DEVELOPMENT, STABILITY TESTING, AND PERFORMANCE OF XPERT MTB/RIF PROFICIENCY TESTING MATERIAL IN NATIONAL TUBERCULOSIS REFERENCE LABORATORY, NAIROBI CITY COUNTY, KENYA

MARGARET WAIRIMU NGANGA (B.Sc. MLS)
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DECLARATION

This thesis is my original work and has not been presented for the award of a degree or any other award in any other University.

Signature: ..... Date: 04/01/2024

MARGARET WAIRIMU NGANGA

P150/CTY/PT/26853/2018

Department of Medical Laboratory Science

Supervisors:

This thesis has been submitted for examination with our approval as the University supervisors.

1. Dr. Abednego Musyoki

   Department of Medical Laboratory Science

   School of Health Sciences, Kenyatta University

   Signature:.....**Date:** 4/1/2024

2. Dr. Nelson Menza

   Department of Medical Laboratory Science

   School of Health Sciences, Kenyatta University

   Signature:.....**Date:** 8/1/24

DEDICATION

This thesis is dedicated to Kenyatta University, National Tuberculosis Reference Laboratory, my family and friends.

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LIST OF ABBREVIATIONS ANDACRONYMS

AFB	Acid fast bacilli
AFRO	African region
AIDS	Acquired Immunodeficiency Syndrome
ATCC	American Type Culture Collection
BD	Becton Dickinson
BSC	Biological Safety Cabinet
BSL	Biosafety level
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
DM	Diabetes Mellitus
DR-TB	Drug resistant Tuberculosis
DST	Drug susceptibility testing
DTS	Dried tube specimen
EQA	External quality assurance
FIND	Foundation for Innovative New Diagnostics
HIV	Human Immunodeficiency Virus
IQC	Internal quality control
INH	Isoniazid
KNH	Kenyatta National Hospital
LPA	Line probe assay
LJ	Löwenstein-Jensen
LTBI	Latent Tuberculosis infection
MDR	TB Multidrug resistant tuberculosis
MGIT	Mycobacteria Growth Indicator Tube

MTB	Mycobacterium tuberculosis complex
NAAT	Nucleic acid amplification test
NACOSTI	National Commission for Science Technology and Innovation
NTRL	National Tuberculosis Reference Laboratory
OADC	Oleic acid, albumin, dextrose, and catalase
PCR	Polymerase chain reaction
PLHIV	People living with Human Immuno-Deficiency Virus
PPE	Personal protective equipment
PT	Proficiency testing
QAS	Quality Assurance Systems
QC	Quality Control
RIF	Rifampicin
TB	Tuberculosis
WHO	World Health Organization
ZN	Ziehl-Neelsen stain

ABSTRACT

Systematic and ongoing proficiency testing (PT) program is a fundamental element of external quality assurance in diagnosis of TB using Xpert MTB/RIF assay. In many resource-limited setting, particularly in Kenya, PT is poorly covered and largely inconsistent following inadequate supply of dried tube specimens (DTS). This has serious negative implications on patients' management and TB control programs. The aim of this study was to develop, assess stability and performance of local DTS panels for Xpert MTB/RIF assay PT in Kenya. This study was conducted at the National Tuberculosis Reference Laboratory (NTRL) and DTS piloting was done in Xpert MTB/RIF sites in Nairobi City County. An experimental study design was adopted to develop and assess stability DTS panels, and a cross sectional study design was adopted to pilot panels. The piloting sites were purposively selected to include all the twenty (20) TB diagnostic laboratories that were using Xpert machine in Nairobi City County, from August 2019 to December 2019.Bacterial strains stored in a -80^{o}C freezer at the NTRL were retrieved and processed following standard bacteriological procedures. The strains were sub-cultured in liquid culture media (MGIT), growth detected by the BACTEC® MGIT 960® system, and their purity confirmed. Bacterial cultures were then heat-inactivated and pre-tested using Xpert MTB/RIF assay before preparing DTS. To assess the effect of drying on the stability of DTS panels, DTS panels were left uncapped inside a biosafety cabinet for 7, 10, and 14 days, and immediately subjected to Xpert MTB/RIF assay. In assessing the stability of the DTS panels at various temperatures as anticipated in TB diagnostic laboratories that use Xpert machine across the Kenya, 36 DTS panels from 7, 10, and 14 days drying periods were held at 18^{o}C, 20^{o}C, 24^{o}C, 33^{o}C, and 40^{o}C for 12 weeks, and subjected to Xpert MTB/RIF weekly. For piloting, a set of 5DTS panels, 5 disposable sterile dispensing pipettes, processing instructions, and PT results evaluation form were delivered to the targeted piloting sites in a sealable transparent bag for testing by the Xpert MTB/RIF personnel who printed the results, entered them into the reporting form, and a scanned image send to the principal investigator for analysis. Data were analyzed using STATA v17 and variables were summed up in medians, interquartile range, means, and standard deviation (SD). Each of the five pilot DTS panels was assigned an accuracy score of 20 points, and individual laboratory scores as follows; incorrect determination of either MTB detection (0 points), unsuccessful result (error, invalid, or no results) (5 points), RIF-indeterminate result (10 points), and correct determination of both MTB detection and RIF resistance (20 points). Total scores for each laboratory were computed, with 100% being considered Excellent, ≥80% Satisfactory, and <80% Unsatisfactory. The DTS panels' probe A Ct values were within the expected mean range (16-23) and SD limit (≤3), with100% concordance between those dried for 7, 10 or 14 days and the pretest results. Except for DTS panels held at -80^{o}C, the probe A mean Ct values of panels dried for 7 and 14 days increased with increasing temperature (from -20^{o}C to 40^{o}C) during the 12 weeks' study period (p= <0.001), with the greatest mean Ct value increment was at 40^{o}C.Ninety-five per cent (18/20) of piloted laboratories reported the expected, with 10% (2/20) of sites giving discordant results, false MTB detection (5%, 1/20) and error (code 5007) (5%, 1/20). 90% (18/20) of the piloted sites had satisfactory, 80% had excellent, and 10% (2/20) had unsatisfactory results. This study recommends adoption of 7-day DTS drying period, -80^{o}C and 40^{o}C storage up to 2 weeks, and up scaling DTS production for proficiency testing in all Xpert MTB/RIF testing in the current study setting and beyond.

CHAPTER ONE: INTRODUCTION

1.1 Background Information of the Study

The disease tuberculosis (TB) continues being in the forefront as the cause of morbidity and mortality from sole infectious agent worldwide, above Human Immunodeficiency Virus / Acquired Immuno Deficiency Syndrome (HIV/AIDS) (Mutinda, 2019). In 2021, TB incidence rate was 10.6 million cases (but only 6 million cases were notified) and 1.6 million deaths, of which 187,000were people living with HIV (WHO, 2022). Of these, 23% of TB incidences and33%of the mortality cases occurred within the 47 countries in the World Health Organization (WHO) African region (AFRO). During the same period, Kenya ranked number fifteen (15) in the WHO top twenty-two (22) high burden TB countries (WHO, 2022). According to the latest Kenyan survey (2016), five hundred and fifty-eight TB incidences for every one hundred thousand persons above 18 years were reported. This was an increment from the estimates reported in 2015 by WHO of 233/100,000 population (Enos *et al*., 2018).

The TB epidemic in Kenya is compounded by HIV/AIDS co-infections and high rates of drug-resistant TB (DR-TB) (Ngari *et al*., 2023). A study on drug resistance conducted in 2015 showed that DR-TB accounts for 3.1% of all newly diagnosed TB patients and 10% of people who had previously been treated for TB but have been re-diagnosed with TB(Ombura *et al*., 2016). Kenya notified five hundred and seventy-seven persons as being resistant to the anti-TB medications in the year 2017, which was an incline from four hundred and forty-four cases reported in year 2016(WHO,2018).

Proper TB diagnosis, especially in endemic areas, is crucial for prevention, control and treatment. The traditional sputum smear microscopy has low and varying sensitivity (20-80%)among active TB cases and has significant limitations in detecting Drug resistance Tuberculosis (DR-TB) (Mnyambwa *et al*., 2017).Culture and drug susceptibility testing require expensive and sophisticated laboratory infrastructure and the process can take 42 days or more(Yusoof *et al*., 2022). Line Probe Assay (LPA) is dependent on smear results and is prone to contamination and human error(Ramani,2016).To resolve issues pertaining TB diagnosis and in agreement with the goals of controlling and ending TB in the world, to attain fast TB testing methods and evaluation techniques for anti-TB drugs susceptibility in every TB infected person, the WHO in 2010 endorsed TB diagnosis using Xpert machine(manufactured by Cepheid Inc.; Sunnyvale, CA, USA)as a faster and superior TB testing tool(WHO, 2021). However, efficiency of Xpert technique depends on internal and external quality controls, using the proficiency testing (PT) materials in form of dried tube specimens (DTS) (GLI, 2017).

Implementation of Xpert MTB/RIF assay in resource-limited countries has met obstacles arising from weak quality assurance (QA) programs, involving evaluation of laboratories performance in terms of undertaking competency assessment and site visits to under-performing laboratories. Proficiency testing can be done by: dried tube specimens (DTS), artificial sputum, dried culture spots or lyophilized and liquid samples(Dowdy *et al*., 2019). A country may produce its PT materials or obtain them through participation in a commercial PT scheme. The exorbitant prices of the

commercial PT panels together with delays in acquisition processes frequently disrupts PT programs in the low middle income countries, with potential detrimental effects on the quality of the Xpert MTB/RIF results. As such, production of locally made PT materials stands out as the main option in efforts to ensure continuous QA programs and realization of the full benefits of Xpert MTB/RIF assay in treatment of TB infected persons(Gumma *et al*., 2019).

1.2 Statement of the Problem

Despite the great efforts to control TB, many people are continuing to die due to delayed diagnosis and treatment initiation. The World Health organization (WHO) endorsed Xpert MTB/RIF assay in 2010 as a robust diagnostic method for fast detection of TB and drug resistance. Following the endorsement, Kenya procured and rolled out 226 Xpert machines to all counties as the primary TB diagnostic technique for suspected TB cases. However, implementation of this method has been challenged due to weak external quality assurance (EQA), including proficiency testing (PT), equipment maintenance and onsite supervision to underperforming laboratories.

A well-structured and an ongoing EQA through PT, using dried tube specimen (DTS) is required to evaluate staff competence, as well as equipment performance. However, in Kenya, DTS- proficiency testing materials are not locally available. They are obtained externally as a donation from Center of Disease Control and Prevention (CDC) Atlanta, United States of America(USA). Unfortunately, their supply is insufficient (only 158 receive the panels among all the 226 Xpert diagnostic sites). Furthermore, supplies are unreliable due to delays in shipment thus disrupting EQA programs in the whole country, with a potential negative impact on TB patients'

management and infection prevention interventions. Therefore, this study focused on developing locally made dried tube specimen (DTS) panels for checking quality of diagnosing TB using the Xpert machine at National Tuberculosis Reference Laboratory, Kenya. In addition, the stability and performance of the developed DTS were evaluated.

1.3 Justification

Locally made DTS will guarantee timely, adequate and reliable quality assurance (QA) program for all Xpert MTB/RIF testing sites in Kenya. This ensure improved management of TB patients, through reliable test results, and quality epidemiological data that can inform TB prevention strategies in-line with the WHO End TB strategy through a 90% reduction in deaths caused by tuberculosis deaths by 2030.In future, the locally produced PT panels can be scaled up and sold to neighboring countries; thus, serving as a foreign income.

Evaluation of the optimal drying period and suitable temperature for storage and transportation of the DTS panels is crucial to ascertain stability and viability of the proficiency testing materials when distributed all over the country where climatic conditions are varying. This is critical to ensuring this study DTS are handled appropriately to achieve the desired purpose as quality checker for Xpert MTB/RIF. Piloting of the developed DTS in Nairobi City County was important to evaluate the performance in assessing laboratory staff competency and verification of Xpert instruments, and inform decision to up scale production to cover other Xpert MTB/RIF testing sites in Kenya.

1.4 Alternative Hypothesis

i. Dried tube specimen **(**DTS) panels for Xpert MTB/RIF proficiency testing can be developed at National Tuberculosis Reference Laboratory (NTRL).
ii. DTS panels developed at NTRL are stable for Xpert MTB/RIF proficiency testing.
iii. There is excellent performance of in-country-developed DTS panels as Xpert MTB/RIF proficiency testing materials in Nairobi City County.

1.5 Objectives

1.5.1 General Objective

To develop, evaluate stability, and pilot locally made proficiency testing panels for Xpert MTB/RIF proficiency testing at National Tuberculosis Reference Laboratory, Nairobi City County, Kenya.

1.5.2 Specific Objectives

i. To develop DTS panels for Xpert MTB/RIF proficiency testing at National Tuberculosis Reference Laboratory.
ii. To evaluate stability of in-country-developedDTS panels at National Tuberculosis Reference Laboratory.
iii. To evaluate the performance of in-country-developed DTS panels as Xpert MTB/RIF proficiency testing materials in Nairobi City County.

1.6 Significance of the Study

This study data will be availed for consumption by the decision makers to inform decisions on supporting the production of locally made PT panels instead of procuring the commercial panel from international countries which are unreliable due to the shipment delays and insufficiency. Locally developed DTS PT panels will ensure an external quality assurance system that is reliable, sufficient and sustainable to all Xpert TB diagnostic sites throughout Kenya. Findings of the stability assessment will help inform the healthcare providers on the storage conditions of these proficiency testing materials.

1.7. Limitations of the study

The study did not investigate the performance of the DTS proficiency testing materials when piloted in other counties across the country. It was only conducted in one county (Nairobi County) due to financial constraints.

CHAPTER TWO: LITERATURE REVIEW

2.1 Tuberculosis Epidemiology

Tuberculosis is an infectious disease caused by a single infective agent, a rod-shaped bacillus, *Mycobacterium tuberculosis* (MTB). Taxonomically, MTB belong to the genus *Mycobacterium*, family Mycobacteriaceae and order Actinomycetales. Human beings are the only reservoir for the species MTB which is the etiologic agent of TB is in humans, although many animals are susceptible to infection. Other *Mycobacterium* species include; *M. africanum* that causes TB in humans is endemic in West Africa regions; *M. bovis*, *M. caprae* and *M. pinnipedii*, are the causative agents of TB in wild and domestic animals; and *M. microti* causes TB in voles only (Bennett et al., 2019).

MTB is transmitted by way of breathing in the tuberculous materials from the air which goes to attack the lungs and any other body organs (Ngari *et al*., 2023). Approximately, twenty-five percent of people globally have latent tuberculosis infection (LTB) (asymptomatic and it's not transmissible). About a quarter of the population worldwide have Tuberculosis infection though they exhibit no symptoms and cannot transfer the disease to other people (WHO, 2021). About 10% of these dormant TB forms progress to potent TB disease, with principal signs mainly being weight loss, fever, cough and extreme night sweats. People infected with active form of TB can infect 10–15 people with whom they have close contact with over a long period of time. Without proper treatment, TB mortality rate among HIV negative people is 45% in HIV-negative TB patients, but higher mortality rate is reported among HIV-positive patients (Jilani *et al*., 2023).

Generally, TB occurs worldwide and can affect anyone anywhere, differing in various places. Report from the WHO for the year 2017 indicated that 10.4 million persons became sick of TB with 1.6 million deaths occurring from the same. This implies that there was a decline of 1.8% of TB incidences with approximately 4% in mortality rate from what was recorded in the year 2016 (MacNeil et al., 2019)Which showed that mortality due to TB in People Living with Human Immuno-deficiency Virus (PLWHIV) was 1.7 million with more than fourth of these mortality cases reported from the WHO member states in Africa (WHO, 2018). In the WHO AFRO region and the WHO member states in South-East Asia, mortality due to TB was about 82% among HIV-negative people. Additionally, this has been intricated by resistance of Tubercle bacilli to multiple anti-tuberculous drugs. In 2016, about 451,551 new TB incidences of rifampicin resistance were notified (WHO, 2016).

Outbreak of Corona Virus Disease (COVID-19) a human virus caused by severe acute respiratory syndrome–associated corona virus (SARS-CoV), which emerged in Wuhan, China. The first known case was identified in December 2019 which rapidly spread all over the world. On 30 January 2020 COVID-19 outbreak was declared by WHO as a Public Health Emergency of International Concern (PHEIC) and on 11 March, it was classified as a pandemic (Cucinotta *et al*.,2020).COVID-19 pandemic globally brought about disruption in the provision of essential TB services thus leading to increase of the burden of TB disease which reversed decade of gains made over the years(Martin-Hughes *et al*.,2022).There was a sharp drop of the notified new and relapse TB cases in the year 2020, this was attributed to decrease in case detection and reporting which was due to the introduction of public health and social measures

that were put into place by countries as a control measure following the COVID -19 pandemic(WHO,2022).

Also, a marked reduction in access to TB diagnosis and treatment caused a surge in undiagnosed (delayed or missed TB diagnosis) and untreated TB cases which fueled the spread of the diseases resulting in a marked increase in mortality due to tuberculosis for the first time over a period of ten years (Wu *et al*., 2020). This was evident in 2021 where WHO report indicated that globally, TB incidence cases were10.6 million, an incline from the 10.1 million cases reported in 2020. Interestingly, about 90% of those who developed the disease were adults. In the 2021 report, 6 million of the cases were from men, 3.4 million were women and 1.2 million children (WHO, 2023). Of this cases, Tuberculosis resistant to anti-tuberculous drugs was reported in about four percent new TB cases whereas 18% were from relapse patients and about six percent were from all other categories of tuberculosis cases (Molla *et al*., 2022).

