various clinical presentations of gastrointestinal

TB when there are infectious (for example, *Burkholderia pseudomallei*) and non-infectious aetiology (such as inflammatory bowel disease and lymphoma), among others (Lowbridge *et al*., 2020).

Diagnostic tests include computerised tomography scans, acid-fast staining, histology, and culture. Acid-fast staining, cultures and nucleic acid amplification tests have been shown to have low sensitivity. Radiological findings are also not specific to the disease (Jin *et al*., 2017). The QuantiFERON test (the conventional interferon-gamma release assay technique) may yield false-negative results in EPTB patients (Kim *et al*., 2018). In detecting gastrointestinal TB, the commercially available interferon-gamma release assay is said to have higher sensitivity and specificity than the standard tuberculin skin test. However, the former cannot differentiate between current and latent infections (Chakinala and Khatri, 2021).

2.5.1.7 Tuberculosis peritonitis

This form of TB is often a consequence of the reactivation of TB in the peritoneum, which is spread via blood from abdominal organs such as the genitals, urinary tract, and intestines. This TB is normally seen in patients with weak immune systems, end-stage chronic renal failure, and heavy drinkers with cirrhosis (Srivastava *et al*., 2014). The visceral and parietal peritoneum develop an increasing number of tubercles as the disease worsens. The "exudation" of protein-rich fluid from the tubercles leads to the development of ascites. Over 90% of individuals suffering from tuberculous peritonitis present with ascites, while the remaining 10% show a more progressed "dry" phase, a fibroadhesive type of illness (Ramírez-Lapausa *et al*., 2015).

People who suffer from TB peritonitis may have mild symptoms with pain in the abdomen, tenderness and fatigue (Davis, 2018). In order to diagnose TB peritonitis, microbiological testing and paracentesis (which involves the removal of peritoneal fluid to determine the level of adenosine deaminase which is a highly sensitive and specific diagnostic marker for the disease) are essential. The yield from AFB microscopy using ascitic fluids is dismally poor; it has a reported sensitivity of 0% to 6%. But, a high sensitivity of 80% was obtained when using

culture as the diagnostic method of TB in peritoneal fluids (Ramírez-Lapausa *et al*., 2015).

2.5.1.8 Tuberculosis pericarditis

This is the inflammation of the pericardium due to *M. tuberculosis*. The tubercle bacilli from pleural fluids, hematogenous dissemination from the primary focus, and mediastinal lymph nodes are believed to cause infections of the pericardium (Mayosi *et al*., 2005). Several immunological events trigger the inflammatory process, such as the formation of fibrinous with a predominance of leukocytes, followed by the development of a granuloma due to a vast number of tubercle bacilli. This will lead to serosanguineous pericardial with abundant lymphocytic exudate, absorption of the effusion along with caseous granuloma, and thickening of the pericardium, which subsequently contribute to fibrin deposits, collagenosis, and eventually fibrosis, whereafter scarring occurs and the pericardium constricts. Calcification of the pericardium and cardiac encasement hampers the diastolic filling (Mayosi *et al*., 2005; José Miguel, 2019; Isiguzo *et al*., 2020).

Some of these abnormalities are shown in Figure 2.7. Clinical features include pericardial rub, fever, and pleurisy (Walter and Darley, 2012; Jones-Lopez and Ellner, 2011; Thwaites, 2014). Other complications may be present, such as shortness of breath, low blood pressure, and quiet heart sounds due to cardiac tamponade (Tierney and Nardell, 2018). Heart failure is usually a result of TB pericarditis (Little and Oh, 2012). Despite the greatest efforts, 15% to 20% of cases with pericardial illness go undiagnosed, which indicates a general lack of reliable, cost-effective, novel diagnostic assays that can improve quick clinical judgement (Isiguzo *et al*., 2020). Despite the fact that it is typically not a rule in tests, adenosine deaminase remains the most extensively used biochemical test. The most often used diagnostic test for TB pericarditis is pericardial fluid culture, which has a sensitivity of 53% to 75%, but it takes at least three weeks for results to be available, and yields from microscopy remain poor (Isiguzo *et al*., 2020).

A biopsy of the pericardium or epicardium may be considered if there is diagnostic uncertainty despite biochemical adenosine deaminase and fluid culture, or if pericardial fluid is difficult to collect. When pericardial fluid and biopsy specimens

are evaluated early in the effusive stage, the chances of achieving a definitive bacteriological result are the highest (Jung, 2016; José Miguel, 2019; Isiguzo *et al*., 2020). The DNA of the tubercle bacilli in pericardial fluid can also be identified using PCR such as the GeneXpert, and better sensitivity was obtained in several studies (Yu *et al*., 2017; Saeed *et al*., 2017; Ullah *et al*., 2017; Khan *et al*., 2018, Song *et al*., 2018; Andrianto *et al*., 2020; Yu *et al*., 2021).

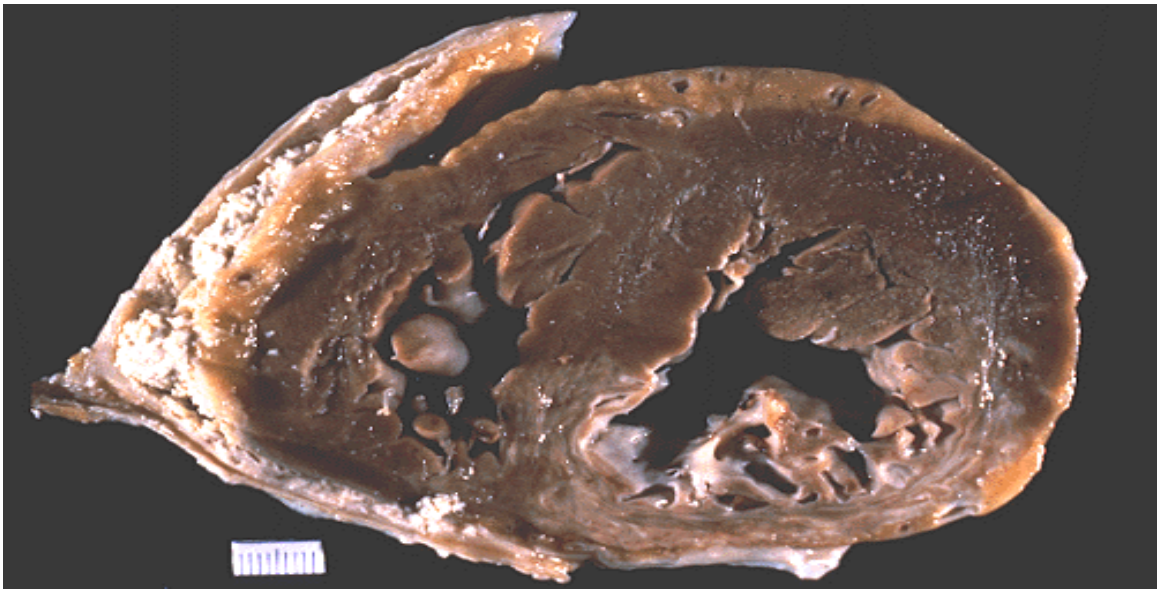

Figure 2.7: Cross-section of an abnormal pericardial cavity of a patient with tuberculosis pericarditis

Source: Veinnot (n.d.)

Due to clinical manifestation variations, EPTB remains a challenge to diagnose for doctors and microbiologists worldwide. Specimens obtained from probable infection sites ultimately need to be cultured to make a definite diagnosis of EPTB. Due to the difficulties of collecting particular samples in some sites, paucibacillary samples lower the sensitivity of traditional diagnostic methods. Present laboratory diagnostic approaches are primarily culture-based (liquid and/or solid culture media), which take many weeks to produce observable bacteriological growth. Traditional EPTB diagnostic techniques are ineffective, require a long time to diagnose, and frequently need invasive and arduous procedures and specialised knowledge. Extrapulmonary tuberculosis's prevalence is likely underestimated as a result of these diagnostic problems. The introduction of molecular diagnostics appears to have made a significant difference.

The Xpert *Mycobacterium tuberculosis*/rifampicin (MTB/RIF) assay (hereafter referred to as Xpert MTB/RIF) is a fast and automated molecular test with high sensitivity and specificity for PTB identification. Although regulatory bodies have validated Xpert MTB/RIF for testing TB in sputum samples, it is deemed “off-label” in some non-respiratory samples. The Xpert MTB/RIF has, however, been studied in multiple trials due to the limitations of traditional tests for EPTB diagnosis. The overall diagnostic accuracy using the Xpert MTB/RIF assay for EPTB in both young and elderly populations has been reported by several systematic reviews (Tortoli, 2012; Solomons, 2015; Kim *et al*., 2015; Marouane *et al*., 2016; Bholla *et al*., 2016; Culqui-Lévano *et al*., 2017; Metaferia *et al*., 2018; Das *et al*., 2019; Tadesse *et al*., 2019; Uppe, 2020; Mokaddas *et al*., 2020; Habous *et al*., 2019; Seo, 2020; Binjomah *et al*., 2021; Kohli *et al*., 2021).

2.5.2 *The GeneXpert Systems and Xpert MTB/RIF Assay*

Culture remains the gold standard method in diagnosing TB despite its longer TAT compared to other methods, such as microscopy and newly found molecular tests that use nucleic amplification. The Xpert MTB/RIF assay is one of the latest diagnostic tools that use nuclei amplification to detect the organism *M. tuberculosis* in clinical specimens. It utilises the multiplication of nucleic acids to detect the DNA in the TB bacilli and simultaneously determine the resistance of such bacilli to Rifampicin (WHO, 2011; Cepheid, 2012; Sedky *et al*., 2018). Various versions of this GeneXpert system are used worldwide, and the NTRL utilises GeneXpert IV and GeneXpert XVI to perform the Xpert assays, as shown in Figures 2.8 and 2.9.

Figure 2.8: GeneXpert XVI, which is a version that has 16 modules and a desktop computer

Source: Primary investigator

Figure 2.9: Two GeneXpert IV systems at the National Tuberculosis Reference Laboratory

Source: Primary investigator

The assay utilises both real-time and reverse transcriptase PCR during sample extraction, processing of the sample, amplifying the *rpo*B gene of *M. tuberculosis*, and, lastly, during identifying the target sequences. The cartridges are a closed system, used only once and are disposable, thus inhibiting cross-contamination of samples during processing. Not only is the Xpert MTB/RIF assay fast (releasing results in less than two hours), but it is also easy to use as it is automated, thus limiting the need for highly skilled personnel to run it (Cepheid, 2012; WHO, 2014).

2.5.2.1 The principle of the Xpert MTB/RIF Assay

The GeneXpert instrument frameworks incorporate automated sample processing, nucleic acid amplification, and detection of target sequences in simple (sputum) or complex specimens (in concentrated sputum residue) for the detection of MTBC and rifampicin resistance. This occurs in a qualitative manner by utilising PCR and melt peak detection (Cepheid, 2020).

The system also comprises an instrument, bar code scanner, computer, and preloaded programming to run specimens and to review the outcomes of the tests. The assay requires the utilisation of single-use Xpert MTB/RIF cartridges containing the reagents and processes of PCR. Since the cartridges are independent, cross-contamination between samples is limited. The cartridge is also composed of the sample processing control, along with a probe control check. The sample processing control is available for satisfactory processing of the objective microorganisms, to monitor if there are inhibitors in the PCR tests, and for subsequent melt peak detection. Moreover, the reagent rehydration, PCR tube filling in the interior of the cartridge, the integrity of the probes, and stability of the dye are confirmed by the probe control check (Cepheid, 2019; 2020).

The primers in Xpert Ultra assay multiply a part of the *rpo*B gene, which contains the 81-base pair “core” region and areas of the multi-copy IS1081 and IS6110 insertion elements target sequences. The melt analysis with four *rpo*B probes is intended to discern the conserved wild-type sequence from mutations linked to rifampicin resistance in the core region. The two additional insertion element probes improve the MTBC detection of the specimen due to the multi-copy insertion

element target sequences in most TB strains. Results are displayed after approximately six minutes for a negative specimen and after 77 minutes for a positive specimen (Cepheid, 2019; 2020).

2.5.2.2 Overview of the performance of the Xpert MTB/RIF Assay

The WHO advocated the Xpert MTB/RIF assay as a first-line diagnostic method for people with clinical suspicions of TB that are linked with HIV or MDR cases. The assay could be of great use in countries with a high TB/HIV burden as it can diagnose TB even in HIV-positive patients without decreasing its specificity compared to AFB microscopy (WHO, 2014). The limit of detection of Xpert MTB/RIF is much superior than that of microscopy but comparable to that of culture (Pang *et al*., 2017).

In the past, there was a lack of evidence regarding the instrument's specificity and sensitivity to diagnose TB using other samples such as CSF; hence the WHO advised the use of other standardised methods such as culture, microscopy, and DST for extrapulmonary samples. However, as the years passed, it was recommended that the GeneXpert platform and Xpert MTB/RIF assays could also be beneficial for diagnosing EPTB from samples such as CSF for TBM and lymph nodes and other tissues (WHO, 2014; Cepheid, 2014). Due to its high sensitivity, the new Xpert Ultra cartridges are better able to detect EPTB (Wu *et al*., 2019; Sekyere *et al*., 2019). Moreover, the NTRL is currently using these advanced Xpert Ultra cartridges for PTB samples only.

A study by Li *et al*. (2017) reported that the Xpert MTB/RIF assay had 70.6% sensitivity and 91.9% specificity on TB detection in EPTB samples (pleural fluid, CSF, urine, ascitic fluid, and joint cavity fluid). These findings were lower than those Vadwai (2011) reported, which showed 80.6% sensitivity and 99.6% specificity of this assay on EPTB samples (biopsies, CSF, body fluids, and pus). Similarly, in a study conducted by Tortoli *et al*. (2012), the Xpert MTB/RIF assay showed better results in diagnosing EPTB using samples such as urine, pus, and biopsies compared to other methods, namely culture and microscopy.

Thus, with its high specificity and sensitivity in diagnosing PTB, the GeneXpert also has promising results for diagnosing EPTB. It is, therefore, essential that the

GeneXpert in the NTRL be validated, as previous reports show that the GeneXpert can indeed have a vital impact on the rapid diagnosis of EPTB globally. Although the specificity and sensitivity of many reports may differ, the use of new GeneXpert Ultra cartridges will save many EPTB patients from delayed diagnosis.

2.6 CULTURE

This method of diagnosing TB in clinical samples is still declared the gold standard by the WHO. Culture results provide a conclusive diagnosis as organisms are identified to the MTBC level and tested for their drug sensitivity (Ministry of Health, Lesotho, 2015). Furthermore, this method can be used as a monitoring tool for patients already taking treatment. Culture is reported to have a higher sensitivity for PTB samples as a sputum sample containing an estimation of approximately 10 live bacilli/ml is required compared to microscopy, which requires a sputum sample with ~5 000 bacilli/ml (Achimi, 2019).

For cultured samples to be declared negative on liquid and solid media, they must be subjected to an incubation period of six to eight weeks. Factors that influence time to detection of mycobacterial growth include the growth rate and concentration of the bacilli in the clinical samples (Ministry of Health, Lesotho, 2015). Since *M. tuberculosis* is a slow-growing microorganism (replicates after 15 to 20 hours), it needs a special culture media for optimal growth. On that account, *M. tuberculosis* can be routinely grown on a solid or liquid medium. Solid media can either be egg-based or agar-based (Idh, 2012; Achimi, 2019).

2.6.1 Egg-based culture media

Egg-based culture media is commonly used due to its long shelf life, low cost, ease to prepare (it can be prepared in-house) with minimum contamination, stability of chemicals used, and easy visualisation of the colonial morphology. This medium has an advantage when it comes to the differentiation of biochemical characteristics, and it supports the growth of *M. tuberculosis*. However, cultures take as long as eight weeks to become positive based on the rate of growth and quantity of AFB in the specimen (NTRL, 2015).

The WHO recommends it for both culture and drug susceptibility testing. Lowenstein-Jensen (LJ) is a selective medium that contains malachite green, which acts as an inhibitor of contaminants such as bacteria and fungi. Mould is the most observed contaminant in solid media, and such cultures are discarded. Various ingredients are added to the medium to support the growth of different MTBCs; for example, the addition of glycerol to the LJ medium supports the robust growth of *M. tuberculosis*, while the growth of *M. bovis* is promoted by the addition of pyruvate to the LJ medium. When stored in a refrigerator, the LJ media can be used for six to 12 months, and this type of medium enhances the growth of the majority of MTBC (Achimi, 2019).

2.6.2 Agar-based culture media

Middlebrook media can serve as a selective medium with the addition of antimicrobial agents to prevent the growth of undesired bacteria, thus ensuring selectivity. The colonies formed on the media are visualised and quantified better as the media's appearance is clear. In comparison to the egg-based media, the growth of mycobacterial colonies is detected faster in this type of medium. These media can be prepared as plated agar or slant (tubed) (Achimi, 2019) and are named Middlebrook 7H9, 7H10, and 7H11, depending on the agar used.

The 7H9 is a culture medium developed in the late 1940s by Dubos and Middlebrook. This medium was composed of oleic acid albumin, which plays a vital role in the growth of the tubercle bacilli and its protection against toxic agents. Around the late 1950s, the innovators of 7H9 modified the media to a non-selective 7H10 media enriched with oleic albumin dextrose catalase (OADC), which provides a better environment for the growth of mycobacteria. The constituents of 7H10 include different organic salts, co-factors, biotin, malachite green, catalase, sodium citrate, albumin, vitamins, oleic acid, and glycerol. The inorganic cations in the medium are held together by the chemical reaction formed by converting sodium citrate to citric acid. Vital growth elements are obtained from inorganic salts, found in 7H10 media. Glycerol serves as the *Mycobacterium* species' key source of both carbon and energy. Malachite green is a selective agent that allows the growth of mycobacteria and prevents that of other microorganisms. The recovery of

damaged bacilli is facilitated by biotin and catalase (HiMedia Laboratory, 2012; Hardy Diagnostics, 2020).