Kenya has been categorized among high TB burden countries. By the year 2017, approximated TB incidences were 319 cases per 100, 000 population while deaths due to TB disease were estimated at 50/100, 000(Gichuki *et al*., 2021). In the 2016, a nationwide incidence survey demonstrated that in a population of 100,000 about 558 people had TB infection of which was increment from the 266 reported by WHO, this indicates that underestimation lead to omission of 40 per cent of the cases each year. In determination to readdress control of TB in Kenya, the government through Ministry of Health has put measures to enhance diagnosis of Tuberculosis, enhance private sector collaboration by carrying out mobilization of the community to increase

their understanding of TB disease thus making TB everybody's business (Enos *et al*., 2018).

2.2 TB Control Challenges

Global control of TB remains a formidable challenge for decades to come despite the global TB control strategies being put into practice in various nations to help diminish Tuberculosis incidences and prevalence of latent infection. However, in spite of all these encouraging efforts, actualization of the TB-free world has remained an uphill task for many National TB programs globally (Macneil *et al*., 2019).This is due to challenges experienced in the following areas: Lack of early recognition and efficient treatment of both drug-susceptible and -resistant TB cases, inadequate diagnostics and treatment, impediment of drug-resistant TB (DR-TB) and MDR-TB, managing and controlling TB promoting factors such as HIV infection, diabetes and smoking to minimize the risk of progression of active TB from latent form of TB disease, targeted preventive chemotherapy and an effective vaccine-based approach (Li *et al*., 2017).

Delayed detection and patient management is one of the reasons for failure in TB control because they lead to increased infectivity, disease burden and fatality. Available data in Sub-Saharan Africa shows that delayed TB diagnosis could be caused by long travels TB clients make to get to a healthcare provider as well as first sourcing help from a traditional healer. Also, for most HIV patient co-infected with TB, some patients suffer from fear and stigma and financial constraints upon acquiring the disease, thus this becomes forms of access barriers to attaining diagnostic and treatment services (Getnet *et al*., 2017).

Across the globe, there is lack of adequate knowledge of the best means to implement and integrate of Active Case Finding (ACF) as a TB screening tool which brings diagnostic services closer to patients; thus, greatly intensifying early case detection among marginalized person who would not present themselves voluntarily for care, as well as, linking diagnosed patients to care. This is happening both at patient's point of care and health system level in diverse cultural, socio-economic as well as national circumstances thus the constant problems of delay in patients seeking early diagnosis and treatment makes Tb control process to be a daunting task (Saini *et al*., 2020). On the other hand, in settings where passive case-finding is in use TB control has been challenging thus missing out on early case detection. With documented delays in notification and treatment initiation for the diagnosed cases, 8–38% of TB cases suffer delayed or failed linkage to care (Saini *et al*., 2020).

Inadequate diagnostic methods for timely detection and effective management of tuberculosis are also a major challenge to Tb control globally. This is mainly because most techniques used for diagnosis of TB are traditional and not robust. For example, the traditional sputum-smear microscopy is not effective in detecting extra-pulmonary TB or sputum smear-negative TB, and this is even worse in children and among people living with HIV. More times than not, smear results are often negative. Most countries have only reference laboratory as the only culture laboratories identifying MDR-TB. Therefore, the lack sufficient diagnostic facilities cause delay in detection, as well as delayed initiation of treatment leading to increased morbidity, mortality and transmission; thus, jeopardizing TB control efforts (Abdu *et al*., 2020).

Prevention of DR-TB or MDR-TB is delaying factor to TB control. DR-TB occurs when the bacteria MTB becomes resistant to the drugs used to treat TB. This state occurs when the drugs used for the treatment of TB are misused or mismanaged. Through the following: non-adherence to the prescribed treatment, health care providers giving wrong prescription by either by dosage or the duration of treatment, unavailability of the proper treatment for TB or when the quality of the drugs are poor (Phelan *et al*., 2018). DR TB is transmitted in a similar way as drug-susceptible TB.MDR-TB occur when there is resistance more than two most potent first-line TB treatment regime namely isoniazid and rifampicin. Of the one-third of persons infected with TB worldwide, presence of drug resistance genes in MTB is the greatest test of mitigating Tuberculosis. Even though drug resistant TB is curable, the cure rates still are practically below 100% both in low income and high-income (LMIC) settings; thus, posing a major public health problem among immune suppressed persons. Almost half a million new cases MDR-TB emerges every year (Phelan *et al*., 2018).

Another technical challenge for TB control is the management of risk factors, such as human immunodeficiency virus (HIV) infection, diabetes and smoking which predispose the people to the risk of progression from latent infection to active TB disease and also contributes to poor tuberculosis treatment results. A diagnosis with diabetes mellitus (DM) that is poorly controlled triples the possibility of developing from the initial infection to active TB (Silva *et al*., 2018). About 15% of TB cases worldwide have a probable link to Diabetes Mellitus. The load of bacteria at presentation is high in patients with Diabetes Mellitus than those without diabetes mellitus, and they relatively their conversion to culture negativity is prolonged, and

their chances of dying from TB or getting a relapse are also higher than non-diabetic patients. Thus WHO is advocating for bidirectional screening for TB in all DM patients and vice versa (WHO, 2016). Smoking whether passive or active exposure to cigarette smoking plays a significant role in the pathogenesis of TB in that it is associated with the dysfunction of immune cells such as macrophages which are responsible for immune response to infections, increasing susceptibility to infection with MTB and of the development of active TB.

Additionally, the psychosocial aspects of smoking have been linked to desertion of anti-Tb drugs; thus, increasing the threat of death owing to TB to be over nine (9) times for tobacco smoking TB patient than for non-smokers. Upon quitting, the risk of death among the tobacco smokers drops drastically by 65% in comparison to what is seen in those who carry on with smoking after contracting TB. As a control strategy, therefore, TB programs should encourage patients with TB to go through smoking termination treatment, which remarkably improves the quality of their life (Silva *et al*., 2018). HIV rapidly lowers immune systems; and thus, predisposes patients to progression of active TB when exposed to the bacilli. There is rapid deterioration of the immunity thus progression to active TB during their lifetime is 100 times higher in people have no HIV. A progressed HIV-TB co-infection stage, on the other hand aggravates the troubles of achieving quality TB results, as well as efficient patient management because sputum smears test negative and with no typical chest radiography features, this means that coexistent TB infections continue to be untreated. Also, increased relapse rates among HIV-positive people are related to the use of some TB treatment (Takhar *et al*., 2018).

Unsuccessful implementation and scale up of the public–private mix (PPM) strategy, which is one of the component of the second pillar (patient-centered care) as an intervention for use in ending TB by the year 2035 has been reported in several countries as an obstacle to TB control. Private pharmacies play a very important role in Tb control. With most of them having the advantage of; long operational hours, varieties of drugs in their inventory, little or no queues as well as no consultation charges. These retail outlets are common areas where people go to seek medical care (Daftary *et al*., 2019). In India, 40% of symptomatic patient's initial point of medical contact is the pharmacy and 25% of these patients continue to seeking advice from pharmacies even after diagnosis, preferring to keep away from doctor consultations. Studies have, however, shown that pharmacy providers commonly dispense drugs (cough syrups, anti-histamines, bronchodilators and antibiotics) over-the-counter (OTC) without referring the patients to a doctor for TB testing and treatment; hence, this self-medication and poor referral practices can delay TB diagnosis (Daftary *et al*.,2019).

Additionally, continued use of broad-spectrum antibiotics, such as fluoroquinolones can as well result to the development of drug resistance TB (Daftary *et al*., 2019). In Zambia, a report was published that indicated that most facilities which tested and offering management of TB patients in the profit making health facilities did not notify the TB cases to the National TB Control Program, citing a frail partnership between the National TB program and the private sector (Chongwe *et al*.,2015). In Kenya, the PPM collaboration in the past had various limitations with profit making health facilities citing inadequate resources, poor governance and lack of frequent sensitization of private-sector health workers as the challenges that were causing

inability to successfully embrace the spirit of PPM where communities, civil society/organizations and all public and private care providers are engaged and well informed on the benefits of intensifying the steps between thorough assessment of patients on indication of presence of TB disease, laboratory test and timely reporting of the cases. Mailu and others in their study on assessing TB control activities in the private and public health sectors in Kenya from 2013 to 2017 demonstrated that, in past 10 years, the profit making Tb management health facilities has been greatly improving even though there are various programmatic hindrances that require to be addressed (Mailu *et al*.,2019).

2.3 Global TB control Action Plans and Strategies

Global efforts to control TB have been a journey which began in the nineties (90s) after World Health Organization acknowledged Tuberculosis as a disease of international concern which was declared a global emergency in 1993. Control of Tuberculosis using the directly observed therapy strategy (Directly Observed Therapy Short-course (DOTS) with a short-course regimen was recommended in 1994 following the 2000 United Nations (UN) Millennium Development Goals whose targets were to be achieved by 2015 (Sotgiu *et al*., 2017). The DOTS strategy was implemented through five key elements which were considered essential for global TB control, including commitment of political forces, sputum microscopy as the main tool for case detection in care seeking persons with prolonged cough, standardized short course chemotherapy under proper case-management conditions including directly observed treatment (Sotgiu *et al*., 2017).

In 2001, the WHO and the global advocacy organization Stop TB Partnership launched bold and ambitious plan as efforts to control TB, the initial Global Plan to Stop TB 2001–2005 mostly focusing on expanding the DOTS strategy and the rising challenge of drug resistance in TB and HIV infection. Later, the second Stop TB Strategy 2006–2015 came into place targeting to attain worldwide access to high-quality diagnosis and treatment, lessen the number of patients suffering and the socioeconomic burdens related to TB, protect poor and vulnerable populations from TB, and support development of new tools and enable their timely and effective use thus dramatically reduce by halving TB prevalence and deaths by 2015 (Matteelli *et al*., 2018).

In 2014, the WHO's End TB Strategy was adopted. This came in as new and holistic master plan that was approved by World Health Assembly in May 2014, where a wide array of partners in the ministries of health, other governmental authorities, civil society representatives, development and public health experts, as well as researchers from 194 member countries were involved in developing the strategy(John,2019).This global plan served as a blueprint for countries to patching up the efforts and greatly accelerate the fight against TB among those most affected, such as the poorest, most vulnerable, socially marginalized and those that are inequitably served and be able to successfully bring to an end the global TB epidemic as part of the newly adopted Sustainable Development Goals. The specific goals included reducing the number of TB incidence by 80%, mortality due to TB by 90%, and to get rid of catastrophic costs for TB-affected households by 2030 (Matteelli *et al*., 2018).

A forth global plan, End TB 2016–2020 strategy came into place in 2015. The plan gave guidance and the road map to be followed to set the world on the right path towards achieving the goal to end TB by the year 2030 as directed in the UN Sustainable Development Goals. This strategy outlines milestone of 50% reduction in incidence and 75% reduction in mortality by the year 2025, and an overall target of 90% reduction in incidence and 95% reduction in mortality by 2035 (Matteelli *et al*., 2018). To reach these targets, countries need to redouble their TB control efforts and as well as adopt new TB control strategies such as active case finding and contact tracing approaches were to timely detect cases in the community, re-direction of TB support and attention to endangered and marginalized groups to ensure that there is early TB diagnosis and the diagnosed patients are linked to care, intensification of timely reporting through development and distribution of new tools for TB control and advocate for the implementation of TB services packages that are extensive and can be used in different types of epidemic and socioeconomic environments(Matteelli *et al*., 2018).

The current plan to end TB as a global health threat was unveiled in 2022 and name Global End TB 2023-2030.It is a costed plan indicating priority actions and estimated financial resources required to eliminate TB by 2030. The Global Plan clearly illustrate how a global financial investment of US$250 billion can be used from 2023 to 2030 to save millions of lives through timely diagnosis and treatment of 50 million TB cases, improve development, establish and support approval and distribution of a new TB vaccine and intensify efforts to fight TB to avoid disruptions by emerging crisis, such as COVID-19 pandemic or conflicts which cases derail on TB programs (Matteelli *et al*., 2018).

Owing to the intricacy of TB and the increased MDR-TB cases globally, timely identification of active pulmonary TB at an early stage remains a public health priority for the treatment of infected cases, control and formulating public health policies to minimize the spread of this disease. Initial testing includes a chest X-Ray then identification or isolation of MTB bacilli from bodily secretions using sputum evaluation tests such as smear microscopy, growth of mycobacterium in solid and liquid medium, and molecular tests (Jilani *et al*., 2023).

2.4 TB Diagnosis

About 30 percent of high risk group who are exposed to MTB will develop latent TB, and if left untreated, about 10 percent progress to active TB at an advanced age. The Centers for Disease Control and Prevention (CDC) advocates for screening anyone showing symptoms for active TB (Jilani *et al*., 2023).

2.4.1 TB Diagnostic Algorithms and Guidelines

Symptoms of TB are often confused with those of other conditions therefore careful testing is required. The Centers for Disease Control and Prevention (CDC) recommend screening anyone with symptoms for active TB to undergo triaging by a medical officer for exposure history, thorough physical examination of signs and symptoms as well, physical assessment of the lymph nodes to check for swellings and listen to the lungs for possible significant health risks are done before ordering any tests (Jilani *et al*., 2023).Diagnostic investigations are done according to the revised guidelines and algorithm of WHO which have been adapted by Ministry of Health - National TB Program, Figure 2.1.

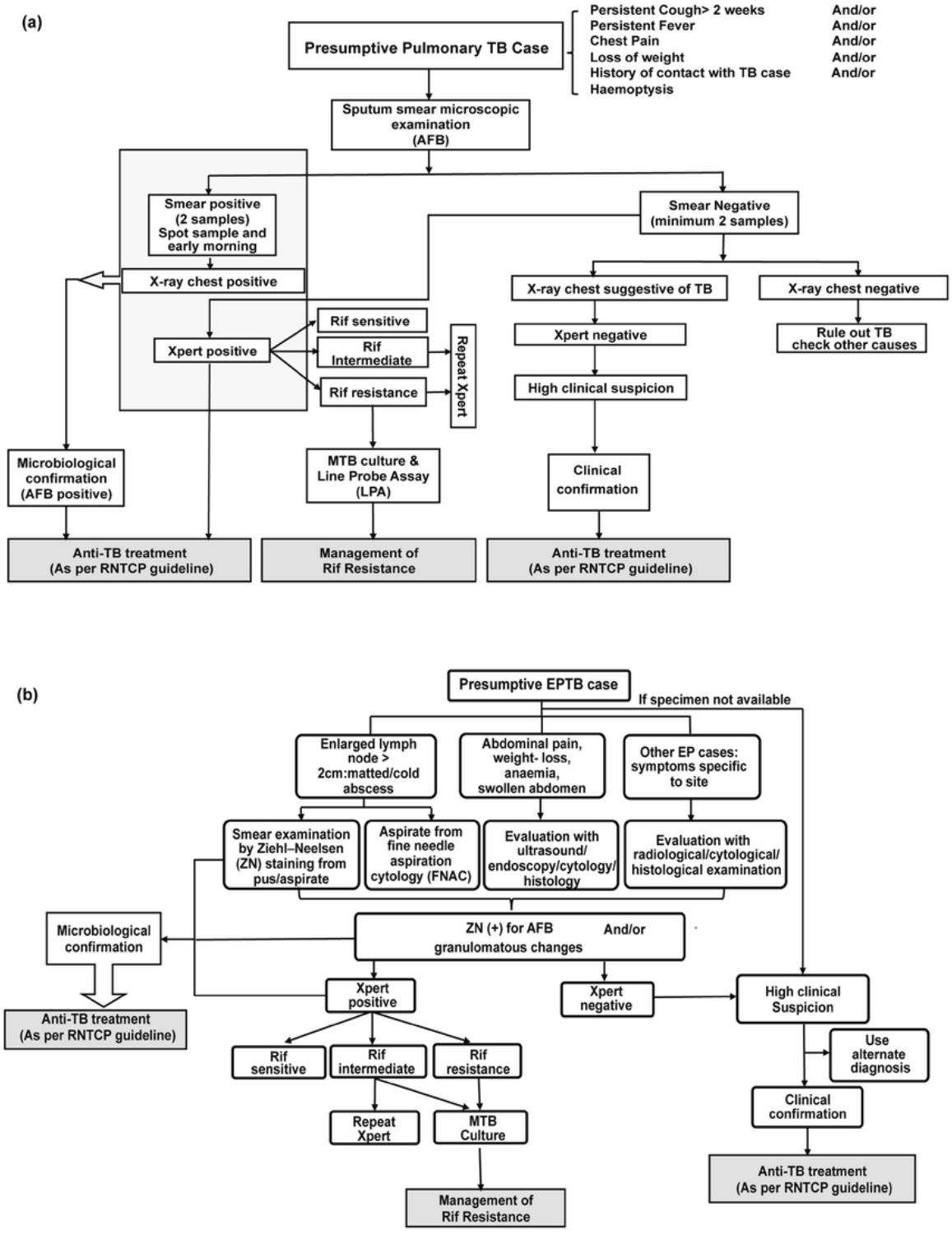

Figure 2.1 Revised National Tuberculosis program algorithm for diagnostic investigations of both pulmonary tuberculosis (PTB) and Extra- Pulmonary Tuberculosis (EPTB) (Lee, 2015).

2.4.2 Tuberculin Skin Test

This method is also known as Mantoux test. It is performed in two-steps where an intra-dermal injection of the Tuberculoid purified protein derivative materials made into the patient's skin and in-duration is observed in 48-72 hours. The result depends on the size of the raised, hard area or swelling. Interpretation of the patient's result is what is considered as possible risk of exposure. Exposure to patients is classified according to the measurements of the in-duration as well the threats of contact to a TB infected person. It is classified as follows; *little risk* patients, have a minimal likelihood of exposure to tuberculosis (Pahal *et al*., 2023).

The finding s is considered positive if considerable in-duration of 15 mm or higher is evident after the intra-dermal injection of the Tuberculoid. *Moderate risk* patients, have an medium likelihood of contact to TB and in this case positive results are indicated by an in-duration greater than 10 mm. *High risk* patients, have great possibility of exposure to tuberculosis, the positive results are indicated when the measured in-duration is greater than 5 mm. Mantoux test positively reported indicates exposure to TB or latent tuberculosis. Nevertheless, Mantoux test has no specificity, and patient's need to have chest x-ray and additional confirmatory test to establish if the person has latent TB infection or active TB. Negative skin test means the person's body did not react to the test, and that latent TB infection or TB disease is not likely. The TB skin test is the preferred TB test for children under the age of five (Pahal *et al*., 2023).

2.4.3 Smear Microscopy

Despite the technique being a simple and fast (takes about 3-5 minutes) and low cost and efficient (results are available within 24 hours of specimen collection) in detecting those cases of pulmonary tuberculosis in sputum of persons who have had coughs for or exceeding a fortnight, this method only permits speculative diagnosis of TB because the AFB in a smears may be other acid-fast organisms other than MTB. It's imperfect in differentiating tubercle bacilli from other *Mycobacterium* and live bacilli from dead ones; thus, reliance on the method may possibly affect patient outcomes as well as under-reporting of the disease (WHO, 2018).

Smear microscopy is a traditional method, which has been widely used to diagnose pulmonary tuberculosis (TB) in most developing countries. It employs the detection of acid-fast bacilli (AFB) in stained and acid-washed smears examined microscopically to provide the initial bacteriological evidence of the presence of MTB in clinical specimens. Specimen used is early morning sputum, deeply coughed specimen with a volume of 2ml collected in a clean specimen container for three (3) consecutive days for best initial TB diagnosis (Bayot *et al*., 2022).

An acid-fast staining technique employs the use of two types of procedures, the Ziehl-Nielsen methods which mainly employ the use of Carbol-fuchsin to stain the AFB and directly microscopy to scan smears, and fluorescent microscopy method which uses auramine-O or auramine- rhodamine dyes to stain the bacilli before scanning the smears in a fluorescent microscope. Bacteria must be present in high concentrations to be visible, typically over 10,000 AFB per ml to produce a positive smear result. The

likelihood of observing the bacilli in a smear is less than 10% if AFB concentration is below 1000 bacilli per ml of sputum (Bayot *et al*., 2023).

Smear microscopy has limitations such as low and varying sensitivity (20% to 80%) hence it may fail do detect all active TB cases, and since its yield is highly dependent on individual skills and experience of the Microscopist. A smear that may be negative requires meticulous screening to identify low numbers of AFB. The method is cumbersome, costly for patients and does not detect DR-TB (Mosissa *et al*.,2016).

2.4.4 MTB Culture, Identification and Drug Susceptibility Testing

2.4.4.1 Culture

TB culture is a highly sensitive technique for growing, identifying and isolating MTB as well as drug resistant strains in specimens. It is the Gold standard method. Specimen of choice are collected in accordance to the clinical history presented which indicate the site of infection as either pulmonary or extra-pulmonary infection. These specimens include, sputum, urine, cerebrospinal fluid (CSF), blood, or other tissue. MTB culture is supposed to be done on both the Lowenstein Jensen (LJ) and Mycobacterium Growth Indicator tube (MGIT) medium (Jilani *et al*., 2023). Patients specimen are processed by specialized decontamination and inoculation procedures and inoculated on Lowenstein Jensen culture medium which are incubated in temperature of 37 degrees centigrade in a supplementary incubator. Specimens are also inoculated into liquid broth, Mycobacterium Growth Indicator Tube incubated in programmed BACTEC MGIT 960 liquid culture equipment that's manufactured by Becton, Dickinson and Company (Caulfield *et al.*,2016). Despite the liquid medium being quite expensive, this culture method is more sensitive to detect very low bacterial load and permits growth of bacterial colonies fast, within 10-14 days, and for

this reason, it is termed as the gold standard and the fundamental culture method for drug susceptibility testing. On the other hand, solid media is more cost effective but takes up to 8 weeks to grow the organisms. Culture method is very effective in differentiating MTB from Non Tuberculous Mycobacterium (Jilani *et al*., 2023).

Even though culture is the gold standard for TB diagnosis, this technique has limitations: it is normally centrally located as a national reference laboratory, where a set of level three bio-containment protection measures are adhered to. The method takes prolonged duration for the *Mycobacterium* to produce colonies in the media whereas antimicrobial responsiveness tests to be done prior to results being ready for patient management. The increased turnaround time associated with Antimicrobial susceptibility testing (AST) eventually allows TB transmission and extended periods of ineffective therapy (Caulfield *et al*., 2016).

2.4.4.2 MTB Culture Identification

Identification of MTB in culture can be done by the fast technique of a rapid kit (SD TB Antigen MPT64) and conventional biochemical reactions. Currently, use of rapid test methods of identifying MTB is more in use because they are rapid and precise in comparison with traditional techniques of biochemical reactions. The test in use Tuberculosis Antigen MPT 64 Rapid kit manufactured by Standard Diagnostics, Seoul, South Korea, is an Immuno-chromatographic test (ICT) using Monoclonal Antibodies against MPT64 antigen for affirmation of MTB in isolates (Cao *et al*., 2021). The Mycobacterium Protein Tuberculosis (MPT64) protein is produced during growth of MTB complex and is never present in other atypical and *Mycobacterium*. The Mannose Binding Protein 64 (MPB64) / MPT64 encoding genes are in deleted

regions of difference (RD), so RD1, RD2 and RD3 are used to specifically identify MTB through antigen-antibody bonds, as well as to differentiate between MTB Complex (MTB-C) from a *non-mycobacterium tuberculosis* (Cao *et al*., 2021).

A study done by Kumar VG and others on demonstrated that the rapid SD MPT 64 TB Ag kit has high sensitivity and specificity, had no untrue positivity and the low tech rapidity scores compared to molecular methods. Its sensitivity, specificity, positive predictive and negative predictive values were 97%, 100%,100% and 92%, respectively (Kumar et al., 2017). With the sluggish growth of MTB accompanied by laborious, risky cumbersome tests together has led to long delay in drug susceptibility testing. This prolonged time needed for correct identification of Mycobacterium Tuberculosis (MTB) isolates could be one of the factors causing increase in TB cases. Automated culture systems like BACTEC 960 MGIT have notably minimized the turn-around-time for culture, but it is limited in that it doesn't differentiate between Mycobacterium Tuberculosis (MTB) and Mycobacterium other than TB (MOTT) /Non tuberculous mycobacterium (Cao *et al*.,2021).

Hence confirmatory identification of MTB continues to be done by traditional biochemical techniques which are tedious, lengthy and need intricate safety measures. All this while, the infected person keeps on spreading the disease to many other vulnerable individuals. WHO endorsed the Rapid SD TB AgMPT64 to cater for the need for TB to be detected with this fast and accurate method for confirming MTB, to enhance robust TB case management. The use of MPT64 Rapid SD TB Ag is expected to be able to touch peripheral health services, as well as be easier in monitoring and monitoring the community towards TB (Cao *et al*., 2021).

At National Tuberculosis Reference Laboratory, there are various methods of identifying *Mycobacterium tuberculosis*. First through acid fast bacilli smear staining where the characteristic rods are observed. *Mycobacterium* is acid fast (stains red) and its rods are beaded. Because the smears require a high bacterial load (100/ milliliter of specimen) for the AFB to be visualized in a light microscope. The method is further supported by performing identification through cultures. For solid media (rough tough and buff colonial growth characteristics are observed) and for liquid media, *Mycobacterium* growth indication is within 7–21 days. Other MTB identification include Molecular assays (Line probe assay, Xpert MTB/RIF assay) and Rapid MTB antigen identification tests which are substitutes for Molecular test rapid Immuno-chromatographic test kit (MPT 64 TB Ag detection ICT) (Campelo,2021).

2.4.4.3 Drug Susceptibility Testing

Antimicrobial Susceptibility Testing for Mycobacterium Tuberculosis Complex (MTBC) involves testing of a single, critical concentration (CC), so as to distinguish between resistant from susceptible isolates of MTBC and is peculiar for each anti-TB agent and test method. Laboratory findings of the susceptibility of tubercle bacilli to anti-tuberculosis agents play three crucial roles; guiding the choice of treatment to be administered to the patient; helps in confirming emergence of drug resistance when a patient fails to show adequate response to treatment; helps in surveillance of emerging drug resistance (Tiberi *et al*., 2022).

Drug susceptibility testing (DST) is paramount for early detection and effective management and control of DR-TB. This comes with the increasing need for surveillance and proper treatment of MDR-TB whose cases has been rising slowly in

most parts of the world. Also increasing risk of development of reluctance to respond medication, such as fluoroquinolones and aminoglycosides injectables and the spread of drug-resistant strain has necessitated DST (Tiberi *et al*., 2022).

With exception of DST in isoniazid and rifampicin, results obtained through other widely used DST methods are not reliable. To obtain reliable and highly predictive DST outcome, susceptibility should be thoughtfully determined with representative MTB clinical isolates from patients who have not been on anti-tuberculosis drugs, and from infected persons who fail to respond to drugs with a therapy that has the assessed medication (Tiberi *et al*., 2022).

There are two MTB DST methods. First is the indirect DST method. It is carried out on liquid medium following culture growth from a clinical specimen. The method is time consuming from isolation from cultures and to performing DST. The second method is the direct DST method, the conventional method where solid media containing susceptibility testing drugs is used to culture the raw clinical specimens. This method is disadvantaged with prolonged duration before obtaining LJ culture medium results because the colonies growth-rate is relatively low (Amini *et al*.,2019).

Direct DST has successfully been tried using BACTEC 460 liquid medium which was a radiometric method. But due to the emission of radio-active waste materials, the method has been phased out. Studies done have shown direct DST using liquid medium to be successful and that duration of the test incubation time ought to be prolonged from two weeks to three weeks because MTB clinical specimen takes time to grow compared to isolated culture (Amini *et al*.,2019).Thus a proposed protocol

was suggested that the express Drug Susceptibility Testing be set up from sputum samples of high bacterial load smear positivity because an innoculum from a specimen with a low bacterial count may have prolonged duration of over two weeks before it attains necessary growth unit for detection capacity of drug susceptibility (Amini *et al*.,2019)

2.4.5 Molecular Techniques

Molecular techniques for detection of TB are currently being widely used due to their ability to diagnose and confirm drug resistance in TB cases in a faster and more cost-effective ways as opposed to diagnosing using traditional culture methods which takes long to give results (Nurwidya *et al*., 2018). WHO advocates for sputum based resource adjusted diagnostic alternatives as replacement test for sputum smear microscopy as the preliminary laboratory evaluation for diagnosing TB and promptly check for drug sensitivity at the peripheral laboratory level (Nurwidya *et al*., 2018).

In line with the WHO strategy of ending TB by 2035, detecting TB early and offering universal DST highlights the importance of laboratories timely and correct detection of drug susceptible and drug resistance Tuberculosis in patients. Molecular techniques based on nucleic acid amplification techniques (NAAT), such as Polymerase Chain Reaction (PCR) produced for real-time detection of TB are being implemented in resource limited Nations. Annually, the three million TB infected people who missed out on TB diagnosis or non-reported have the potential to be reached out using molecular tests for TB (MacLean *et al.*, 2020*).*

While the conventional methods are indispensable in diagnosis of TB, with aim to achieve the End TB strategy goals by 2035, innovations of new tools have been

endorsed and implemented in many countries including Kenya to help to fight TB. These include revised molecular tests that are delivered even at the lowest level of levels of the healthcare system. These tests offer feedback promptly for fast linkage to care hence avoiding delay to treatment as well as patient loss-to-follow-up especially for the Drug resistant TB patients. In the recent years exciting developments have been made in the molecular tests available for diagnosing Tuberculosis, this includes acknowledgement of WHO for the following diagnostic techniques; Xpert MTB/RIF, Xpert MTB/RIF Ultra, loop-mediated isothermal amplification (TB-LAMP) and lateral flow Lipoarabinomannan (Ignatius *et al*.,2020).

The WHO recommended these sputum based resource adjusted diagnostic options to substitute AFB smear screening as the preliminary technique for TB diagnosis, and prompt check for drug sensitivity at the peripheral laboratory level since then, molecular methods have become the most popular tests in finding and detecting MTB Complex and also for detection of MDR-TB cases (Eddabra *et al.*, 2018), because they are faster and more effectively especially in pauci-bacillary disease patients as well as those living with HIV (Nurwidya *et al*., 2018).

2.4.5.1 Line Probe Assays

A rapid PCR strip technology that was approved by the WHO in 2008 to identify MTB and drug resistance profile of isoniazid (katG and inhA genes) and rifampicin (rpoB genes) (Santos *et al*., 2017).This distinct method faces various challenges that may limit execution at low healthcare settings in contrast with Xpert. Due to its reliance of AFB microscopy outcome, the duration it takes for the patient to obtain results after the sample has been collected is prolonged especially when hitches in

release of AFB findings arise. The technique may likely suffer from contagions as well as adverse effects from technologist's mistakes; and being a semi-automated system, its laborious, requiring about 3 rooms part for undertaking of the various steps to avoid microbial contamination (MacLean *et al*., 2020).

Technical errors and occurrences of contamination during the procedures lead to adverse effects on this method. Additionally, the technique has a notable level of untrue alarm in RIF / INH resistance causing disparity between Using Line Probe Assay and traditional Drug Susceptibility Testing reports or clinical features. Using Line Probe Assay for the purpose of diagnosing Tuberculosis and detecting resistance is expensive in contrast to Xpert that can play similar role in detection of Rifampicin resistance (which is the main treatment drug for TB disease). The technology is intricate and is intended for trained personnel working under specialized moderate risk-level containment laboratories. It also takes more time to carry out as compared to Xpert MTB/RIF (MacGregor-Fairlie *et al*., 2020).LPA has limited detection in smear negative patient due to low MTB cells below the detection. Other limitations which can block its implementation in most health facilities in limited-resource settings, including requirement for specialized equipment, well skilled personnel and infrastructure issues (MacGregor-Fairlie *et al*., 2020).

2.4.5.2 Whole Genome Sequencing

Whole genome sequencing (WGS) is a molecular diagnostic tool where genomic sequence information of an entire bacteria is obtained and compared with that of a known reference. WGS is significant in detection of low frequency mutations, finding key deletions and insertions, and identifying other genetic changes among microbial

strains thus building a constructive understanding of its evolution, genetic diversity and the mechanisms involved in drug resistance (Wang *et al*.,2022). From the initial complete genomic series H37Rv was declared toward end of the 19th century, WGS has been significantly advanced in TB research, with the sensitivity and specificity reported for predicting single drug resistance genes being over 80%. Lugi Wang and other in their study where they used WGS to predict Drug resistance in MTB isolates from samples from China and Russia noted that Whole Genomic Sequencing sensitivity and specificity for predicting resistance to primary treatment for tuberculosis unlike with second-level of treatment towards TB. WGS helped identify novel mutations in *katG* and *rpoB* genes which along with frequent mutations can give a reference for clinical microbiology laboratory diagnostic techniques for detecting DR-TB (Wang *et al*., 2022).

2.4.6 Interferon Gamma Release Assays

Interferon gamma release assays (IGRAs) are blood-based tests which are laboratory alternatives to the Mantoux technique intended for testing of latent tuberculosis infection (LTBI). The IGRAs are ex vivo assays which measure the response of T-cell after overnight stimulation with antigens that are proportionally specific for MTB. It is s a very specific and sensitive screening test which quantitatively evaluates the level of inflammatory cytokines such as interferon-gamma (Banaei *et al*.,2016). IGRAs commonly used tests include the Quantiferon-TB Gold In-Tube (QFT-GIT) assay (Cellestis/Qiagen, Carnegie in Australia) and the T-SPOT.TB (T-SPOT) assay (Manufactured by Oxford Immunotec, Abingdon in United Kingdom). New four-tube version of QFT (called QFT-Plus) has been launched by Qiagen. The method is advantageous in that only a single blood draw is required thus returning of the patient

to the health facility for interpretation of result is unessential. However, the method has several limitations in that, it is expensive, requires professional knowledge to do the test and has lots of untrue positive and negative results (Banaei *et al*., 2016).