The OADC enrichment is made up of oleic acid, which plays a role in the metabolic activities of the bacilli, albumin, and sodium chloride, of which their importance is the same as mentioned in 7H9 Middlebrook. Dextrose provides energy, while catalase kills toxic peroxides. Middlebrook 7H11 is a highly selective version of Middlebrook 7H10, which provides optimum conditions for the rapid growth of mycobacteria through the addition of casein hydrolysate, which promotes the growth of resistant strains of mycobacteria. Other selective additives include carbenicillin and polymyxin B, which inhibit the growth of almost all Enterobacteriaceae; trimethoprim lactate, which hinders the growth of *Proteus* species; and amphotericin, which suppresses yeast's growth in the medium (Hardy Diagnostics, 2020; Aryal, 2022).

2.6.3 Liquid media

In the early 1990s, the CDC advocated that liquid media be used in collaboration with solid media for the isolation of *M. tuberculosis*. The BACTEC Mycobacteria Growth Indicator Tube (MGIT)^{TM} 960 system, as seen in Figure 2.10(i), is a commercially and commonly used instrument that uses liquid media. The MGIT is composed of 4.0 ml of 7H9 broth and 0.25% glycerol contained in a 16x100 mm tube. Embedded at the bottom of the tube is a silicone indicator that fluoresces light due to the growing *M. tuberculosis* organisms that consume oxygen in the tube. Prior to inoculation of sputum in the MGIT, 0.5 ml of OADC mentioned above and supplements of an antimicrobial mixture consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin, which is known as PANTA is added (Heifets *et al*., 2000; Siddiqi and Rusch-Gerdes, 2006).

Liquid media are reported to have a superior performance outcome compared to solid media in terms of mycobacterial recovery rate, time to detection, and drug resistance. Another added advantage of liquid culture is that some MTBC isolates can only survive in liquid media and not on solid media. One chief drawback of liquid media is that they are more likely to be contaminated than solid media; hence, it is crucial to add antimicrobials to liquid media. Liquid media provide better

detection time and recovery rate compared to solid media. The shelf life of this type of medium is also longer. Slow-growing *M. tuberculosis* takes 12 to 16 days to be detected. Liquid media are also known as broth media. (Siddiqi and Rusch-Gerdes, 2006; Ministry of Health, Lesotho, 2015; NTRL, 2018).

2.6.4 Automated systems

Among the Food and Drug Administration (FDA)-approved automated (liquid media) systems utilised for faster detection of mycobacteria globally, the three most commonly used are:

1) Becton Dickinson BACTEC MGIT™ 960 system (see Figure 2.10(i));
2) Biomerieux BacT/ALERT ®3D; and
3) Thermo Scientific VersaTREK™.

The Becton Dickinson BACTEC MGIT™ 960 system and Thermo Scientific VersaTREK™ are also approved for drug susceptibility testing of MTBC.

Figure 2.10(i): Two BACTEC Mycobacteria Growth Indicator Tube™ 960 systems at the National Tuberculosis Reference Laboratory

Source: Primary Investigator

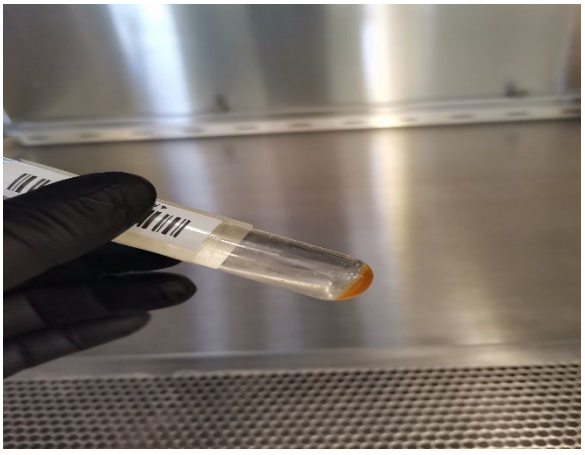

Figure 2.10(ii): A positive Mycobacteria Growth Indicator Tube™ showing white clumps as an indication of growing *Mycobacterium tuberculosis*

Source: Primary Investigator

CHAPTER 3:
METHODOLOGY

3.1 STUDY AREA

This study was conducted at the NTRL in Lesotho. The laboratory is located on the outskirts of the capital city of Maseru (approximately 6 km from Maseru) in a small village called Lithabaneng. The NTRL is a full-service mycobacteriology laboratory that receives primary and referral samples from various health facilities around the entire country for culture, identification, and susceptibility testing of *M. tuberculosis*.

3.2 STUDY POPULATION, SAMPLE SIZE, SAMPLE TYPE, STATISTICAL ANALYSIS, AND ETHICAL CONSIDERATIONS

The sample size comprised 176 extrapulmonary specimens from patients suspected to have EPTB. The specimens were from different testing sites, specifically busy hospitals such as the Berea, Maluti, Mafeteng Regional, Queen Elizabeth II, Motebang, Queen 'Mamohato Memorial, and Seboche hospitals from 2017 to 2019. Each specimen was aliquoted twice (one specimen was used for GeneXpert testing while the other was used for culture testing), which totalled 352 aliquots.

3.2.1 Inclusion criteria

The clinical diagnosis of patients was scrutinised, and suitable patient specimens that met the selection criteria were used in this study (the selection criteria are discussed below). The inclusion criteria were as follows:

- Specimens from patients with suspected EPTB.
- There was no limitation on age or sex.
- The non-respiratory specimens included pleural fluid, ascitic fluid, CSF (only collected at hospital level), pus swab, body fluid, lymph node aspirate, urine, scrotal box, abdominal fluid, synovial fluid, gastric aspirate, throat swab, FNA, auxiliary lymph node FNA, and others (e.g., pericardial fluid, peritoneal fluid, and bone marrow aspirate).

3.2.2 Exclusion criteria

The exclusion criteria were patients not diagnosed/suspected of EPTB and insufficient specimens (samples of less than 3 ml were considered insufficient). Moreover, specimens with particles that could block the GeneXpert probe, such as faeces and blood, were not accepted as per the GeneXpert manufacturer's instructions.

3.2.3 Statistical analysis

Descriptive and inferential statistical analysis (DISA) was conducted on IBM's Statistical Package for the Social Sciences (SPSS) (version 26). No patient's identity was disclosed; instead, laboratory numbers were provided as specimens were logged in the DISA system. All laboratory request forms generated during the study remained strictly confidential by compiling them monthly and archiving them in safe lockable shelves for one year, whereafter they were shredded; however, the electronic DISA system has the ability to safely store logged information indefinitely.

3.2.4 Ethical considerations

Ethical approval was applied for from and granted by the Ministry of Health Lesotho, with clearance ID 174-2019 (see Appendix A).

3.3 PROCEDURE

3.3.1 Sample transportation and storage prior to testing

Specimens were transported immediately to the NTRL whenever possible or were kept at 2 °C to 8 °C or in a cool place (not a freezer) in remote health facilities if the specimens could not reach the NTRL on the same day they were collected. Specimens were transported to the nearest peripheral TB laboratory, which sent the specimens to the NTRL within three days using triple packaging systems for optimal results.

Cerebrospinal fluid specimens were treated as exceptions as they were not refrigerated but transported immediately to the NTRL. Specimens that could dry

out, such as swabs and tissues, were kept moist during transportation by adding 1 ml sterile 0.9% saline. No additives or preservatives were added to the extrapulmonary samples. Delays exceeding seven days and improper storage would interfere with the final culture results, thus causing an increased contamination rate in the specimens, according to the NTRL (2015). Proper sample handling, collection, preservation, and storage lead to high-quality results, which are paramount in laboratory testing and, ultimately, patient care and treatment. In this study, retrospective results found on the NTRL database were also used as part of the analysis and the experimental part was conducted using the methods discussed in the following section.

3.3.2 Sample processing

The specimens were routinely tested using the culture method as the gold standard, with GeneXpert (using both Xpert MTB/RIF and Xpert Ultra cartridges) as the method to be validated. The first aliquots of the specimens were processed using the Xpert Ultra as follows:

The procedure for running specimens was per the manufacturer's instructions for testing sputum samples, which was used as the reference method. Briefly, sample reagent was added to each specimen at a ratio of 2:1 in a biosafety cabinet. The mixture was swirled vigorously for 15 seconds and incubated for 15 minutes and later shaken again, with another incubation of 10 minutes thereafter. Once the mixture was liquefied, a 2 ml specimen was dispensed slowly into a labelled Xpert Ultra cartridge via a port using a sterile pipette and inserted into the GeneXpert instrument for testing; the results were displayed on the monitor in less than two hours. The remaining specimen and buffer mixtures in the specimen container were kept in the refrigerator at 2 °C to 8 °C to preserve them should a repeat test be needed. Once the results were displayed and were satisfactory, the cartridges were discarded in a biohazard bag, which was fastened and placed in the biohazard waste bin. When the biohazard bag was three-quarters full, it was tightly sealed and taken out of the biohazard waste bin for disposal by means of incineration.