2.4.7 TB-Lipoarabinomannan Technique

TB-Lipoarabinomannan (TB-LAM) technique is an upcoming key urine-based TB diagnostic technique, used in PLWHIV where Human immunodeficiency virus-associated TB has several noncontiguous sites arising from MTB Lympho-hematogenous dissemination. The disseminated TB infection is mostly missed because of diagnosis is demanding due to limitations in obtaining specimen from the infected site, and this has been thought to be a contributor to increased death rate in Human Immunodeficiency Virus-infected persons with neurological co-infections (Lawn *et al*., 2017).

Therefore, to minimize undetected TB cases and eventually minimize deaths in patients with advanced Human Immuno-Deficiency Virus disease (urine sample is obtained and subjected to lateral flow assay (LFA) for TB-Lipoarabinomannan (TB-LAM) or the Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, CA). First-generation Alere Determine TB-LAM Ag assay (TB-LAM; Alere, Waltham, MA) has been documented to have sensitivity of 40%–70% and a specificity of ≥98% in TB/HIV-co-infected patients with CD4 counts<100 cells/μL (Lawn *et al*., 2017). However, extra-pulmonally TB (EPTB) diagnosis by Xpert MTB/RIF assay testing of urine specimens using has limited sensitivity therefore not recommended by the WHO (Atherton *et al*.,2018).

A powerful model of Xpert MTB/RIF (Xpert MTB/RIF Ultra) with technical improvements giving high sensitivity using the lowest bacillary load has currently been introduced by Cepheid. A case study documented by Atherton LL and others on "Detection of MTB in urine by Xpert MTB/RIF Ultra: a useful adjunctive diagnostic tool in HIV-associated tuberculosis'' demonstrated that urine is an additional viable clinical sample for use with Xpert Ultra for detection of disseminated TB (Cresswell *et al.*,2020).

Another study as well done in Uganda assessing the prevalence of disseminated TB infection by testing urine using the Alere TB-LAM LFA and Xpert Ultra in HIV-positive adults presenting with suspected meningitis, demonstrated that Ultra was significantly more sensitive with 50% sensitivity in patients with CD4<100 cells/µL than Xpert MTB/RIF against a composite reference standard. It showed a prevalence rate of 26% of urine samples which turned positive for TB tests from patients presenting with HIV-associated meningitis. This clearly indicates and promotes the expansion of TB urine diagnostics in patients with meningitis to intensify prompt detection and management of TB, more so sub-Saharan Africa where TB prevalence is extremely high (Cresswell *et al*., 2020). However, use of TB-LAM records reduced sensitivity for detection of EPTB; hence blending both TB-LAM and Xpert MTB/RIF assay techniques improves the detection of EPTB mainly for countries with high EPTB and HIV cases (Simieneh *et al*., 2022).

2.4.8Xpert MTB/RIF Diagnostic Technique

Xpert MTB/RIF assay is a computerized, instantaneous cartridge based nucleic acid amplification test (NAAT) assay using on the Xpert machine system (Cepheid, Sunnyvale, private company based in Califonia which is a complementary of Danaher Corporation). It is probe-based, completely automatic polymerase chain reaction (PCR) technique which concurrently detects MTB and resistance to rifampicin (mutations in the *rpoB* gene) and gives results within two hours(Forbes *et al*., 2018). Xpert MTB/RIF (GX) estimates mycobacterial load by measuring the threshold-cycle (Ct) of five molecular beacon probes (A through E) that target the rifampin resistance determining region of *rpoB* gene (Gotham *et al*.,2021).

In comparison with traditionally methods such as culture techniques (solid and liquid media), studies show that Ct values is equivalent to the bacillary load in respiratory specimens (colony counts on solid agar growth media or measures of *Time-to-Detection* (TTD) in liquid culture for example lower Ct values represent a presence of higher starting concentration of DNA template whereas higher Ct values represent a lower concentration of DNA template. The mean Ct values are also categorized by the Xpert system semi-quantitatively in relation to sample positivity as follows; very low (>28 cycles), low (23–28 cycles), medium (16–22 cycles) and high (<16 cycles), (Fradejas *et al*., 2018).

The probes in the Xpert machine contains wild-type sequences, therefore any variability occurring in the sequence between a probe and the test organism prevents hybridization and indicates that a mutation is present. This testing platform is a fast, automated PCR device which carries out sample processing through concurrent

nucleic acid amplification and detection of the pathogen in a single cartridge. With sensitivity of 99.1%, Xpert MTB/RIF assay identifies *Mycobacterium tuberculosis* complex(MTBC) in 99% of patients with AFB positive smears, over 80% whose AFB results are negative cases and 92% of patients with positive cultures as well as resistance to rifampicin (Fradejas *et al*.,2018).

This diagnostic method has several advantages; with all processing stages being undertaken inside one cartridge, it minimizes applied technical time and opportunities for contamination. The method is fast with a turnaround time of 2 hours. The sample reagent for this assay has a tuberculocidal activity hence decreasing biosafety concerns during the test procedure making it safe for tests to be performed directly to raw clinical specimens without decontamination of samples specimens making it appropriate for lowest level of medical facilities (e.g., minimal danger). It is also performed on clinical samples that have non-contagious microbes (heat-inactivated or chemically deactivation) (Fradejas *et al*.,2018).

With the approval and endorsement of Xpert MTB/RIF assay by US Food and Drug Administration for direct testing on smear-positive or -negative sputum specimens in patients who have been on anti-tuberculosis therapy for more than 3 days thus no interruption of the test outcome as it is with culture method(Dicks *et al*.,2019).Therefore due to this high throughput, these distinctiveness are mainly helpful in increasing accessibility of Xpert MTB/RIF assay in health facilities where there are few equipped diagnostic centers and wherever provision of rapid diagnostic methods are essential near the treatment centers, as opposed to holding back for long to obtain results by use of traditional diagnostic methods (Dicks *et al*.,2019).

Since 2010 after WHO endorsed the Xpert MTB/RIF assay and recommended its use as the initial sputum check test for all subjects with medical suspicion of MDR-TB and HIV-associated TB (MacLean *et al*., 2020), the platform has gained lots of favor in being embraced in resource limited countries for diagnosis of TB and HIV diseases well as receiving various approvals by WHO to be expanded as the primary diagnostic test of choice for all presumptive pulmonary or extra pulmonary TB cases (Joshi *et al*., 2018).Ministry of Health (MOH) as well endorsed it as the preliminary diagnostic test for all suspected TB cases (Enos *et al*.,2018).

Time and time again great strides have been made into the development of powerful versions of the Xpert MTB/RIF test. From 2017 when the MTB/RIF Ultra, a more sensitive improvements have been made on version of the Xpert test was endorsed by WHO, in 2020, WHO strengthened its endorsement as the primary test for pulmonary and extra-pulmonary TB and the following year it authorized an improved Xpert MTB/RIF (Xpert ultra) test for detection of resistance to the main second-line anti-TB drugs regimen (WHO, 2021).

2.3 Challenges Facing Xpert MTB/RIF Implementation

The short-coming related to implementation of Xpert testing vary remarkably between countries with high-class economy and low-class economies. Globally, the impact of the effectiveness of Xpert in LMICs has been hindered due to implementation challenges which often make many of this LMIC fail to achieve more significant public health outcomes (Brown *et al*., 2021). Quality results for samples tested with Xpert MTB/RIF highly depends on the quality of the sample collected. To accomplish

that, samples should be collected in special falcon tubes with a tight lid to avoid leakage and or are either stored in a refrigerator if transportation to the testing sites takes few days or are transported immediately (Brown *et al*.,2021). These factors in the resource-limited countries are not easily accomplished due to challenges such as: shortage of sputum collection containers, poor power supply in most remote areas, causing power surges and other areas totally lack electricity to enable refrigeration storage of samples until they get transported to the testing sites. Other sites totally lack refrigeration equipment and therefore most of their samples are rejected at the testing sites due to poor quality samples (Nalugwa *et al*., 2020).

According to study published on the challenges with scale-up of Xpert MTB/RIF in Uganda, 35% of the assessed sites experienced shortage of sputum mugs for sample collection while other 43 % experienced problems with electricity thus refrigerating stored samples until the day they are transported to testing sites was a major challenge. Also, for the sites that lacked refrigeration equipment, efforts for the staffs to collect quality samples were turned futile when they ask patients to go with the specimen collection containers at home to collect the sample on the anticipated specimen transporting day and return it to the facility but the patients they fail to do so (Hanrahan *et al*., 2016).

Delay in transporting samples for testing or delayed test result notification minimizes the chances to advance in treatment initiation. Comparing patient's turnaround time using the former smear microscopy system and Xpert, some studies have shown that TB diagnosis using Xpert machines is taking long duration to treatment or thus loss to follow-up (Nalugwa *et al*.,2020). Intervention for early TB case detection is a

constant challenge generally due to limiting testing algorithms that minimize access to testing for people with presumptive TB who are not within the scale of the testing algorithm. However, in resource-limited settings, removing these restrictions comes with huge cost implications. Hence no single intervention will result in achieving the End TB goals (Hanraham *et al*., 2016).

Creations taken to foresee the effects of a variety of interventions indicates that making developments in diagnostic testing will give a great reward towards early identification of TB and MDR-TB thus allowing cases to be triaged early and be given appropriate case management is done leading to improvement in patient outcomes (Brown *et al*.,2021). Common stock-outs of Xpert commodities is another constrain hindering execution of Xpert MTB/RIF in developing countries. In Kenya, despite great efforts and resources being put into place to widely avail Xpert machines in the country, their use still remains low, especially in children. A study carried out in Kenya by Oliwa and others on "Perspectives and practices of health workers around diagnosis of pediatrics tuberculosis in hospitals in a resource-poor setting" showed that health facilities which are both very busy and came from counties that reported a high incidence of TB experienced frequent stock-outs of Xpert cartridges and reagents thus hindering diagnosis of TB among the population (Oliwa *et al*., 2020).

Obstacles to the detection of TB disease, low utilization of TB diagnostic services and the deficiency in identification of qualified patients were the implementations challenges identified through a systematic review study by Scott Brown and others on the "Implementation of Xpert for TB Testing in Low- and Middle-Income Countries". These obstacles mostly occur due to lack of proper dialogue or referral channels

between health centers and laboratories (Brown *et al*., 2021). Constraints of Low staffing and heavy patient flows in health facilities situated where there is high burden impacts heavily on the capability and motivation of individual health workers to diagnose TB in the population TB experience lots of challenges in diagnosing TB. Also, despite the high population frequenting these health facilities, there were serious issues of low staffing and heavy patient flows which impacted on the capability and 'motivation of individual health workers to diagnose TB in children. Hence the use of these Xpert machines for diagnosis of TB in the population is wanting (Oliwa *et al*., 2020). Consistent negative Xpert results obtained from the test procedure as well as the difficulties in obtaining specimen for MTB/RIF for confirmation of TB in children is a recognized age-old dilemma that may have been a contributor of reduced interest in the diagnostic technology by the individual health care workers leading to its under-use (Oliwa *et al*., 2020).

2.4 Xpert MTB/RIF Quality Assurance

Accurate diagnosis is an elementary component of TB revolutionized care and management. With WHO current recommendation for Xpert MTB/RIF as a rapid TB diagnostic test, technique is most pronounced in developing nations, particularly in secondary medical management centers and close to the point of care (Kabugo *et al*., 2023). Nonetheless, many facilities encounter hitches due to their weak quality assurance systems, staff development and proficiency, insufficient equipment certification and service maintenance, and in-adequate technical support. To guarantee quality and reliability of Xpert MTB/RIF assay, regular assessment of quality of this technique ought to be performed by an outside laboratory through provision of schedules Proficiency Testing (PT) (Kabugo *et al*., 2023).

Independently, a Proficiency Testing system can assess conformances in various stages of laboratory testing; before testing of samples, during processing of samples and after the testing procedures are completed done. This helps to recognize priority needs for upgrading (Kabugo *et al*., 2023). Excellence principles measures are requisites which are fulfilled to ensure achievement of quality Tb diagnosis. The Xpert MTB/RIF system has its set standards that have been set to assess the functioning of the TB diagnostic system. This then forms the foundation of the benchmarks used for the Xpert MTB/RIF QA system. Quality checkers are made from standards intended for ensuring the quality of testing and for evaluating diagnostic networks. There are practical steps to be undertaken to ascertain quality is maintained in Diagnosis of TB using the Xpert MTB/RIF assay at all levels (national level and at the testing sites implementing this system). At the national and supervisory levels, the quality assurance activities majorly target on developing processes and systems required to reinforce quality testing in all facilities (Albert *et al*., 2016).

2.5 Xpert MTB/RIF Systems Key Quality Assurance Activities

Global Laboratory initiative (GLI) a practical guide on implementation of quality in the Xpert MTB/RIF testing has given guidance on how to set up and execute quality assurance (QA) system for Xpert MTB/RIF diagnostic facilities in the diagnostic networks. The identified quality assurance activities to consider include, forming national policies and guidelines, standard operation procedures, performance indicators for undertaking monitoring and evaluating, coordinating external quality assurance (EQA) and proficiency testing (PT) programs as well as providing

supportive onsite visits (Abebaw *et al*.,2022). Also, Quality assurance in the Xpert MTB/RIF implementing testing sites focuses on the proceedings and activities undertaken to ensure there is production of quality and reliable results through the test procedures performed in the laboratory as well as ensuring that there is a functional laboratory-medical interface to enhance efficiency in referral for testing, prompt reporting and linkage of diagnosed patient's management (Gumma *et al*., 2019).

Findings from a study done in Vietnam by Gumma V and others found that offering orderly PT rounds, accompanied by feedback and tailored onsite supervision visits to poorly performing laboratories greatly support improved testing accuracy and to reductions in the number of sites achieving unsatisfactory results. Regardless of the feedback, as the laboratory personnel keep doing various rounds of proficiency testing, they become more and more experienced on the task, hence improve the testing services. By undertaking PT, this becomes a gesture to laboratories that their performance is paramount and is being monitored and therefore the technical staffs become more diligent following gained experience in proficiency testing. They identified that after undertaking about five round of PT culminated into demonstration of perfect scores by the laboratories evaluated; however, they emphasized on the need for continued effort to sustain the laboratories performance (Gumma *et al*., 2019).

In another study done in Ethiopia by Abebaw and others to assess "Quality assurance practices in tuberculosis diagnostic health facilities in Ethiopia" showed that implementation of quality assurance practice in Xpert MTB/RIF diagnostic laboratories plays a crucial function in meeting the goals of the End TB strategy 2035.From their findings, of the 79.4% Xpert diagnostic sites were enrolled in the

SLMTA program (Strengthening Laboratory Management towards Accreditation) implementation, a robust QA system was formed resulting in the production of quality-results for patient care as well the SLMTA program assisted health facilities laboratories to achieve ISO certification. They also noted that most of Xpert diagnostic laboratories assessed never monitored turn-around-time of samples from collection to reaching at the laboratory thus it becomes difficult in tracing early problems that may arise prior to sample processing (Abebaw *et al*.,2022).

Several studies have shown that 48%-62% of errors obtained in Xpert MTB/RIF assay originate from most in the pre-analytical phases. Also, the lack of a quality control new reagents and verification of the method verification in use, standard operating procedures (SOPs) not being in use in most laboratories diagnosing TB using Xpert machine was observed. Owing to the fact that SOPs are vital for every test procedure in the laboratory in elaborating how a give technique is performed and ensuring the accurate and precise laboratory findings are produced, this posed as a huge quality assurance system gap in the TB diagnostic health facilities which would impact heavily on the quality of results produced (Abebaw *et al*., 2022).

Against this background, all Xpert MTB/RIF diagnostic sites should be sustained by EQA program and also incorporate supervision visits to underperforming laboratories and proficiency testing (PT) activities. According to Klein and others, the dried tube specimens, dried culture spots, artificial sputum, liquid samples as well as lyophilized are available proficiency testing panels for Xpert MTB /RIF assay that do exist and have assured quality for use in Xpert MTB/RIF EQA program (Klein *et al*.,2020). A nation may choose to develop its panels locally or procure them; nevertheless, for PT

panel's development, a country has to meet the required capacity in terms of infrastructure and resources. As for commercial panels they are readily available with assured but are costly for most Low and Middle Income Countries to sustain thus commercial acquisition of quality checkers for Xpert MTB/RIF testing sites is a major challenge and creates a cracks in External Quality Assurance program testing (Gumma *et al*., 2019).

There is an existing Xpert MTB/RIF EQA program in the country coordinated by the NTRL where 157 high sputum smear microscopy testing sites are enrolled and Xpert MTB/RIF machine placed. Even though the TB reference laboratory has the capacity to produce proficiency testing panels to assess TB testing using Xpert machine, the Kenya EQA program relies on panel donations from CDC which are inadequate for all Xpert testing laboratories country-wide, therefore they are given out selectively. For that reason, locally produced quality checkers are urgently needed to ensure there is no EQA program services interruptions which may occur when there is delays in shipment of the panels hence the quality of testing isn't assessed and eventually may lead to missed and incorrect diagnosis of patients which may impact negatively on the management and control of TB in the country (Gumma *et al*., 2019). Also consistent supplies of panels will guarantee quality in all aspects of Xpert MTB/RIF testing are tacked hence meeting the goal of End TB strategy in the country.