3.3.3 Quality assurance

Two known controls (positive and negative sputum samples) were run like ordinary sputum, following the manufacturer's instructions on a weekly basis as internal quality control. Manufacturer's kit controls (lot-to-lot verification of reagents) were performed with every newly shipped order (to check if the travelling/shipping conditions such as temperature were maintained, and ultimately the integrity of the controls for quality results) or every new lot change in order to ensure the quality and consistency of lots prior to their use. Records were documented in the quality control logbook.

3.3.4 Culture

The LJ medium was prepared as proposed in the Mycobacteriology Laboratory Manual (Stinson *et al*., 2014).

The second aliquot of each specimen was used for culture purposes as described in the above-mentioned manual. Briefly, a 10 µl inoculating loop was used for tapping and striking the sediment on two slopes of LJ media, and two to three drops were inoculated on each medium. The inoculated culture media were put in a slanted position in a rag placed in an incubator set at 35 °C to 37 °C aerobically. The media tube was loosely closed for a week to allow the inoculum to spread and absorb evenly. After a week, the culture tubes were screw-capped tightly in order to reduce the media's evaporation and drying. Tubes were then put in an upright position if there was limited space in the incubator. Growth was monitored weekly (twice in the first week to observe contamination faster and request repeat specimens timely if needed) up to the eighth week, and results were reported and documented in the appropriate register and worksheet.

Results were declared negative after eight weeks of incubation, and negative slants were discarded in the biohazard bin. Each positive LJ slant was identified, the results were reported immediately as preliminary results, and further testing was carried out. Two to three colonies were picked from each positive growth and checked for AFB by smear microscopy. This is where individual slides were labelled with the unique laboratory number assigned to each specimen, stained with the ZN stain, and examined. If the AFB were detected by microscopy, a further

identification test was performed using several colonies scraped from the positive LJ slants. The scraped colonies were added to 200 µl of extraction buffer of the MPT/MPB 64 antigen test kit for the identification of *M. tuberculosis* at the species level as per Stinson *et al*., (2014). The results were declared culture positive after two lines formed in the test kit cartridge. The *M. tuberculosis* isolates are kept at -80 °C in sterile 10% skim milk for many years.

3.3.5 Quality control

Quality control was performed by a trained laboratory technologist on duty or the principal investigator of this study under supervision of experienced laboratory technologists for each run. Negative controls were prepared using distilled water/buffer, while for positive controls, known H37RV strains were used and incubated for two to eight weeks and checked for growth, as shown in Figure 3.1(I and ii).

Figure 3.1(i): Commercially prepared Lowenstein-Jensen medium that is negative

Source: Primary Investigator

Figure 3.1(ii): In-house prepared Lowenstein-Jensen media that are positive

Source: Primary Investigator

CHAPTER 4:
RESULTS

4.1 INTRODUCTION

The purpose of this study was to validate the GeneXpert instrument for TB diagnosis of extrapulmonary samples. Three hundred and fifty-two samples were analysed from 2016 to 2019. From the 352 samples 166 were processed by Xpert Ultra,10 by Xpert MTB/RIF and 176 by culture method. Statistical analysis was conducted on SPSS (version 26), and the results are presented and interpreted systematically. This includes the demographics of the respondents, descriptive statistics (frequency), and inferential statistics (analysis of variance), which are presented below.

4.2 RESULTS

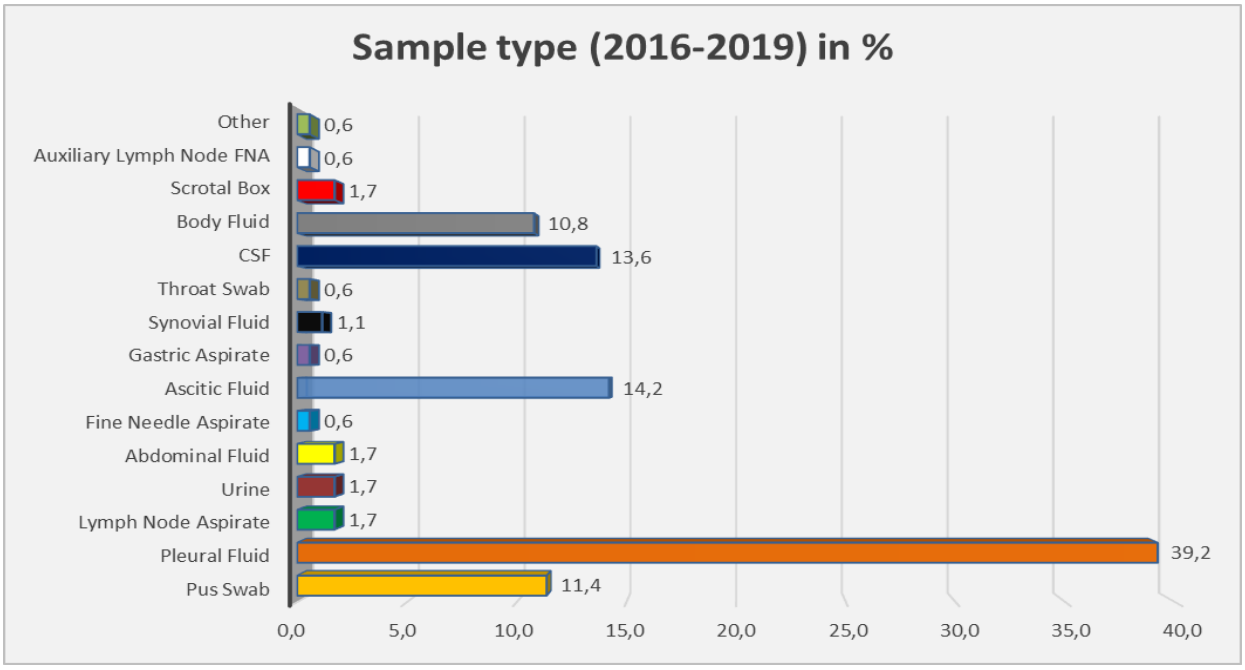

Figure 4.1: Sample types brought to the National Tuberculosis Reference Laboratory for extrapulmonary tuberculosis diagnosis from 2016 to 2019

Figure 4.1 delineates that pleural fluid was the most collected sample type in the different health facilities, which represents 39.2%, followed by ascitic fluid (14.2%), and CSF (13.6%). Extrapulmonary samples that were rarely tested included gastric aspirates, throat swabs, auxiliary lymph node FNA, and synovial fluids, among

others. Table 4.1 illustrates the detection rate of the two methods being compared, namely the GeneXpert and culture. The GeneXpert detected *M. tuberculosis* in more specimens than the culture method.

Table 4.1: Comparison of the detection rate of the GeneXpert and culture

Specimen type	GeneXpert		Culture	
	Number of positive	Positive %	Number of positive	Positive %
Pleural fluid	13/69	19%	4/69	5%
Pus swab	12/20	60%	6/20	30%
FNA	2/2	100%	1/2	50%
Throat swab	1/1	100%	1/1	100%
CSF	3/28	11%	1/28	4%
Ascitic fluid	1/25	4%	0/25	0%
Lymph node aspirate	1/3	33%	0/3	0%
Urine	1/3	33%	0/3	0%
Total	**33/176**	**19%**	**13/176**	**7%**

Figure 4.2 compares cases detected by the GeneXpert and culture in the four years (2016 to 2019).

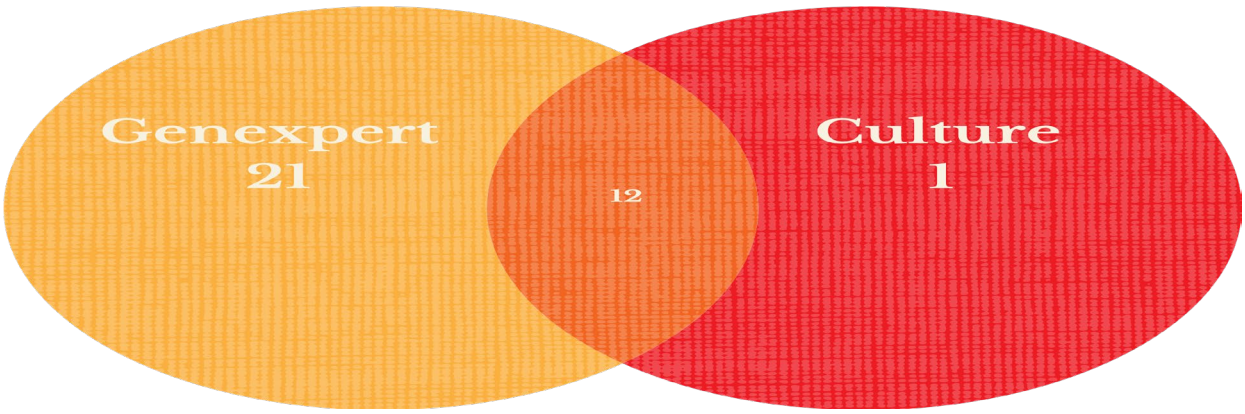

Figure 4.2: Comparison of cases detected by the GeneXpert and culture (2016 to 2019)

From this study, 34 EPTB cases were positively detected between 2016 and 2019. A total of 33 cases were detected by the GeneXpert, while only 13 cases were detected through the culture method. Both methods were able to detect 12 cases, as demonstrated in Table 4.1. The culture method failed to detect 21 cases in comparison to the GeneXpert, which missed only one case. The results further show that the sensitivity and specificity of the GeneXpert are 92.3% and 87.1%, respectively (see Table 4.2).

Table 4.2: GeneXpert diagnosis test vs culture diagnosis test cross-tabulation

GeneXpert test		Culture test		Total
		Positive	Negative	
Positive	Count	12	21	33
	% within culture diagnosis	92.3%	12.9%	18.8%
Negative	Count	1	142	143
	% within culture diagnosis	7.7%	87.1%	81.3%
Total	Count	13	163	176
	% within culture diagnosis	100%	100%	100%

The dominant age group diagnosed with EPTB was young adults aged 21 to 40, while the elderly (>60 years) were few (three). It is also worth mentioning that three patients' ages were not provided, which led to unknown ages (not indicated). Table 4.3 illustrates age distribution among patients with EPTB specimens tested.

Table 4.3: Age distribution among positive cases detected by the GeneXpert

Age of patients diagnosed with EPTB using the GeneXpert	Number of patients
<20	1
21-40	14
41-60	12
>60	3
Unknown (not indicated)	3

NB: <20 included a female aged 17. >60 included a male and female aged 63 and a male aged 69.

CHAPTER 5:
DISCUSSION OF FINDINGS

5.1 DISCUSSION

In this study, the diagnostic yield of the GeneXpert assay for detecting *M. tuberculosis* in non-respiratory specimens (CSF, pleural fluids, gastric aspirates, throat swabs, synovial fluids, ascites, and more) was compared to AFB culture, which was used as the gold standard. The results of this study revealed the broad variety of sample type tested, as indicated in Figure 4.1. The most prevalent specimen type was pleural fluid (39.2%), followed by ascites (14.2%) and CSF (13.6%). The rarely collected specimens included auxiliary lymph node FNA (0.6%), gastric aspirate (0.6%), and throat swabs (0.6%).

In a study conducted in Dhaka by Islam *et al*. (2021), pleura was shown as the most frequent site of collection for specimens for EPTB diagnosis, which represented 21.2%, followed by urine (18.6%) and CSF (15.4%). Similarly, in a study conducted in India by Ravikumar and Bai (2017), their findings also revealed that pleural fluid was the most commonly collected EPTB specimen (29.9%), then from the meninges (22.5%) and the abdomen (19.6%). The probable reason for more pleural fluids than other specimens could be that most people suspected to have TB in high TB burden countries have pleural effusion as an early presentation of primary TB infection (Metaferia, 2018). When the primary disease manifests, the pleural cavity is the nearest site to the lungs.

Table 4.1 shows the results of *M. tuberculosis* detection using culture and the GeneXpert from various non-respiratory specimens. It was observed that the GeneXpert was able to detect MTBC in all the specimen types, as shown in Table 4.1. Of the 69 pleural fluids tested using the GeneXpert, 13 (19%) of them were detected as *M. tuberculosis* positive, while only four (5%) were positive using culture. Low detection for tubercle bacilli in pleural fluids using the GeneXpert was also reported by Metaferia *et al*. (2018) at 11.9%, by Uppe *et al*. (2020) at 35%, by Tortoli *et al*. (2012) at 44.4%, and by Kohli *et al*. (2021) at 49.5%. Huo and Peng (2018) reported similar results that exhibited a poor sensitivity (30%) for *M. tuberculosis* in pleural fluids. Lower detection of MTBC in specimens such as

pleural fluids through the GeneXpert and culture may be due to the paucibacillary character of EPTB disease. Moreover, the ideal specimen for pleural TB diagnosis is a pleural biopsy rather than pleural fluid (Li *et al*., 2020). Low Xpert MTB/RIF sensitivity could also be caused by PCR inhibitors in the pleural fluid or blood that may have contaminated the sample following invasive sample collection.

The literature reported moderate to high sensitivities ranging between 50.9% and 89.3% when Xpert/RIF was used to diagnose TB in pleural fluids (Kohli *et al*., 2018; Khan *et al*., 2018; Tadesse *et al*., 2019; Binjomah *et al*., 2021; Nataraj *et al*., 2016). The degree of specificity in these reported trials varied from 42% to 100%. This could have been due to the author's use of a concentration step in studies that indicated higher sensitivity. In order to improve the sensitivity of a technique, sample concentration is a common step conducted prior to specimen analysis. This is whereby after the specimens are added to the suitable decontaminant, they are span in a refrigerated centrifuge at 4 °C to 10 °C (to protect the *M. tuberculosis* from dying due to the high amount of heat generated during centrifugation). The centrifuged deposit (sediment) is preferable for analysis as it is believed to have a high bacteria yield (Denkinger *et al*., 2014; Bahr *et al*., 2015; Yoshimatsu *et al*., 2015). It is also essential that the sediment is resuspended using 2 ml phosphate buffered saline to reduce the concentration of any toxic components that may inhibit the growth of the tubercle bacilli. Denkinger *et al*. (2014) found that the concentration step enhanced the sensitivity of 49.1% of pleural fluids compared to 41.6% of pleural fluids that did not undergo the concentration step. In addition, fresh samples were reported to exhibit a higher sensitivity (59%) than frozen ones (31.4%), as reported by Denkinger *et al*. (2014).

In the present study, 12 (60%) positives of all pus swabs were detected using the GeneXpert, while six (30%) tested positive using culture. According to Scott *et al*. (2014), Xpert MTB/RIF performed best mostly on fluid samples with a thick appearance, particularly pus, which had the highest sensitivity of 87%, when contrasted to pleural fluid samples, which had a low sensitivity of 47%. The latter could be due to a further dilution of pleural fluids with a sample reagent (SR) buffer, or a treatment with the SR buffer that was excessively harsh compared to the pus specimen with a thick nature, such as sputa. However, the most likely explanation is that pus and other viscous fluids have a constitution more comparable to sputum

samples, rendering the SR buffer, which was intended to liquefy sputum, also a suitable fit.

Past studies (Vadwai *et al*., 2011; Kim *et al*., 2015) also mentioned lower sensitivity of the Xpert MTB/RIF assay in body fluid specimens than in thicker specimens with solid components, such as tissue biopsies or pus. Lower detection of MTBC in culture compared to the GeneXpert in this study could be due to the dead bacilli, which are also detected by the GeneXpert as it has the ability to detect both dead and live bacilli. Another reason could be the paucibacillary nature of specimens.

Tortoli *et al*. (2012), Marouane *et al*. (2016), Khan *et al*. (2018), and Binjomah *et al*. (2021) all reported good sensitivity and specificity of Xpert MTB/RIF in pus samples, which ranged between 80% and 100%. Mechal *et al*. (2019) found a moderate sensitivity of 64.3% but a high specificity of 96.9% in their study. The mere fact that almost all pus specimens are collected from wounds on the easily accessible epidermis' superficial surface, as well as the fact that pus samples are frequently of sufficient volume, and the GeneXpert's performance is centred on the number of mycobacteria in clinical samples, could explain the high and moderate sensitivity of pus.

In this study, both FNAs tested positive by the GeneXpert, while culture was able to detect MTBC in only one of the specimens. The sample type of these two FNAs was not indicated. For those shown (i.e., source mentioned) the lymph node aspirates, which were positive cases using the GeneXpert constituted 33%, and zero cases were detected using the culture method. This non-detection of *M. tuberculosis* by the culture method could be due to the low bacterial load in the specimens. Also, the rigorous decontamination procedure used in the culture method could have killed the tubercle bacilli or the form of the caseous lesion in the lymph node tissue aspirated could have harboured non-viable tubercle bacilli, which the GeneXpert was able to detect. Fine needle aspiration and biopsy are two major invasive procedures for diagnosing lymph node TB, and it is worth noting that the sensitivity of specimens collected using different methods varies.

Lymph node biopsies are by far the most invasive when taken, but they are said to have the highest sensitivity compared to FNA specimens (Yu *et al*., 2019; Shen *et al*., 2022). According to Uppe *et al*. (2020), the detection of MTBC in FNAs

exclusively when using the GeneXpert was 57.14% but was found to be lower in lymph node biopsies, where the sensitivity was found to be 46.87%. Polymerase chain reaction inhibitors are believed to be responsible for the lowered sensitivity of Xpert MTB/RIF in body fluids and tissue (Pai *et al*., 2004; Theron *et al*., 2014). For example, blood is one of the most prevalent PCR inhibitors due to the presence of haemoglobin in erythrocytes, lactoferrin in leucocytes, and immunoglobulin G (a protein in plasma) (Sidstedt *et al*., 2018; Cai *et al*., 2018). Salts, proteins, and cellular debris (mostly found in grinded tissues) have all been identified as PCR inhibitors in fluids found after sample treatment, such as centrifugation without subsequent resuspension utilising a buffer solution (Binjomah *et al*., 2021).