2.6 Impact of an Interrupted External Quality Assurance System

In the event that this EQA system is interrupted, it means there will be unreliable results being produced in the lab. This in turn will impact heavily on the individual patients, household members and community, Health care system as well as the

surveillance system. Inability to diagnose the Tb and treat the patients early exposes close relatives and the community at large to the risk of acquiring TB infection. Additionally, there is risk of exposure of the disease to healthcare providers and other patients visiting the healthcare settings due to the close contact patterns. This will therefore result to advanced occupational risk of TB infection and active TB disease amidst the health care workers (HCWs) and practicing medical students. False negative results at the patient level means that there will be delayed detection leading to intense progression of the disease increasing complications as well as death threats to the patient (Assefa *et al*., 2019).

False-positive diagnosis has major negative effects to the patient in that being put into a six-month anti-TB treatment may have adverse effects like increased toxicity in the live due to unnecessary drugs intake and even though TB treatment is free, there are those non-medical cost that the patient incurs such as transport costs during visits to the points of healthcare, increased catastrophic costs such as extra nutritional needs for the patient. There are also possible risks of loss of income due to the patient's prolonged sickness. This will impact heavily on the household member's living standards, education and may lead to poverty if at all the patient was the sole provider of the house hold. The family may also experience a lot of stigma due to the fact that their family member has TB disease (Houben *et al*., 2019).

Also due to missed diagnosis of the main causative factor of the patients' health problems, the missed disease condition (presenting with similar symptoms as TB) being confused with TB will continue progressing thus deteriorating the patients' health and eventually may lead to patients death .To the healthcare system first by

provision of un-necessary treatment to the False positive diagnosed patients, it will bring constrains to the health system leading additional cost of the healthcare system or reduction of treatment services for the rightfully deserving patients. Also, bedridden patients will over-stretched the services i.e. bed, food, increased need for care by the medical staffs (Houben *et al.*, 2019).

Surveillance system will be affected by the TB burden being falsely estimated. The resulting misleading false positive information can be an indication of great success in TB screening. And in case of deaths of the patients, it will be notified as death due to TB yet the true mortality rate may be low but the false TB death leads to false country and global TB burden and mortality reporting (Houben *et al*., 2019).

2.7 Xpert MTB/RIF Assay External Quality Assurance Materials

Following the endorsement and the massive implementation of Xpert MTB/RIF as the initial diagnostic test for TB globally, the need for robust External Quality assurance program through Proficiency testing (PT) is critical to independently check conformance of the pre-analytic, analytic, and post-analytic phases of analysis in laboratory quality management systems and identify areas that require improvement ensuring patients receive accurate and reliable diagnostic testing. PT programs have been hard to set up for many resource limited countries due to cost limitations and technical capacity (Scott *et al.*,2014).

As of 2011, there was no Xpert MTB/RIF external quality assurance program in place globally. In efforts to source for the most appropriate panels, Scotts *et al* in South Africa did a study where five (5) specific Xpert EQA panels consisting of various

matrices were innovated, developed and distributed by both commercial and noncommercial manufacturers as follows; lyophilized samples, liquid, dried culture spot, artificial sputum matrix and dried tube specimen (DTS) which have been mainly implemented in well-resourced countries (Scott *et al* 2011).

Evaluations to assess the performance of all the five panels were conducted in South Africa at 11 Xpert testing sites. Overall, there was no major differences in performance between the Vircell, CDC, GLI, or NHLS panels, indicating that all of these panels are suitable for use in an EQA program. However, for the MMQCI liquid panel, it scored low because it required cold chain maintenance of which is a challenge in many low-resource settings and as well this material could not be transferred easily into the Xpert cartridge (Scotts *et al.,* 2014). Liquid matrix similarly to culture spot required stringent storage and transportation conditions, poor stability and high prevalence rates of cross-contamination during preparation stages. DTS matrix was easy and cost-effective to produce, stable and safe to transport, easy to use and produce accurate and precise results with minimal prevalence of cross – contamination.

This study therefore adopted the DTS techniques used by Degruy et al in United States Atlanta, Georgia, United States between 2013 and 2015 to develop Dried tube specimen's panels for Xpert MTB/RIF (Degruy *et al*.,2020). The method has as well been used in other diseases like Syphilis, HIV PT schemes such as HIV viral load testing. The method is an answer to the critical need for countries to implement a simple and sustainable national PT programme in order to ensure accuracy of Xpert MTB/RIF results by producing their own PT panels for Xpert MTB/RIF assay using

equipment and supplies that are commonly available in laboratories conducting tuberculosis liquid culture (Kabugo *et al*.,2023).

CHAPTER THREE:MATERIALS AND METHODS

3.1 Study Area

This study experiments were conducted at the National Tuberculosis Reference Laboratory (NTRL), while the developed DTS piloting was done in all TB diagnostic laboratories that were using Xpert machine in Nairobi City County. The NTRL is a unit under the division of National Public Health Laboratory (NPHL), which is located in Kenyatta National Hospital grounds off Ngong road in Nairobi Kenya. The NTRL is authorized to offer quality diagnostic services in the country on AFB smear microscopy, TB culture and drug sensitivity testing. The NTRL monitors and improves the quality of laboratory testing through its External Quality Assurance program which oversees laboratories in Kenya undertaking acid fast bacilli (AFB) microscopy and Xpert MTB/RIF testing services through onsite supervisory visits, proficiency testing, blinded rechecking and training.

The NTRL also provides TB operational research support to the health systems within Kenya. The study piloting sites were: Kenyatta National Hospital (KNH), Mbagathi County Referral Hospital, Mama Lucy Hospital, Mathare North Hospital, Mathari Teaching and Referral Hospital, Kibera South Health Centre, Defense Force Memorial Hospital, Nairobi Remand Prison Health Centre, Rhodes TB Clinic, Bahati Multi Drug Resistance (MDR) Health centers, Riruta Health Centre, Nyumbani Children's Home, Eastern Deanery Aids Relief (EDARP), Aga Khan Hospital, Nairobi Hospital, Coptic Hospital, Gertrude's Children Hospital-Muthaiga, Pathologists Lancet Laboratories, International Organization for Migration(IOM), and National Tuberculosis Reference laboratory (Xpert MTB/RIF section).

3.2 Study Design

An experimental study design was adopted to produce and assess stability of dried tube specimen (DTS) panels as quality checkers for TB diagnosis using the Xpert MTB/RIF technique. To assess the performance of the developed-DTS panels in TB diagnostic laboratories that were using Xpert machine within Nairobi City County, a descriptive cross sectional study design was adopted. The Nairobi City County was purposively selected because it had the highest number of Xpert MTB RIF testing sites.

3.3 Sampling Technique

The piloting sites to evaluate the performance of the developed DTS panels were purposively selected to include all the twenty (20) TB diagnostic laboratories that were using Xpert machine in Nairobi City County, from August 2019 to December 2019.This being an experimental study, sample size calculation was not required.

3.4 Laboratory Experiments

3.4.1 Retrieval of Standard Reference Strains

Standard reference strains (*Mycobacterium Tuberculosis* and *Mycobacterium fortuitum*) used in the research were obtained from the National Tuberculosis Reference Laboratory (NTRL) stock for quality control materials, preserved in Middle-brook 7H9 broth (Becton, Dickinson and Company, USA) preserved in 30% glycerol, and stored at -80^0C. Recovery of the strains was done in reference to standard procedure for reviving glycerol stock of bacterial culture(Addgene.Org, 2014), in a Biosafety Level III(BSL) cabinet. Briefly, the strains were thawed in ice

and inverted several times to mix. The strains were then activated followings procedures described by Kyle and others (Degruy *et al*., 2020).

3.4.2 Characterization of the Clinical Strains

The Mycobacterial strains were phenotypically and genotypically characterized and assessed for purity using Brain Hearts Infusion (BHI) agar (Becton, Dickinson and Company, USA), whereby, upon thawing, each of strains were sub-cultured onto two Lowenstein-Jensen (L-J) agar (Becton, Dickinson and Company, USA) slants and incubated at 37^{o}C for a maximum of 3 weeks while in a slanting position. In the initial week, the culture tube caps were left at a quarter-turn loose and then later tightened. Using a magnifying glass to macroscopically examine colonies, growth was monitored twice in the first week and once in each succeeding week (Degruy *et al.*, 2020). The results were recorded as either Growth Obtained (GO) or No Growth Obtained (NGO).

At the end of 3 weeks, culture slopes that showed no growth were discarded. Among the strains that showed growth, colonial morphology was compared with those of source/parent strains and subjected to Ziehl Nielson straining for microscopic examination of the Acid-Fast Bacilli (Degruy *et al*., 2020). Colonies obtained on the L-J slopes were sub-cultured into liquid medium Mycobacterium growth indicator tubes (MGIT) and incubated in BD BACTEC™ MGIT™ 960 machine (Becton, Dickinson and Company, Sparks, MD, USA) for 42 days. MGIT tubes flagging positive were offloaded from the machine, recorded and incubated further 4 days in an auxiliary incubator at 37°C.

The isolates were then sub-cultured onto BHI agar to check for contamination and confirm the purity of the culture growth, with results recorded as ‘no growth obtained’ (NGO), interpreted as pure colonies or ‘growth obtained’ (GO), interpreted as contaminated with other strains and were discarded (Gumma *et al*., 2019). From MGIT tubes with NGO results on BHI smears were prepared on microscopic slides and subjected to Ziehl Nielson staining for microscopic identification of Acid-fast bacilli by either coded or non-coded characteristic beads.

The AFB-positive strains were subjected to MPT64 antigen test (Standard Diagnostics, Seoul, South Korea) to identify *Mycobacterium tuberculosis* complex (MTBC), with results recorded as positive or negative. The MPT64 antigen test positive MTBC strains were subjected to drug susceptibility testing (DST), whereas the MPT64antigen negative isolates Non-mycobacterium tuberculosis(NTM) were subjected to a Line Probe Assay (LPA) (Hain Life Science GmbH, Nehren, Germany). PCR technique which was performed according to the manufacturer's protocol to confirm strain genotype of the bacteria (Gumma *et al*.,2019). All the isolates were genotypically characterized with Xpert MTB/RIF assay based on mean and standard deviation of the probe A cycle threshold (Ct)values (Mean16-23 and SD <3), forming a baseline(expected) results for comparing strains molecular characteristics after de-activation and development of the DTS panels (DeGruy, 2020).

3.4.3 Bacterial Strains Inactivation and Verification of the Inactivation

Inactivation for positive bacterial cultures in this study was done through heat inactivation, other bacterial inactivation techniques such as the chemical method

(Xpert MTB/RIF Sample Reagent (SR) (Cepheid; Sunnyvale, California, United States) has been used by Degruy in their novel DTS-based Xpert MTB/RIF PT panel methodology where they produced Xpert MTB/RIF DTS panels between 2013 and 2015 for DTS Panel validation (Degruy *et al*.,2020).

Strains were heat-inactivated at 80-85^{o}C as described by Kabugo and colleagues (Kabugo *et al.*, 2023). Briefly, MGIT cultures were placed in a rack inside the oven and timer started when the temperature rose to 80^{o}C. The oven temperature was checked after every 30 minutes up to 60 minutes, to ensure it was at 80-85^{o}C and time recorded on the inactivation verification worksheet without opening the oven. The MGIT culture tubes were removed from the oven and allowed attain room temperature, and labeled "heat-inactivated cultures" with date of inactivation (Degruy *et al*., 2020).

Verification of the strains heat inactivation was done following procedure described by Degruy and colleagues (Degruy *et al*., 2020). All the inactivated culture isolates were homogenized by voltexing the MGIT tubes with 3 mm glass beads inside and letting them stand for 10 minutes. The supernatant was collected into sterile 50 ml falcon tubes to form the Neat stock solutions, labeled with respective name of the isolate and a stock number. From each stock solution, 500µlwas inoculated in MGIT tube labeled with respective name of the isolate, number of the isolate stock solution, and date of culturing. The MGIT tubes were capped tightly and mixed by inversion2-3 times, processing one stock solution separately to limit cross-contamination. The inoculated MGIT tubes were loaded into the BD BACTEC™ MGIT™ 960 machine

(Becton, Dickinson and Company, Sparks, MD, USA) and incubated for two cycles of 42-days each (total of 84 days), (Degruy *et al*., 2020).

After the first 42-day cycle, tubes flagging negative were scanned and a print-out filed in the Dried Tube Specimen Preparation binder. The tubes were then scanned and reloaded into the Bactec machine for the second 42-day cycle. Upon completion of the second 42-day cycle, the tubes were scanned out and the unloaded negative report stored. After 84 days, the samples flagging negative were considered as completely inactivated(Degruy *et al*., 2020). Inactivation Verification Log was generated comprising results for each MGIT tube for the two (2)42-day cycle of the viability check.

3.4.4 Preparation of DTS Panels

For every heat inactivated and verified stock solution, 0.5ml were pipetted into a sterile 50ml conical tubes labeled with respective strain, and to prepare a 1:10dilution of the isolates, 4.5ml of sterile distilled water was added and premixed with 0.5 µl green dye (food color, Kroger brand) to enhance visibility. Five cryovials (4-ml capacity) for each diluted inactivated stock strains were labeled each vial with name of isolate, and aliquot number (A-E). 5 DTS tubes were prepared from each of the 1:10 diluted stock solution by pipetting 100 µl of the diluted stock solution into the respectively 5 labeled cryovials leaving them with caps opened inside Biosafety cabinet at moderate condition, one week, until they appear visibly dried prior to fastening their caps. Dried tube specimens were then kept inside a light-free area under normal conditions (Degruy *et al*., 2020). Remaining 1:10 dilution was discarded and while the remaining stock solutions were stored in the refrigerator set at 2-8^{o}C waiting pretest results.

To determine the reliability of the developed DTS PT panels' accuracy and precision was done through pretesting on a limited scale before producing the DTS in large numbers as well as validation of the developed DTS panels. Pre-testing of the DTS was done to determine which stock solutions to include in the DTS panel production. This was done by subjecting a set of 5 panels for every inactivated strain dilution to Xpert MTB/RIF assay as per the Xpert MTB/RIF testing procedure and results recorded as 'DTS panel pretest results' (Degruy *et al*.,2020). To ascertain whether DTS generated accurate Xpert MTB/RIF assay results, findings of MTB detection and rifampicin resistance from DTS pretest were verified against the expected Xpert MTB/RIF results for the parent's strains (as initially determined through phenotypic and genotypic testing). The results were considered to be correctly matched if the qualitative MTB detection and RIF resistance results matched the parents strain expected results for all the five pretested DTS panels and Probe A Ct values mean was within the semi-qualitative range of medium (Ct of 16-23) and Low(Ct of 22-28) and SD (≤3)The pretest findings formed basis of reference for results of validation and stability assessment test of the developed DTS panels (Degruy *et al*., 2020).

Inactivated strains stock solutions with similar CT value for Probe A in pretest results were pooled together to increase volume of working stock for DTS production. The DTS stock strains were renamed and given new isolate identifiers (e.g., stock #K 1901- corresponding to stock #1; K-country Kenya, 19-year of production and #01-stock number.

Based on the proof of inactivation, pretest results and Probe A mean Ct values showing medium (16-23) to low range(22-28) and a SD of below3, five (5) stock solutions showing the lowest cycle threshold (Ct) value for Probe A were selected to comprise the DTS panels(DeGruy*et al.*, 2020). DTS stock solutions clinical strains selected included three (3) positive controls (Two MTB-detected rifampicin-sensitive and one MTB-detected rifampicin-resistant) and two Negative controls being Non Tuberculous Mycobacterium as shown in Table 4.3. As such, stock isolates (K1911, K1906, K1910, K1903 and K1907) were selected and renamed 2019-PT-1, 2019-PT-2, 2019-PT-3, 2019-PT-4 and 2019-PT-5, respectively.

To determine the number of 4-ml cryovials (aliquots) needed to be prepared per stock, at least 15% extra panels for validation, QC and instrument verification purposes were added. Stock solutions of the 5 selected strains were each dyed with food-color and serial dilutions made at a ratio of 1:3 using distilled water to increase concentration of MTB DNA in the stock solutions as well as attain the semi quantitative range of medium to low reading on the Xpert MTB/RIF assay being required range for concentration of MTB DNA for this experimental research. Cryo-vials with a capacity of 4ml were labeled, respectively, and 100µl of each of the homogenized final dilutions aliquoted. The prepared DTS were then left uncapped at room temperature in a running biosafety cabinet (BSC-II) for one week to facilitate moisture evaporation as well as sterility of the surroundings.

3.4.5 Validation of the Developed DTS Panels

To a certain accuracy and precision of the DTS PT materials, validation was performed to confirm baseline deoxyribonucleic acid(DNA) concentration after drying the developed DTS. This was done by randomly selecting eight (8) tubes from

each of the five (5) dried tubes specimen panels (prepared in final procedure of 3.4.4 above), totaling to 40 DTS panels and testing using Xpert MTB/RIF assay. The target level of detection was treated as low to medium range when Probe A CT value ranged from 16-23 and standard deviation was less than 3. Results were recorded as DTS validation results and were compared with the pre-test results as shown in Table 4.4. Validation findings formed reference for findings from piloting sites where the performance of DTS panels were evaluated.

3.5 Stability of DTS Panels

3.5.1 Effect of Drying Period on the Stability of DTS Panels

Determining effect of drying on the stability of DTS panels was necessary to establish the optimum drying time required for preparing stable DTS panels that can give quality and reproducible results. This way, a set of three (3) DTS from each DTS panel developed were left uncapped inside the BSC for 7days, 10 days, and 14 days, and immediately subjected to Xpert MTB/RIF assay. The results obtained were recorded as 'Day Zero' validation results and were compared with the pre-test results. 'Day zero validation results' were also used as a reference standard for results obtained in subsequent testing of DTS panels for temperature stability.