These particles additionally obstruct the PCR by interfering with the amplification enzyme (Theron *et al*., 2014). As a result, any method that eliminates blood from these specimens before processing will enhance the sensitivity of the test. MTBC was detected in the specimens by both methods (GeneXpert and culture) for throat swabs. This could be due to enough bacilli to be detected in these specimens by both methods and this shows that both methods were highly sensitive (100%) for the throat swab samples. However, the results could be overrepresented due to the small sample size, i.e., one sample.

Of the 28 CSF samples tested using the GeneXpert, three (11%) were positive, while only one (4%) CSF specimen tested positive by culture. Culture has been observed to have limited sensitivity for various EPTB samples, including CSF and pleural fluid, given the low bacillary load in such non-respiratory specimens (Hoel *et al*., 2020; Mokaddas *et al*., 2021). Seo *et al*. (2020) and Uppe *et al*. (2020) also found GeneXpert sensitivities at 41.7% and 30%, respectively, when using CSF specimens for detection of TB meningitis. This could have been attributed to specimens with lower volumes (due to complex and invasive sample collection), which could have impacted the number of bacteria, which is crucial for the GeneXpert assay's sensitivity. Another reason could be that CSF specimens with xanthochromic appearance, owing to subarachnoid haemorrhage or jaundice, can lead to false-negative results because of the bilirubin in the specimens as bilirubin is a known PCR inhibitor (Chin *et al*., 2019).

Bahr *et al*. (2015) and Denkinger *et al*. (2014) indicated that centrifugation and the use of greater volumes of CSF increase the yield of the tubercle bacilli that lead to TBM, which show a positive yield of 26% for CSF specimen volumes of 6 ml or more, compared to a 7% positive yield for lower volumes. Furthermore, Nhu *et al*. (2014) and Bahr *et al*. (2015) demonstrated that Xpert MTB/RIF assays could significantly improve TBM diagnosis confirmation, particularly when the volume of CSF analysed by Ultra is increased instead of being split among numerous tests. Kohli *et al*. (2018) reported a pooled sensitivity of 71.1% and specificity of 98% for CSF samples when Xpert MTB/RIF was utilised as the method to be evaluated and culture as the reference method in a meta-analysis.

However, there was one study in a Cochrane review by Kohli *et al*. (2018) comprising 129 study participants that employed Xpert Ultra in the diagnosis of TB meningitis using CSF specimens, and achieved both a high sensitivity of 90% and a specificity of 90%. Kohli *et al*. (2021) found that Xpert MTB/RIF seemed to have a lower sensitivity of 71.1% and a high specificity of 96.9% for CSF samples. In contrast, Xpert Ultra exhibited a higher sensitivity of 89.4% and a relatively reduced specificity of 91.2%. These two studies demonstrated that the Xpert Ultra assay has enhanced sensitivity in paucibacillary samples like CSF when evaluated against the phased-out Xpert MTB/RIF assay, despite the newer version's (Ultra) limitation of reduced specificity (Kohli *et al*., 2018; Kohli *et al*., 2021).

According to the literature, the enhanced Xpert Ultra's performance in terms of sensitivity for sputum samples is due to the advanced chemistry, as well as cartridge design. It contains two distinct multi-copy amplifying targets, IS6110 and IS1081, and a bigger PCR reaction chamber capacity (50 µl) compared to the Xpert MTB/RIF (25 µl). Additionally, Ultra features entirely nested nucleic acid amplification, faster thermal cycling, and enhanced fluidics and enzymes. The limit of detection (LOD) was thus improved tenfold as a result of these modifications (LOD of 15.6 cfu/ml per sputum for the Xpert Ultra compared to 112.6 cfu/ml for the phased-out Xpert MTB/RIF) (Chakravorty *et al*., 2017; Hoel *et al*., 2020; López-Roa *et al*., 2021). The Xpert Ultra cartridge has a lower specificity than the Xpert MTB/RIF cartridge, likely owing to previous TB infection, with non-viable bacilli leading to positive Xpert Ultra findings in some individuals who do not present with active TB disease (Caws *et al*., 2018; Kohli *et al*. (2021).

Several studies also found high sensitivities of 100% (Mokaddas *et al*., 2021), 97.2% (Nataraj *et al*., 2016), 85.7% (Tortoli *et al*., 2012), 83% (Khan *et al*., 2018), 82% (Binjomah *et al*., 2021), 75% (Tadesse *et al*., 2019), and 75% (Kim *et al*., 2015) in CSF specimens for identification of MTBC. Habous *et al*. (2019) found an average sensitivity of 66.67% for CSF samples. The GeneXpert technology is an obvious choice for improving TB meningitis diagnosis when there are few bacilli present in the CSF, as the culture method takes several weeks and performs poorly in such a sample type. Furthermore, HIV-positive patients with TB meningitis had higher CSF bacillary loads than HIV-negative patients suffering from TB meningitis (in PTB, nevertheless, the aforementioned is reversed in HIV-positive patients with a lower bacillary load in their sputum).

This study detected MTBC in ascitic fluids (4%) when using the index test. When employing the GeneXpert to test for TB found in ascitic fluids, a similar low MTBC detection of 27.8% (Alvarez-Uria *et al*., 2012), 28.57% (Ahmad *et al*., 2018), and 30% (Uppe *et al*., 2020) was reported. Moreover, Rufai *et al*. (2017) examined the efficacy of the GeneXpert approach for the detection of TB utilising ascitic specimens, using MGIT-960 as the standard reference. Only 12 (17.9%) out of 67 tested positive when using the GeneXpert, while the other 82.1% showed negative results. In the same study, the GeneXpert assay was found to have inaccurate diagnostic importance in 70.5% of culture-positive samples. This indicates that negative GeneXpert cases from highly proteinous body fluids, such as ascitic fluid, should be investigated further through other phenotypic approaches.

The GeneXpert's limited sensitivity in this study could be due to the paucibacillary characteristic of ascitic fluid and the small sample size. Conversely, Xpert MTB/RIF appears to provide a high positive predictive value, which suggests that if the GeneXpert reveals positive results, therapy can begin without awaiting the results of additional tests. It has the fastest TAT (<2 hours). Other investigations demonstrated excellent sensitivity and specificity of 100% for ascites, as reported by Allahyartorkaman *et al*. (2019) and Khan *et al*. (2018). In contrast, Scott *et al*. (2014) reported modest sensitivity of 59% and high specificity of 97%. Using Xpert Ultra cartridges instead of the formerly utilised Xpert MTB/RIF cartridges could cause the increased sensitivity (which was not stated by some researchers).

Since urinary tuberculosis (UTB) symptoms are much the same as those of several bacterial infections, it is difficult to diagnose UTB. Furthermore, this seems to be a chief reason for diagnosis delays and poor treatment outcomes in patients suffering from UTB. The most important procedures utilised in laboratory settings for the diagnosis of UTB are acid-fast staining and mycobacterial cultures. According to Pang *et al*. (2017), when the GeneXpert, mycobacterial culture, and microscopy were used to compare urine results for the diagnosis of renal TB, the former technology surpassed the two conventional procedures. Positive TB cases detected in urine specimens in this study demonstrated the detection of 33% when using the GeneXpert, but none were detected when using culture.

When employing culture as a standard reference for UTB, the GeneXpert had a high sensitivity (94.6%) and specificity (86.5%), whereas microscopy had a much lower sensitivity (40.5%), yet high specificity (99.2%). It should be emphasised that the GeneXpert's sensitivity is dependent on its detection limit (LOD). According to Pang *et al*. (2017), AFB microscopy has an LOD of around 5 000 bacilli/ml of sample, while the Xpert MTB/RIF has a lower LOD of 131 cfu/ml of specimen. Although mycobacterial culture has a lower LOD compared to the Xpert MTB/RIF, the latter's sensitivity was not comparable to that of mycobacterial culture.

The higher sensitivity of the GeneXpert could be attributed to the identification of residual DNA from non-viable bacteria. The non-viscous quality of urine, also combined with the low LOD, has a massive effect on lowering bacterial fixation on the smear; thus reducing the ability of AFB smear microscopy in the detection of tubercle bacilli in urine samples. Compared to sputum specimens with a high viscosity, urine specimens are far more homogeneous. According to Pang *et al*. (2017), the digestion procedure in culture, which is designed exclusively for sputum samples, the very same treatment approach when employed in urine specimens may have appeared excessively harsh.

Consequently, one possibility for the lower detection of MTBC in cultured urine specimens in this study was that tubercle bacilli in urine samples were overexposed to extreme alkaline conditions, which inactivated a portion of the tubercle bacilli and resulted in a low recovery rate by conventional culture method. Furthermore, identifying UTB is complicated because of its possibly unusual clinical

manifestations and the tendency for UTB to be concealed mostly by urinary tract infections. Further research needs to be conducted to define the best digesting environment for urine and other non-respiratory specimens based on the findings of this study.

Regarding the diagnosis of urogenital TB utilising urine specimens, one comprehensive study revealed the better diagnostic value of the GeneXpert assay in comparison to mycobacterial culture when taken as the gold standard (Kohli *et al*., 2018). The systemic review, however, contained case-control studies and trials with fewer than five specimens, which may have resulted in an overrepresentation of the Xpert MTB/RIF's diagnostic ability (Kohli *et al*., 2018). According to the literature, the sensitivity of the Xpert MTB/RIF when using urine specimens was very high and ranged between 88.9% and 100% (Kim *et al*., 2015; Allahyartorkaman *et al*., 2019; Habous *et al*., 2019; Binjomah *et al*., 2021; Mokaddas *et al*., 2021). The high sensitivity results could be attributed to dead bacilli recognised by the GeneXpert.

It must be taken into consideration that although pleural fluids were mostly collected and had more positive specimens, followed by the pus swabs using the GeneXpert, the value of *M. tuberculosis* detected in the pleural fluids was not statistically significant; that is, the p-value was greater than 0.005, while for pus swabs the value for MTBC detected was statistically significant (p-value was less than 0.005). The statistical accuracy of less frequently collected specimens may have been affected due to the smaller specimen size, which may have overrepresented the proportions; for instance, throat swabs, urine, and FNA.

The prevalence of MTBC in extrapulmonary samples when using the GeneXpert was 19% and 7% when using culture as the diagnostic method, as shown in Table 4.1. The results of this study varied from a study conducted in 2016 by Fanosie *et al*. at the University of Gondar Hospital, northwestern Ethiopia, where the overall prevalence of EPTB cases confirmed using culture method was 29.8%, and the prevalence of EPTB cases confirmed using GeneXpert was 26.2%, which were both higher compared to the findings of this study. Ethiopia is among the high TB burden countries, and the high prevalence of TB could be due to poverty, malnutrition, and co-infection with HIV.

In this study, the culture sensitivity could be affected by dead bacilli that could not be detected using this method (culture) compared to the GeneXpert, which can detect both dead and live bacilli. According to Vadwai *et al*. (2011), another probable reason for the low sensitivity of the culture method could be the decontamination step necessary for doing the culture of non-sterile specimens, which may have led to a drop in bacillary load. The decontamination process was specifically designed for sputum specimens; it could thus have provided harsh conditions to other specimen types and, as a result, a significant decrease in test sensitivity.

These could be false-positive results by the GeneXpert and may have been brought about by the suspected EPTB patients have already started taking anti-TB drugs, hence the negative effects on culture. Another reason could be the delay in the processing of cultured specimens and the low nature of bacterial load in such specimens. Mycobacterium tuberculosis complex was detected as very low, low, and medium in 18 of the 21 samples that were only detected positive by the GeneXpert specifically using Xpert Ultra catridges. Cross-reactivity between the GeneXpert and various bacteria, viruses, fungi, or nontuberculosis strains has not been observed and should not be the cause of false-positive results (Barnard *et al*., 2015). Positive GeneXpert results that appear culture negative should be interpreted in the context of the patient's medical history and, preferably, confirmed by another molecular test and/or follow-up.

Figure 4.1 shows the number of EPTB cases detected by each method and shared cases. Generally, the GeneXpert was able to detect 33 cases (31 using Xpert Ultra cartridges and 2 using Xpert MTB/RIF cartridges), while culture was able to detect 13 cases. The one case only detected by culture was of a pus swab of a 34-year-old male. The probable explanation of this positive culture yet negative GeneXpert result could be that the latter test has the ability to detect *Mycobacterium* species, whereas culture can detect both MTBC and non-tuberculous mycobacteria, which are also known as mycobacteria other than tuberculosis (Le Palud *et al*., 2014; Barnard *et al*., 2015; Norin, 2015). Another reason the GeneXpert did not detect *M. tuberculosis* in the pus specimen could be the low LOD of the GeneXpert using Xpert Ultra cartridges(~16 cfu/ml of sample) and Xpert MTB/RIF cartridges (131 cfu/ml of sample), while culture has a relatively lower LOD of at least 10 cfu/ml of

specimen. This means that the pus sample could be paucibacillary and, due to a lower LOD of culture, led to negative results in the index test.

This study further revealed that the GeneXpert test detected 12 positive *M. tuberculosis* cases, which were also detected through the gold method, namely culture. The reason for this could be that the specimens had a sufficient bacterial load to be detected by both methods, or perhaps the samples were fresher for MTBC detection, especially for the culture method. The overall sensitivity of the diagnostic test is 92.3%, which also represents the true positive rate, which means that the GeneXpert test accurately identified patients with positive *M. tuberculosis* 92.3% of the time.

The results also show that there was one case where the GeneXpert test failed to detect *M. tuberculosis*, which was detected by culture. However, the false result rate for the diagnostic test was 7.7%. This could be due to the presence of nontuberculous mycobacteria (NTM) strains not coded by the GeneXpert assay. The infections caused by NTM sometimes lead to false positive culture results and worse, might be misdiagnosed as the lethal multidrug-resistant TB (Mnyambwa *et al*., 2017). Furthermore, this study shows that when the GeneXpert tested negative for *M. tuberculosis*, there were 142 cases where the culture also tested negative for *M. tuberculosis*. The specificity (true negative rate) of the diagnostic test is thus 87.1%. This result implies that 87.1% of the time, the GeneXpert correctly tested negative for patients without *M. tuberculosis*. The sensitivity and specificity of the GeneXpert in diagnosing EPTB in this study were higher compared to a study conducted in Egypt by Elbrolosy (2021), which revealed a sensitivity of 81.6% and specificity of 78.9%. The false-negative rate of the study in Egypt was 18.5%, while the false-negative rate in this study was 7.7%.

The reason for the variation in these two studies could be the use of the Xpert Ultra, which is reported to have a better diagnostic performance in detecting the presence of MTBC in EPTB samples than the older version (Xpert MTB/RIF), which was used in the Egyptian study. In a study conducted by Sekyere *et al*. (2019), the sensitivity of the Xpert Ultra was 69.23%, and the specificity was 49.25% in EPTB specimens. The Xpert Ultra is reported to have increased sensitivity, resulting in a

shortfall or decrease in specificity in some studies (WHO, 2017; Lessem, 2017; Kohli *et al*., 2021; Gopalaswamy *et al*., 2021).

Table 4.3 indicated that pleural EPTB was predominantly detected in males aged 21 to 40 years. This demonstrated that pleural EPTB was mainly detected in young adults compared to the elderly and children/infants. According to Chander *et al*. (2012), the most prevalent form of EPTB was pleural TB and males aged between 15-24 were affected by EPTB.

The Xpert Ultra has been proven to have superior sensitivity in EPTB diagnosis compared to other methods, such as culture and microscopy (Sekyere, *et.al*., 2019; Habous *et al*., 2019; Donovan *et al*., 2020; Hoel *et al*., 2020; Cresswell *et al*., 2020; Mekkaoui *et al*., 2021; Gopalaswamy *et al*., 2021; López-Roa *et al*., 2021). The findings of this study revealed that the difference between the results obtained from the GeneXpert (using Xpert and Xpert Ultra catridges) and the results obtained from culture is statistically significant. The GeneXpert in this study showed better sensitivity of 92.3% and 87.1% specificity. This confirms the efficiency and reliability of the GeneXpert machine in rapid diagnosis of extrapulmonary TB.

CHAPTER 6:
CHALLENGES, RECOMMENDATIONS, AND CONCLUSION

6.1 CHALLENGES AND RECOMMENDATIONS

This study had low specimen numbers of throat swabs, urine, FNA, and synovial fluid, among others. Some specimens, such as blood and stool, were not included in the study, as recommended by the manufacturer. Sample handling and storage of specimens before analysis could have affected the integrity of the specimens.

Future clinical studies of this nature should be conducted in different settings in the country with more samples and types for better precision and accuracy. Reflex testing using increased the volume size and increased the sample buffer ratio for GeneXpert ultra. The need for further investigation of the use of suitable decontaminants for extrapulmonary specimens other than sodium hydroxide and N-acetyl-l-cysteine-sodium hydroxide as they provide harsh conditions for some EPTB specimens, which kill some tubercle bacilli, which ultimately produces negative results. Clinicians should use the results from the GeneXpert cautiously and together with the patient's clinical presentation.

6.2 CONCLUSION

The aims of this study were to assess the diagnostic performance efficiency and reliability of the GeneXpert assay in the diagnosis of EPTB. Various non-extrapulmonary specimens were analysed using the GeneXpert when using culture as the reference method. The results showed that the GeneXpert was able to detect MTBC from more sample types than culture. The results further revealed that the GeneXpert test is significant at a 95% confidence interval when using pus swab, FNA, ascitic fluid, throat swab, and auxiliary lymph node aspirates, which can now be tested for EPTB using the GeneXpert instruments across the country after the findings of this study. In this study, the GeneXpert had a better sensitivity of 92.3% and a specificity of 87.1%.