3.5.2 Effect of Temperature on DTS Panels' Stability

To assess the effect of varying temperatures to the stability of DTS panel, parent isolate/neat stock (K 1906) was selected and panel 2019- PT -2 prepared in large numbers (756 panels and additional 10% for weekly IQC/ instrument verification). A set 252panels were dried at each of the 7 day, 10 days and 14 days' periods. Thirty-six

(36) DTS panels from each of the 7 days/10 days and 14 days drying periods were stored at -80^{o}C, -20^{o}C, 4^{o}C for 12 weeks to assess their stability at storage temperatures (Parekh *et al*., 2010). Similarly, to assess the stability of the DTS panels at various room temperatures as anticipated in TB diagnostic laboratories that use Xpert machine across the Kenya, these panels (36 DTS panels from each of the 7 days/10 days and 14 days drying periods) were placed undisturbed for 12 weeks in each of 18^{o}C, 20^{o}C, 24^{o}C, 33^{o}C, and 40^{o}C test trial points based on meteorological records of average temperatures.

On weekly interval, a set of three (3) DTS tubes from each of the drying periods (7, 10, 14days) were retrieved from test trial temperature points and subjected to Xpert MTB/RIF assay (Parekh *et al*., 2010). Results were recorded effect of temperature on the stability of DTS. They were compared with the optimal drying period results ('Day Zero' validation results) of 2019-PT-2 upon drying at 7 days, 10 days and 14 days.

3.6 Performance of DTS Panels

Ten percent (10%) of developed DTS PT panelswere pretested at Kiambu County Referral Hospital, Xpert TB Laboratory section in June 2019.To evaluate the performance of individual laboratories in producing accurate and reliable results using the developed DTS PT panels as the quality checker, DTS panels and testing kits were sent to all 20 TB diagnostic laboratories that use Xpert machine within Nairobi City County. DTS PT panels were packaged using the standard packaging of non-infectious agents. A set of five PT panels,5 disposable sterile dispensing pipettes, processing directives, and Proficiency Test results evaluation form were placed in a

sealable transparent bag and delivered to the targeted piloting sites. At the laboratories, the personnel testing patient's samples at the moment performed the processing of the DTS panels as per the given instructions. Results were printed from the Xpert MTB/RIF machine, entered into the reporting form, scanned and sent to the principal researcher for analysis and scoring.

3.7 Data Analysis

Obtained data was manually entered and analyzed with Microsoft Excel (Microsoft Corp, United States) whereas stability data was analyzed using STATA v17 and variables were summed up in medians (Interquartile range) and means, Standard Deviation. The means of the various DTS PT panels subjected at different test trial points were compared with validation means (drying point at 'Day Zero' results).

Paired t-test was used to classify the mean as statistically different when the p-value<0.005. Box-plots were formulated to detect dissimilarity in the probe A Ct, median values of the different DTS PT panels at respective test trial temperature point over the 12 weeks' period. For DTS piloting, a descriptive data was generated and presented in tables and figures. Each of the five pilot DTS panels was assigned an accuracy score of 20 points, and individual laboratory scores as follows; incorrect determination of either MTB detection (0 points), unsuccessful result (error, invalid, or no results) (5 points), RIF-indeterminate result (10 points), and correct determination of both MTB detection and RIF resistance (20 points). Total scores for each laboratory were computed, with ≥80% being considered satisfactory and those <80% unsatisfactory.

3.8 Ethical Approval and Permit

No human specimen collection was required in this study hence Ethical approval was exempted and research permit was obtained from National Commission for Science, Technology and Innovation (Appendix III). Participation in Xpert MTB/RIF testing was voluntary and free of charge incentives were not provided.

CHAPTER FOUR: RESULTS

4.1 Development of DTS panels for Xpert MTB/RIF Proficiency Testing at National Tuberculosis Reference Laboratory.

4.1.1 Characterization of Parent Strains

Figure 4.1 and Table 4.1 below shows results of phenotypic and genotypic characterization of the parent strains. From the 20 isolates that were retrieved, only16(80%) produced colonies on Lowenstein Jensen (LJ) media,12 (60%)had colonial morphology characteristic of the parent strains and flagged positive in Mycobacterium Growth Indicator tubes (MGIT). Eighty-four percent (83.7%, 10/12) of the parent strains were pure AFB positive isolates. Of these, 80% (8/10) had characteristic coded colonial morphology and wereMPT64 antigen positive. Seventy-five (6/8) %) of the MPT64 positive isolates were pan-susceptible MTB (SS-MTB), whereas 2 (25%, 2/8) were rifampicin-resistant MTB strains (RR-MTB). The MPT64 antigen negative (20%, 2/10) parent strains were LPA- confirmed to be Non tuberculous mycobacterium (NTM). All the strains were genotypically characterized further by Xpert MTB/RIF assay and findings recorded as baseline results.

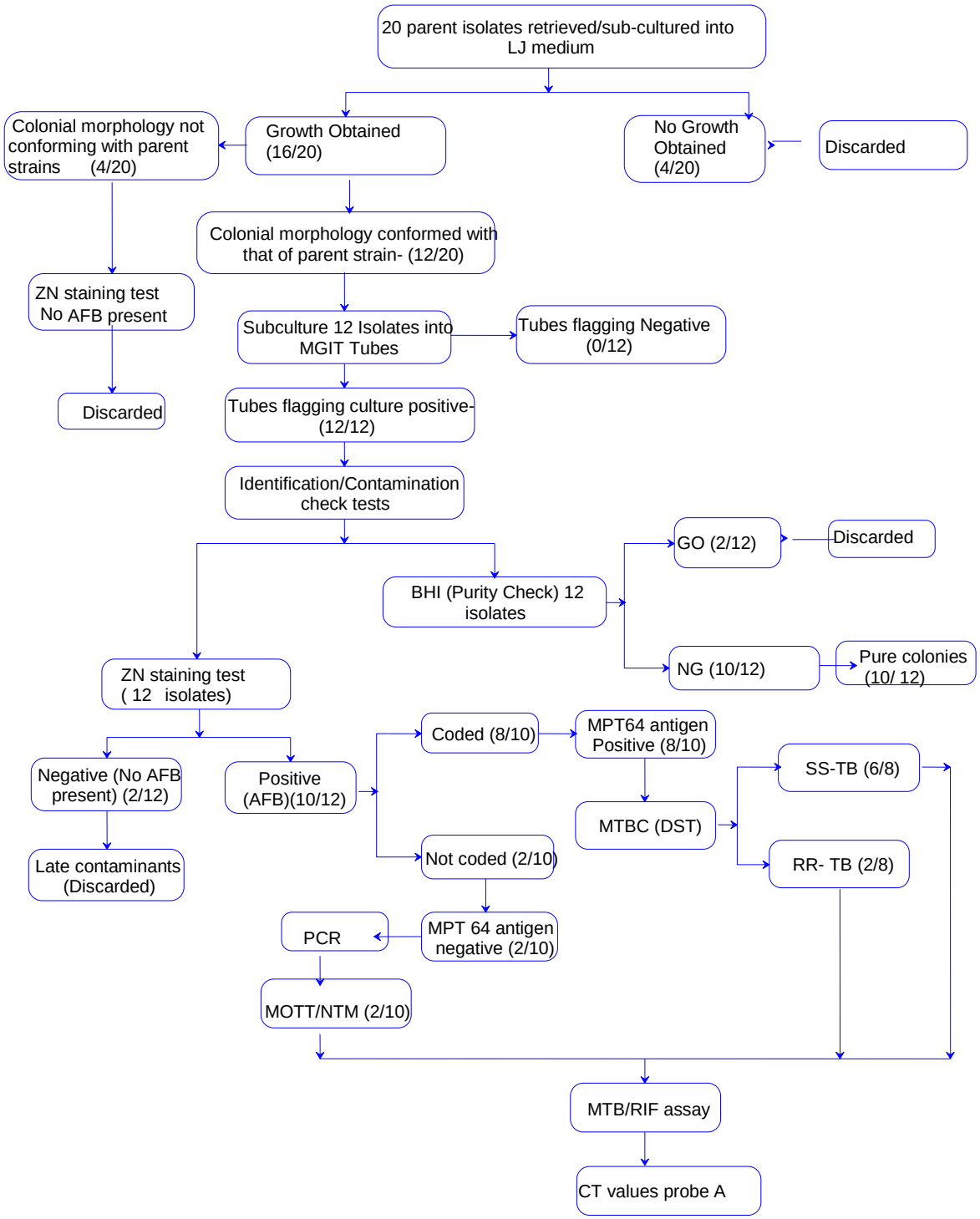

Figure 4.1: Flow diagram on characterization of parent strains

GO- growth obtained, NGO- no growth obtained, MGIT-Mycobacterium Growth Indicator Tube, *Mycobacterium Tuberculosis*/RIF-Rifampicin, CT-Cycle Threshold, NTM-Non *Mycobacterium Tuberculosis*, PCR- Polymerase Chain Reaction, AFB-Acid Fast Bacillus, RR-TB- Rifampicin Resistant Tuberculosis, SS-TB- Pan Susceptible Tuberculosis, BHI- Brains hearts infusion, ZN- Zeehl Nielson, LJ-Lowenstein Jensen

Table 4.1: Characterization of Parent Strains

Parent strains (2016/S/..)	Frequency Total = 20	Isolates Identification and Characterization			
		Bacillus coding	**MPT64 Antigen Test**	**PCR/ DST test**	**Baseline results MTB/RIF ASSAY**
7	1(5%)	Not	Negative	NTM	MTB (ND)/(NA)
003,	3 (15%)	Coded	Positive	MTBC	MTB (D)/RIF (ND)
016	1 (5%)	Not	Negative	NTM	MTB ND)/RIF(NA)
009, 011	2 (10%)	Coded	Positive	MTBC	MTB (D)/RIF (D)
013 and 017	3 (15%)	Coded	Positive	MTBC	MTB (D)/RIF (ND)
No growth	4 (20%)	_	_	_	_
Growth NC Isolates conformed, ZN Negative	4(20%) 2 (10%)	_	_	_	_

NG: No growth, **NC:**not-conformed, **NCD:**Not Coded, **MTB(ND)/RIF(ND):***Mycobacterium tuberculosis* Rifampicin resistance Not detected, **MTB(D)/RIF(D):***Mycobacterium tuberculosis* detected/rifampicin resistance detected, **MTB(D)/RIF(ND):** *Mycobacterium tuberculosis* detected/rifampicin resistance not detected, **NTM** Non *tuberculous Mycobacterium* , **NA:**Not applicable

4.1.2 Heat Inactivation and Verification of Stock Strains

Upon heat inactivation of bacterial strains, there was no growth obtained in MGIT, interpreted as 100% (10/10) inactivation success rate, Table 4.2.

Table 4.2: Heat-inactivation and Verification of Stock Strains

Serial no.	Sample Stock	MTB Baseline results	RIF-R Baseline Results	Bacteria strain	Inactivation	
					Growth MGIT	Remarks
1.	2016/S/3	Medium	ND	SS- MTB	Neg	Passed
2.	2016/S/4	Medium	ND	SS- MTB	Neg	Passed
3.	2016/S/7	ND	N/A	NTM	Neg	Passed
4.	2016/S/8	Very Low	Detected	SS-MTB	Neg	Passed
5.	2016/S/9	Medium	Detected	RR-MTB	Neg	Passed
6.	2016/S/10	High	Detected	RR-MTB	Neg	Passed
7.	2016/S/13	Medium	ND	SS-MTB	Neg	Passed
8.	2016/S/16	ND	N/A	NTM	Neg	Passed
9.	2016/S/17	Very Low	ND	SS-MTB	Neg	Passed
10.	2016/S/ 20	Very Low	ND	SS-MTB	Neg	Passed

RIF-R: rifampicinresistance, **ND:** Not detected, **MTB:** Mycobacterium tuberculosis, **NTM:** Non-tubeculosis mycobacterium, **SS-MTB:** Pan-susceptible *Mycobacterium tuberculosis*, **RR-MTB:** Rifampicin resistant *Mycobacterium tuberculosis*, **Neg:** Negative

4.1.3 Pre-testing and Preparation of DTS Panels

Of all the well characterized and inactivated strains5 DTS PT panels were prepared and from subjected to Xpert MTB/RIF assay (50 DTS panels in total). Sixty percent (60%, 6/10) of these bacterial strains showed Xpert MTB/RIF pre-testing results conforming to the expected (baseline) results, and were assigned new identifiers based on Probe A Ct values, Table 4.3. Those with similar Ct values were pooled together. Five (5) stock samples of three varying Xpert MTB RIF test results were selected and incorporated for DTS panel preparation phase:2negative control strains- MTB not detected, 3 positive control strains (2 being MTB Detected, Rifampicin Sensitive, one (1) MTB Detected, Rifampicin resistant).

Table 4.3: Pre-testing and Preparation of DTS Panels

Sample Stock	MTB Detection	RIF-R	Probe A (Ct)		Bacteria strain	Isolate ID.	Remarks
			Mean	SD			
2016/S/3	Medium	ND	19.8675	0.4512	SS- TB	K 1906	**Retained**
2016/S/4	Medium	ND	19.275	0.4647	SS- TB	K 1906	**Retained**
2016/S/7	ND	N/A	0	0	NTM	K 1911	**Retained**
2016/S/8	Very Low	Detected	29.2754	0.9647	SS-MTB	K 1908	**Discarded**
2016/S/9	Medium	Detected	18.4561	0.7478	RR-MTB	K 1903	**Retained**
2016/S/10	High	Detected	13.4394	1.7641	RR-MTB	K 1903	**Discarded**
2016/S/13	Medium	ND	21.2751	0.6473	SS-MTB	K 1907	**Retained**
2016/S/16	ND	N/A	0	0	NTM	K1910	**Retained**
2016/S/17	Very Low	ND	30.5095	0.8780	SS-MTB	K1917	**Discarded**
2016/S/20	Very Low	ND	29.0095	1.3945	SS-MTB	K1920	**Discarded**

SD: Standard Deviation, **Ct:** Cyclethreshold, **RIF-R:** Rifampicin resistance, **ND:** Not detected, **MTB:** *Mycobacterium tuberculosis*, **NTM:** Non-tuberculous mycobacterium, **SS-MTB:** Pan-susceptible *Mycobacterium tuberculosis*, **RR-MTB:** Rifampicin resistant *Mycobacterium tuberculosis*, **Neg:** Negative, **N/A:** Not applicable, **ID:** Identifier

4.1.4 Validation of DTS Panels

For all the 40 DTS panels selected, probe A Ct values were within the expected mean range of 16-23 and standard deviation (SD) of ≤3. There was 100% agreement level between DTS testing and pre-testing results, Table 4.4.

Table 4.4: Validation of DTS Panels

Isolates	MTB Detection	RIF-R	Probe A (Ct)		Agreement level (%)
			Pretest	Validation	
K1911 (2019-PT-1)	ND	N/A	0	0	100
K1906 (2019-PT-2)	Medium	ND	19.87±0.45	20.63 ± 1.95	100
K1910 (2019-PT-3)	ND	N/A	0	0	100
K1903 (2019-PT-4)	Medium	Detected	18.4±0.74	17.83 ± 0.40	100
K1907 (2019-PT-5)	Medium	ND	21.3±0.6	19.87 ± 2.06	100

SD: Standard deviation, **K1911 – K1907:** All panels used, **MTB:** *Mycobacterium tuberculosis*, **RIF-R:** Rifampicin resistance, **N/A:** Not applicable, **ND:** Not detected**.**

4.2 Stability of in-country-developed DTS Panels at National Tuberculosis Reference Laboratory

4.2.1 Effect of Drying Period on the Stability of DTS Panels

There was 100% concordance (agreement level) between Xpert MTB/RIF results obtained with DTS panels dried for 7,10 or 14 days and the pretest results in this study, Table 4.5.

Table 4.5: Effect of Drying Period on the Stability of DTS Panels

DTS panel Number	Probe A Ct value reading (Mean ± SD)				Agreement level/ Concordance (%)
	Expected (Pretest) results	7 days	10 days	14 days	
K19011	0.0	0.0	0.0	0.0	100
(2019-PT-1)	±0.0	±0.0	±0.0	±0.0	
K1906	19.89	20.7	19.90	17.83	100
(2019-PT-2)	±0.4	± 1.14	±2.05	±0.40	
K19010	18.28	18.63	18.43	18.17	100
(2019-PT-3)	±0.97	±0.12	±1.10	±0.58	
K1903	0.0	0.0	0.0	0.0	100
(2019-PT-4)	±0.0	±0.0	±0.0	±0.0	
K1907	21.3	19.83	19.8	19.53	100
(2019-PT-5)	±0.6	±2.91	±2.89	±2.56	

SD: Standard deviation, **Ct:** Cycle threshold, **DTS:** Dried Tube Specimens

4.2.2 Effect of Temperature on the Stability of DTS Panels

To determine the effect of temperature on stability of the study DTS panels, a total of 756 DTS panels dried for7-day, 10-day and 14-day exposed to different temperature points, -80^{o}C, -20^{o}C, 4^{o}C, 18^{o}C, 24^{o}C, 33^{o}C, and 40^{o}Cfor 12weeks period. A paired t test was used to compare Probe A Ct value means of the different samples to the day "0" means (Validation). All DTS panels showed Xpert MTB/RIF probe A Ct values within the acceptable limits for medium to low (16-23) and standard deviation (SD) (≤3).Except for DTS panels held at -80^{o}C, the probe A mean Ct values of panels dried for 7 and 14 days increased with increasing temperature (from -20^{o}C to 40^{o}C) during the 12 weeks study period (p= <0.001), Table 4.6.Overall, the greatest mean Ct value increment was at 40^{o}C.The overall accuracy and precision of the DTS panels based on validation results was 100%.

Table 4.6: Effect of Temperature on the Stability of DTS panels

Day	Temp	Level of Agreement/ concordance		Median (IQR)	Probe A Ct Mean (SD)	t-value	*P*-value	MD
		MTB	RIF-R					
7	**Day 0 validation**	**100(100)**	**100(100)**	**17.9(17.5-19)**	**18.1(0.7)**			
	-80^{o}c	36(100)	36(100)	17.8 (17.4-18.8)	18.2(0.8)	-1.493	0.136	0.1
	-20^{o}c	36(100)	36(100)	18 (17.3-18)	17.8(0.6)	5.166	<0.001	0.3
	4^{o}c	36(100)	36(100)	18 (18.5-18.9)	18.4(0.8)	-4.48	<0.001	0.3
	18^{o}c	36(100)	36(100)	18.1 (17.4-19.1)	18.4 (0.9)	-4.777	<0.001	0.3
	24^{o}c	36(100)	36(100)	18.5 (17.5-19.5)	18.6 (0.8)	-7.999	<0.001	0.5
	33^{o}c	36(100)	36(100)	18.8 (18.2-19.2)	18.8(1.2)	-36.199	<0.001	0.7
	40^{o}c	36(100)	36(100)	19.3 (20.8-18.5)	20.7(0.9)	17.33	<0.001	1.1
10	**Day 0 validation**	**100(100)**	**100(100)**	**18.1 (17.4-19.1)**	**18.4(0.9)**			
	-80^{o}c	36(100)	36(100)	18.8 (17.7-19)	18.5(0.9)	-2.785	0.003	0.1
	-20^{o}c	36(100)	36(100)	18 (17.6-19)	18.6(0.7)	-1.247	0.106	0.2
	4^{o}c	36(100)	36(100)	18.3 (18.7-19.6)	18.1(0.8)	-3.955	<0.001	0.3
	18^{o}c	36(100)	36(100)	18.6 (18.1-19.4)	18.7(0.8)	-3.955	<0.001	0.3
	24^{o}c	36(100)	36(100)	18.5 (17.7-18.9)	18.9(0.6)	-3.742	<0.001	0.5
	33^{o}c	36(100)	36(100)	19.2 (18.9-20.1)	19.6(0.9)	-14.967	<0.001	1.2
	40^{o}c	35(97)	35(97)	19.7 (18.8-20.2)	19.8(1.2)	-14.816	<0.001	1.4
14	**Day 0 Validation**	**36(100)**	**36(100)**	**18.8 (18.5-19.3)**	**18.4(0.9)**			
	-80^{o}c	33(92)	33(92)	18.4 (17.6-18.5)	18.5(0.7)	-1.392	0.165	0.3
	-20^{o}c	36(100)	36(100)	17.7 (17-17.9)	18.7 (0.6)	-4.403	<0.001	0.3
	4^{o}c	36(100)	36(100)	18.3 (17.4-18.5)	18.1(0.8)	3.955	<0.001	0.3
	18^{o}c	36(100)	36(100)	19.1 (17.5-18.6)	19(0.7)	-8.354	<0.001	0.6
	24^{o}c	36(100)	36(100)	18.9 (18.6-19)	19.1(0.5)	-10.793	<0.001	0.7
	33^{o}c	36(100)	36(100)	19.2 (19.1-20. 3)	19.5(0.7)	-15.315	<0.001	1.1
	40^{o} c	35(97)	35(97)	19.7 (18.6-20.4)	20.1(0.9)	-21.203	<0.001	1.7

Paired samples t-test, **statistically significant, **MTB:** *Mycobacterium tuberculosis*, **RIF-R:** Rifampicin resistance detection, **SD:** Standard deviation, **IQR:** Interquartile range, **Temp:** Trial Temperature points, **MD:** Mean Difference.

Among the DTS panels (7,10 and 14 days old) the mean Ct values equally varied across all the testing points. There was a common pattern across all DTS panels exposed to 33^{o}C and 40^{o}C for the12 weeks, showing the highest increase in Probe A Ct values compared to other DTS exposed to other temperatures, Table 4.6 and figure 4.1 a, b, c largely, agreement level for detecting M.*tuberculosis* and rifampicin resistance among trial panels examined within 12weeks of the stability study and the parental stocks was 100%.From Table 4.6, no erroneous negative or positive result were reported, and there was no any untrue rifampicin resistance detection or un-interpretable results reported among the three different DTS PT panels that were exposed in varying temperatures. In all the panels tested throughout the stability testing period, only 5 errors encountered all showing 'No result'.

To visualize the results, stability investigation findings were represented in box-plots as shown in figure 4.1a, 4.1b, 4.1c below to represent outcomes of each of the DTS PT panels(7/10 and 14 days). The study sought to investigate the differences in CT values across seven temperature points, -80^{o}c, -20^{o}c, 4^{o}c, 18^{o}c, 24^{o}c, 33^{o}c and 40^{o}c based on three DTS panels (7-day, 10-day and 14-day). The graphs highlights comparison of the Day Zero Validation results with outcomes of each type of panel upon exposure to varying temperature points to ascertain changes in sample stability.

The resulting assessment from the 7 days old DTS panels established a slight disparity in the median Ct values for panels tested under -80^{o}C, 4^{o}C and 18^{o}C temperatures from the validation results. The lower median value of CT for panels tested at test trials tested at -20^{o}C as compared with validation scores. The highest median of CT values was seen in panels stored at 24^{o}C, 33^{o}C and 40^{o}C indicating that there was

deviation from validation median. The box plotting for 10 days DTS panels established that there was a marginal variation in median value of Ct value in panels stored at-20^{o}C with the expected results. However, for all other panels stored at -80^{o}C, 4^{o}C, 18^{o}C, 24^{o}C, 33^{o}C and 40^{o}C their median CT values were higher than median CT values for 10 days old validation PT panels.

Outliers were observed at -20^{o}C, 24^{o}C and 40^{o}C temperatures for the 14 days old DTS panels, which deviated substantially from the validation median Ct values. However, this didn't affect the allocation of single values allocation on every side of the median. Generally, box-and whisker diagrams plotted for all the panels revealed that medians were not far from each other at all trial temperatures within the 12 weeks of the study apart from testing 40˚C storage temperature which had relatively higher medians but this didn't interfere with the Probe A detection of MTB and RIF in DTS PT panels. Overall, the plots showed narrow box-plot ranges and shorter whisker lengths throughout the three DTS PT panels but increase was witnessed as the test trial temperatures increased. The box and whisker diagrams broadened in panels kept at 33˚C and 40˚C indicating elevated median values in comparison to panels at -20˚C, -80˚C, 4˚C, 18˚C (Figure 4.1a-c).

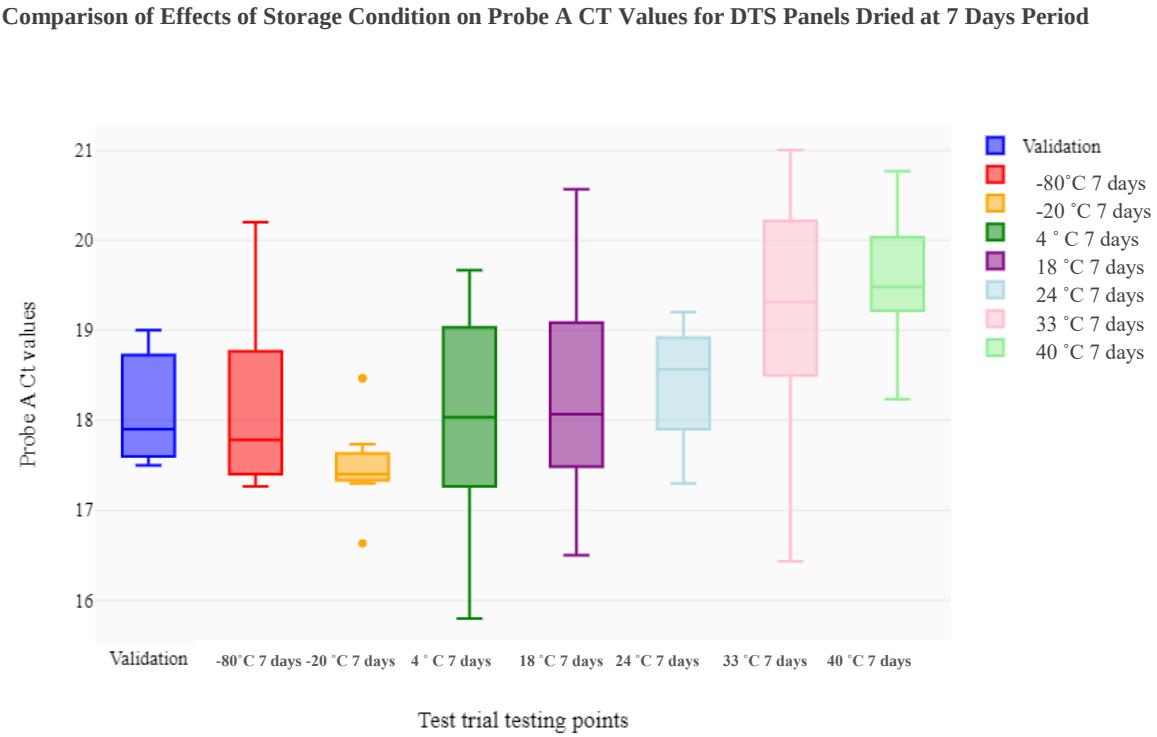

Figure 4.1a: Comparison of Effects of Storage Condition on Probe A CT Values for DTS Panels Dried at 7 Days Period

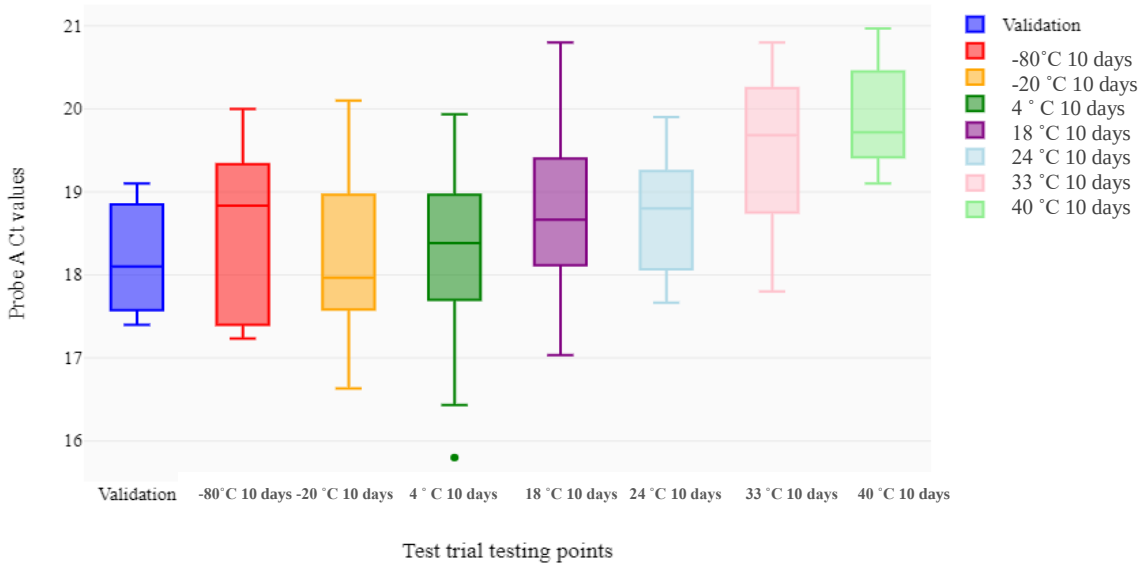

Figure 4.1 b: Comparison of Effects of Storage Condition on Probe A CT Varies for DTS Panels Dried at 10 Days Period

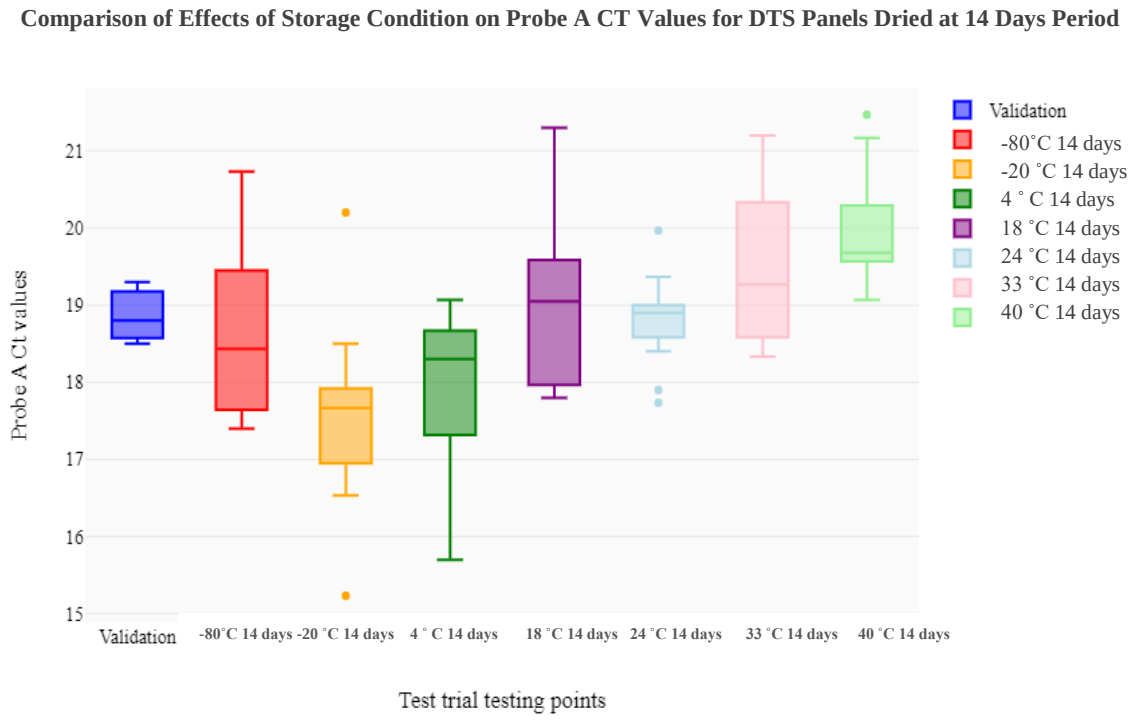

Figure 4.1c: Comparison of Effects of Storage Condition on Probe A CT Values for DTS Panels Dried at 14 Days Period

4.3 Performance of DTS Panels in Xpert MTB/RIF Testing Laboratories, Nairobi City County

4.3.1 Aggregate Performance of Participating Xpert MTB/RIF Testing Sites

There was 100% participation of all selected laboratories in the piloting study. Of these,95% (19/20) successfully detected *M.tuberculosis*and rifampicin resistance as expected, whereas five percent (1/20)of the laboratories gave discordant results. Remarkable performance of 100% was recorded with panel 2019- PT-5 from all participating laboratories reporting expected results of ‘MTB detected’ with ‘No Rifampicin resistance’. Panels 2019- PT-1 and 2019-PT-3, containing Non-tuberculous Mycobacterium (NTM) and formal saline, respectively, were used as negative controls. As such, no 'MTB detection' was expected. However, for

panel2019-PT-1, only 90% (18/20) conformed to this expectation, whereas one site (5%, 1/20) reported discordant results, false MTB detection and another site (5%, 1/20) reporting error (code 5007). For panel 2019-PT-3, 95% (19/20) reported correct results but 5% (1/20) of the sites gave discordant findings, reporting false MTB detection as compared with the expected results and results obtained from all other piloted sites.

Performance of the participating laboratories on panels 2019-PT-2 and 2019-PT-4, Ninety per cent (18/20) of the participating laboratories correctly reported results of MTB detection ('MTB detected'), as expected but a portion of 10% of the sites (2/20, 10%) gave discordant results for panels 2019-PT-2 and 2019-PT-4.Only 95% (19/20) of the piloting sites reported expected results ('MTB/RIF detected')for panel 2019-PT-4, with one site (5%, 1/20) reporting an error (code 5007) in this panel, thus failing to report the expected results.

In all those sites that reported discordant results it was associated with high staff turnover in the facilities hence overload of work leading to technical errors in analytical and post analytical phase. In this study, none of the participating laboratories reported 'un-interpretable result' or 'invalid result' in all the DTS PT panel samples tested (Table 4.7).