Most importantly, it was established that the prevalence of EPTB was 7.7% from 2016 to 2019. The findings from this study demonstrated that pleural EPTB is more common among males and youths aged 21 to 40 years. Early identification of

EPTB is key in successful treatment outcomes of EPTB. The use of Xpert MTB/RIF has a great contribution to the TB control strategy by boosting some limitations of conventional methods of diagnosing TB. However, the inversion of Xpert Ultra has brought about great insight for clinical specimens with a low bacillary load. The instrument can be of great use in diagnosing EPTB even in poor-resource settings in countries where the culture method is not available.

The findings of this study do not replace culture as the reference standard in Lesotho but suggest that the GeneXpert can be used as an add-on test where clinicians can use the results in collaboration with the clinical diagnosis of patients to help them make a faster diagnosis of EPTB through the presence or absence of MTBC as results are produced in less than two hours. This will be beneficial in the early identification of EPTB as it is among the key strategies in a successful treatment outcome of this killer disease that is difficult to diagnose. However, the GeneXpert has a limitation in detecting live (active TB) and dead bacilli; it, therefore, cannot be used in patients who are taking or who previously took TB treatment. More studies should be carried out as the Xpert Ultra is still listed as a conditional recommendation for EPTB diagnosis by the WHO (2021b).

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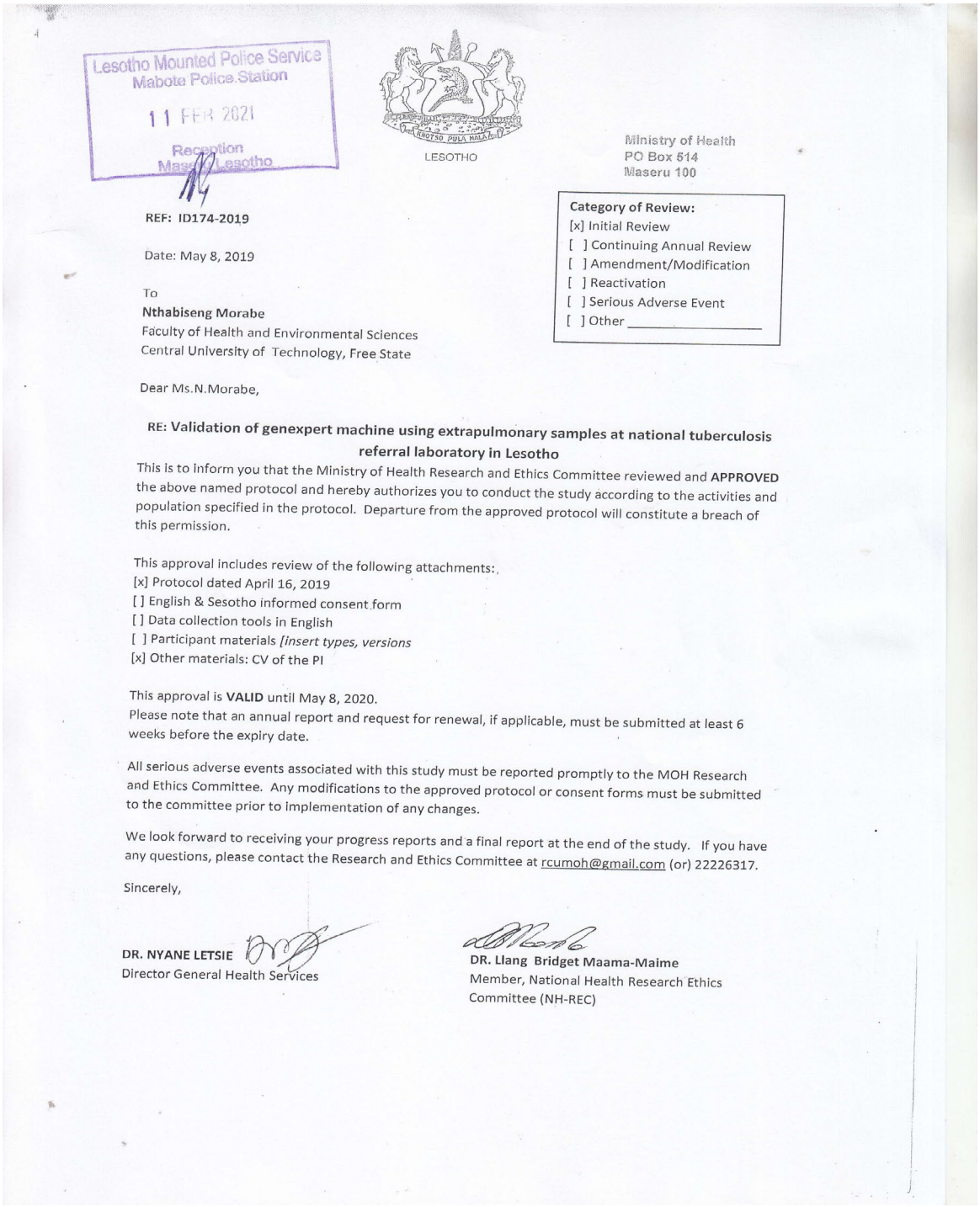
APPENDICES

Appendix A:

Ethical Clearance From Ministry of Health, Lesotho



Lesotho Mounted Police Service
Mabote Police Station
11 FEB 2021
Reception
Masera Lesotho

LESOTHO

Ministry of Health
PO Box 514
Maseru 100

Category of Review:
[x] Initial Review
[ ] Continuing Annual Review
[ ] Amendment/Modification
[ ] Reactivation
[ ] Serious Adverse Event
[ ] Other _____

REF: ID174-2019

Date: May 8, 2019

To
Nthabiseng Morabe
Faculty of Health and Environmental Sciences
Central University of Technology, Free State

Dear Ms.N.Morabe,

RE: Validation of genexpert machine using extrapulmonary samples at national tuberculosis referral laboratory in Lesotho

This is to inform you that the Ministry of Health Research and Ethics Committee reviewed and **APPROVED** the above named protocol and hereby authorizes you to conduct the study according to the activities and population specified in the protocol. Departure from the approved protocol will constitute a breach of this permission.

This approval includes review of the following attachments:
[x] Protocol dated April 16, 2019
[ ] English & Sesotho informed consent form
[ ] Data collection tools in English
[ ] Participant materials *[insert types, versions*
[x] Other materials: CV of the PI

This approval is **VALID** until May 8, 2020.
Please note that an annual report and request for renewal, if applicable, must be submitted at least 6 weeks before the expiry date.

All serious adverse events associated with this study must be reported promptly to the MOH Research and Ethics Committee. Any modifications to the approved protocol or consent forms must be submitted to the committee prior to implementation of any changes.

We look forward to receiving your progress reports and a final report at the end of the study. If you have any questions, please contact the Research and Ethics Committee at rcumoh@gmail.com (or) 22226317.

Sincerely,

DR. NYANE LETSIE
Director General Health Services

DR. Llang Bridget Maama-Maime
Member, National Health Research Ethics Committee (NH-REC)

Appendix B:

Memo of Collection of EPTB Samples at Various Laboratories Across Lesotho

National Reference Laboratories,
P.O. Box 514,
Maseru.
20th May, 2019

To whom it may concern,

Collection of Extra Pulmonary TB samples

TB is classified into pulmonary TB or extra pulmonary TB based on the clinical presentation and the site infected. The former is the most common one type that attacks the lungs and it is easily diagnosed while the latter is found in other parts or organs of the body rather than in the lungs. The common problem with management of this epidemic disease is misdiagnosis or under diagnosis of many cases resulting in high mortality and morbidity rate.

The use of the fast automated molecular assay - GeneXpert was advocated by the World Health Organisation (WHO) in 2010 to diagnose pulmonary TB in adults through testing of sputum samples. In 2013, WHO also recommended that the machine can be used to test for certain specimens for diagnosis of extra pulmonary TB especially TB meningitis using cerebro spinal fluid (CSF) specimens. There are currently 33 GeneXpert functional machines and are distributed in 23 sites. The machines are validated and verified for sputum samples to diagnose PTB. Currently in country, the machines have not been validated and verified for the extra pulmonary samples, therefore, there is a need to validate and verify the machines for extra pulmonary samples before implementing the use of the assay on such samples in order to release reliable and accurate results for patient care.

National Reference Laboratories therefore request your laboratory to keep all extra pulmonary samples so that validation can be performed from 27th May to 20th December 2019. With this validation results, all laboratories will in future be able to diagnose TB on extra pulmonary samples.

The main focus will be to evaluate the performance of the GeneXpert assay on various extra pulmonary specimens tested at National Tuberculosis Referral Laboratory.

Sincerely,

LABORATORY SERVICES
NATIONAL REFERENCE LABORATORIES
20 MAY 2019
P.O. BOX 514
MASERU 100, LESOTHO
TEL: 27008559

'Mathabo Mareka - **National Reference Laboratories Manager**

cc: Director Laboratory Services
NTLP Manager