Table 4.7: Aggregate Performance of Overall Participating Xpert MTB/RIF Testing Sites

	2019-PT – 1	**2019-PT – 2**	**2019-PT – 3**	**2019-PT – 4**	**2019-PT – 5**
Total number of participating sites, n (%)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
MTB Detection					
Sites detecting TB, n (%)	1 (5)	19 (95)	1 (5)	19 (95)	20 (100)
Sites not detecting TB, n (%)	18 (90)	1 (5)	19(95)	0 (0))	0 (0)
Sites reporting an un-interpretable TB result, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sites with missing TB detection result,	0 (0)	1 (5)	0 (0)	0 (0)	0 (0)
RIF Resistance Detection					
Sites detecting RIF resistance, n (%)	0 (0)	0 (0)	0 (0)	19 (95)	0 (0)
Sites not detecting RIF resistance, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sites reporting an un-interpretable RIF result, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sites with missing RIF detection results,	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Invalid Results/Errors					
Sites reporting invalid	0	0	0	0	0
No result	0	0	0	0	0
Sites reporting error, n (%	1(5)	0	0	1(5)	0
Specific codes	5007	-	-	5007	-

MTB: *Mycobacterium tuberculosis*, **RIF:** Rifampicin, **2019-PT–1 to 2019-PT-5**Proficiency testing panels

4.3.2 Performance of Individual Laboratories

To evaluate laboratory performance, sites reporting satisfactory and unsatisfactory scores were individually determined. Satisfactory results, defined as a score of 80% and above score, were obtained in 90% (18/20) of the piloted sites, whereas an excellent score, 100%, was reported in 16/20 (80%) of the laboratories. Ten percent (10%, 2/20) of the sites reported unsatisfactory results, defined as a score less than 80%. These sites were NC-004 and NC-007, each scoring 60% (Figure 4.2).

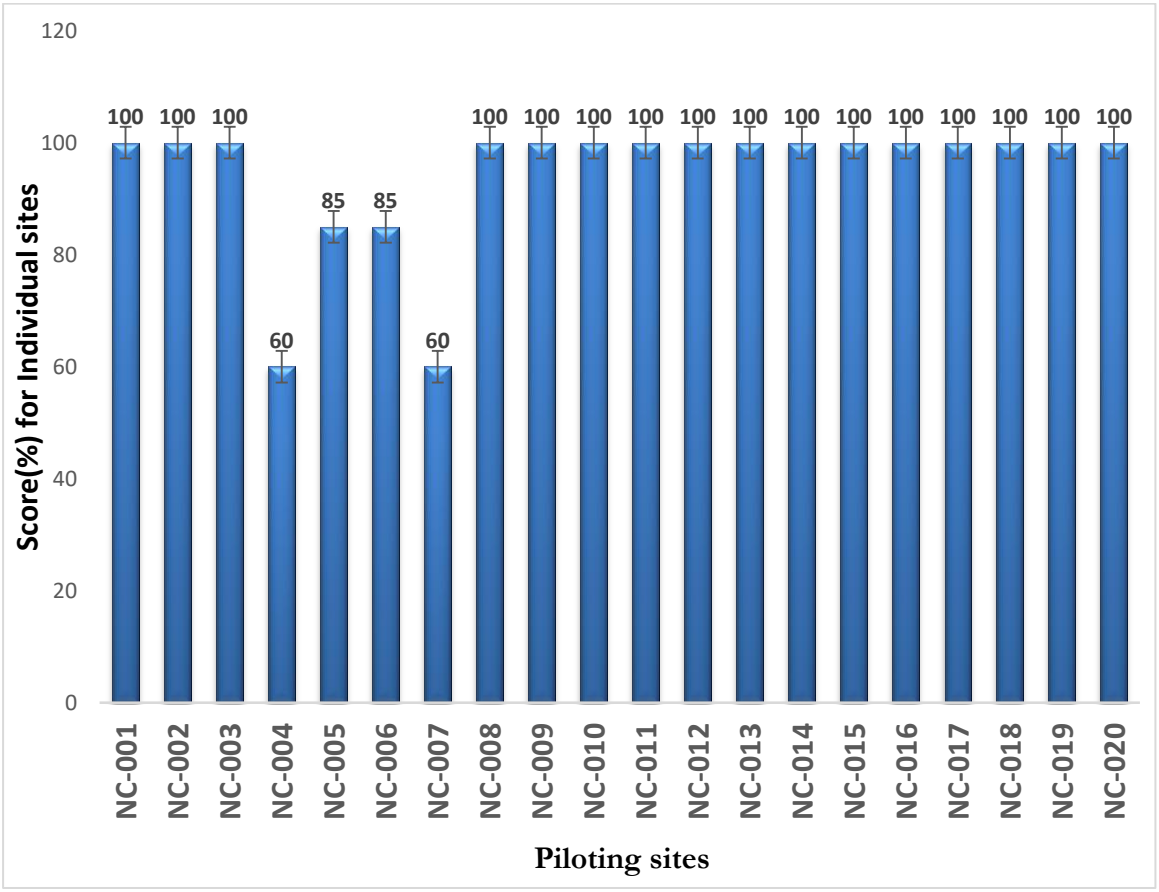

Figure 4.2: Performance of Individual Laboratories

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Development of DTS Panels for Xpert MTB/RIF Proficiency Testing at National Tuberculosis Reference Laboratory

There are no locally- produced DTS panels in Kenya, and since 2014 this country has relied solely on donation the PT materials from CDC Atlanta, USA. However, the supply has been unreliable and insufficient for the over 170 Xpert MTB/RIF testing sites in the country, creating quality control gaps and posing the risk of unreliable diagnosis with negative impact on patient management and TB control programs (Enos *et al*.,2016). To alleviate the global shortage of Xpert MTB/RIF DTS PT materials, CDC Atlanta embarked on a technology transfer program to equip countries in resource-limited settings with the required expertise and capacity to develop quality and cost-effective DTS materials (Degruy *et al*.,2020).

This study findings showed that it was easy to use the locally available materials at TB reference Laboratories to developed DTS panels for Xpert MTB/RIF proficiency testing in Kenya using *Mycobacterium tuberculosis* and *Mycobacterium fortuitum* standard reference stock strains which proofed to be fully Heat inactivated thus ascertaining safety while working with panels at lower levels of healthcare settings (Degruy *et al*.,2023).This study demonstrated total agreement in identification of *Mycobacterium tuberculosis* and rifampicin susceptibility among test trial test trials performed for every developed DTS PT panels with their expected results from the pretest, corroborating those of Scotts and colleagues on “Multicenter feasibility study to assess external quality assessment panels for Xpert MTB/RIF assay in South

Africa"(Scott *et al*., 2014). This means that the procedures undertaken to develop the DTS panels in the current study were homogenous; thus, the panels validation runs gave reproducible, accurate and precise results. As such, the current study demonstrates success in Xpert MTB/RIF DST panels development. Therefore the DTS PT panel development technique can be used boldly to produce quality PT materials which will be used to evaluate quality aspects of *Mycobacterium tuberculosis testing* in all diagnostic facilities which use Xpert machine (Scott *et al*., 2014).

Additionally, with assurance on quality and capacity in the DTS production at the NTRL as evidenced by the 100% concordance of the developed in-country DTS panels with the parent's strains, accuracy and precision of the developmental process is guaranteed and therefore, it is possible to efficiently upscale production of cost effective and reliable quality checkers for TB diagnosis using the Xpert machine in the entire county. Similar findings have been reported elsewhere by Degruy and colleagues in Atlanta Georgia, USA where they demonstrated that DTS production process is scalable to obtain large numbers of PT materials from a single heat-inactivated liquid bacterial culture(Degruy*et al*., 2020).But in most countries with pre-existing Xpert MTB/RIF proficiency testing (PT) programs, coverage is usually confined at the national and regional laboratories because of unavailability or limited supplies of DTS production materials in other levels of TB culture laboratories (Klein *et al*., 2020).

5.1.2 Evaluating the stability of in-country-developed DTS panels at National Tuberculosis Reference Laboratory

5.1.2.1 Effect of Drying Period on the Stability of DTS Panels

DTS panels dried for 7 days, 10 or 14 days all subjected to XPERT MTB/RIF assay agreed 100% with the pretest expected results. This study agrees with that of Parekh and other where they developed DTS PT panels for HIV by drying their specimens inside a biosafety cabinet at ambient temperature overnight. Although their dying testing period was short, but they employed similar technique (Parekh et al 2010). This suggests that the developed panels were stable regardless of the number of days they have taken to dry; and therefore, preparation of DTS panels can be made by drying for 7 days to shorten the production period (Parekh *et al*., 2010).

5.1.2.2 Effect of Temperature on the Stability of DTS Panels

Findings demonstrated that DTS panels heldat-80˚C, -20˚C, 4˚C,18˚C, 24˚C, and40˚C remained stable for upto 12 weeks. Our study agreed with that of Germanio and colleague who employed the same technique while evaluating the stability of HIV Dried tube specimen panels for 12 weeks but differed with our study by their addition of disaccharide trehalose during the development process to stabilize the panels. The current study finding suggests that DTS can be stored at this temperature for a period of up to three months (Di-Germanio *et al*.,2023). However, DTS panels with high mean Cycle threshold values depreciated faster when compared to DTS panels with lower Cycle threshold value readings. This finding corroborates that of Kabugo *et al* using a modified Xpert MTB/RIF (Ultra) which employs the same technique as the Xpert MTB/RIF (Kabugo *et al.,* 2023). They observed that panels held at 2–8˚C

remained consistently steady and be capable of being used efficiently as proficiency testing materials in about a year maintained at that temperature (Kabugo *et al*.,2023).

The standard deviation variation recorded from the different trial temperatures tested in this study were less than three (<3); thus, conforming to the parental stock strains results (validation at Day zero) which is a marker of homogeneity as well as stability of deoxyribonucleic acid inside DTS panels. The results demonstrate that despite exposing the DTS panels to extreme temperature condition, the consistency in DTS integrity was still maintained at high level. This was also demonstrated by Kyle and others (Degruy *et al*., 2020).

There was no statistical difference in the overall detection of MTB and rifampicin resistance or susceptibility in the stability evaluation of DTS panel which was calculated as a change in Ct values, with all DTS panels within the mean range of 16-23 and SD of < 3. Additionally, there was 100% concordance with the validation results across all the 3 DTS panels tested across the 12 weeks' study period. Similar findings have been reported in Uganda by Kabugo and collegues where they associated this with rpoB gene amplification (for detection of MTB strains and rifampicin receptive and defiant genes) being uniformly acted upon by test trial conditions and holding durations of the panels at this conditions across all the DTS panels but still maintained its integrity (Kabugo *et al*.,2023).

Overall comparison between DTS panels evaluated at storage temperature -80˚C, -20˚C and 4˚Cat varying climatic conditions in our country as represented by temperature 18˚C, 24˚C, 33˚C and 40˚C showed marginal variation of median Ct

values from validation results for storage temperature -80˚C, -20˚C and 4˚C compared to the highlighted higher variation among the 18˚C,24˚C,33˚Cand 40˚C DTS. This probably is because it is hard to achieve consistency with routine room temperature storage. Constantly, mean Ct value increase with over 0.5 among the panels stored at 24˚C, 33˚Cand 40˚C suggested ambient temperatures influence or lower MTB DNA accumulation in contract to DTS panels stored at -20˚C, -80˚C and 4˚C.This finding corroborates with those of Kabugo and collegues in Uganda who observed that DTS Panels held at 2-8˚C remained reliably stable compared to panels held at room temperature (Kabogo *et al*.,2023).

Degeneration of DTS panels occurred most in those exposed to 40˚C for three months' period, causing more scattered Ct values and giving the greatest mean Ct value increment across all the DTS PT panels used. This finding could be attributable to heating of the DTS panels leading to increased mean difference of about 1.7. However, the probe A Ct mean difference and mean range for all DTS panels were within the expected values of 16-23 and no inhibition of MTB and RIF detection was witnessed among all the tested DTS panel. This data agrees with that of a study done by Al-Griw *et al*. on Life Science postulations which emphasizes on degeneration of deoxyribonucleic acid upon exposure to increased temperatures which lowers deoxyribonucleic acid accumulation and decreasing DTS stability (Al-Griw *et al*., 2017).

The overall concordance for the detection of *M.tuberculosis* and rifampicin resistance between DTS panels tested within 12 weeks of the stability study and the parental stocks were 100%. This means that the DTS panels were prepared in an appropriate method and the MTBDNA in the DTS panels can remain intact even after exposure to

varying temperature conditions (Di-Germanio *et al*.,2023). For that reason, this clearly shows that the DTD panels are stable and can be used as quality checkers across the country as quality checkers of TB diagnosis using the Xpert MTB/RIF method. The current study findingsubstantiates the fact that this technique has proven to be the backbone through which coverage gaps in provision of proficiency testing materials and internal quality control materials in Xpert MTB/RIF testing laboratories can be realized (Kabugo*et al*.,2023).

5.1.3 Performance of DTS Panels in Xpert MTB/RIF Testing Laboratories, Nairobi City County

This study evaluated the performance of the developed dried tube specimen (DTS) materials externally by the end users, with 100% of the piloted site participating. Ninety-five per cent (95%) of the piloted sites reported the expected DTS panel results, suggesting DTS production technique employed was proper and directives on how to carry out the test were convenient whereas 10% (2/20) had discordant outcomes. These discordant findings have also been reported elsewhere in Uganda (Kabugo *et al*., 2021) and Viet Nam (Gumma *et al*., 2019) and have been attributed to technical errors in the testing facilities majorly related to high rate of staff turnover in many institutions in resource-limited countries is a common challenge that contributes to staff being overwhelmed by work and, in turn, compromising the quality of results (Klein *et al*., 2021). In the current study, the discordant results were attributable to analytical errors such as technical errors during sample processing, cross-contamination, switching of samples or transcriptional errors during reporting of results. However, onsite supervisory visits were undertaken to the underperforming laboratories to support them to promptly identify gaps and provide speedy

interventions through corrective and preventive action plans that mitigate the risk of TB misdiagnosis (Klein *et al*., 2021).

Ninety per cent (18/20) of the participating laboratories correctly reported MTB detection ('MTB detected') results, as expected for panels 2019-PT-2 and2019-PT-4. These findings corroborates those of Klein and others (Klein *et al*., 2021), whereby at in their study, least 90% of the participating laboratories demonstrated the ability to produce satisfactory results that conformed with the predicted results. These findings can be attributed to the competency of staff and adherence to standard operating procedures, as well as regular Xpert MTB/RIF equipment maintenance and servicing (MacGregor-Fairlie *et al*., 2020;Tamil *et al*., 2015).

The errors (code 5007) reported in this study (sites NC-004 and NC-007), were associated with poor equipment maintenance, including adequate cleaning of the plunger rods, as the potential factors that could have led to probe integrity issues. This agrees with findings reported by Klein *et al*. in Atlanta during performance evaluation of their developed PT panel across 14 countries which were using Xpert MTB/RIF testing for TB diagnosis (Klein *et al*., 2021). Unforeseen financial constraints in most resource-limited setting countries make it hard to maintain service warranties for Xpert MTB/RIF equipment. Also, the reported errors in the current study could be attributed to poor pipetting skills, potentially introducing air bubbles and putting insufficient samples into the Xpert machine cartilages (Hanrahan *et al*., 2016). These would suggest the staff training status is wanting and poor adherence to MTB/RIF assay standard operating procedure (SOP). The staff capacity building, onsite supervisory visits, and proficiency testing are paramount and indispensable

components of external quality assessment in Xpert MTB/RIF diagnosis to promptly identify gaps and risks that can potentially affect the patient diagnosis and treatment outcomes. Strict adherence to schedules for equipment maintenance and servicing ensures reduced Xpert MTB/RIF equipment error, thus avoiding delay in service delivery or TB diagnosis that can negatively impact the patient management and TB control programs (Scott *et al*., 2014).

Ninety per cent (18/20) of the laboratories reported a satisfactory score (80% and above), while 10% (2/20), site NC-004 and NC-007, reported unsatisfactory results (60%). These findings are lower than those documented for round 1 proficiency testing in Uganda (Kabugo *et al*., 2021). The discrepancy between the two study findings could be due to the difference in the number of participating laboratories, whereby the study in Uganda involved 490 laboratories, while in the current study, 20 sites participated. However, ours was a pilot study, and 20 piloting sites were successfully used in another study by Parekh and others in evaluating the performance of dried tube specimens HIV proficiency for use in resource-limited settings (Parekh *et al*., 2010).

5.2 Conclusions

i. All DTS panels developed in this study showed Xpert MTB/RIF probe A Ct values within the acceptable limits for medium to low (16-23) and standard deviation (SD) (≤3), and 100% agreement level between testing and pre-testing results.

ii. The developed in-country DTS panels remained reliably stable regardless of the drying period and the testing temperatures they were exposed for the 12 weeks' study period.

iii. The probe A mean Ct values of panels dried for 7,10 and 14 days increased with increasing temperature from -20^{o}C to 40^{o}C during the 12 weeks' period, with the greatest increment occurring at 40^{o}C

iv. The overall performance of in-country-developed DTS panels as proficiency testing materials for Xpert MTB/RIF assay in Nairobi City County was 90% (18/20), with two (10%, 2/20) discordant results being reported.

v. The majority (80%, 16/20) of Xpert MTB/RIF assay sites in Nairobi City County reported excellent results (100% score), with only two (10%, 2/20) laboratories, NC-004 and NC-007, documenting unsatisfactory results (<80% score).

5.3 Recommendations

This study recommends;

i. Seven (7) days drying period in development of DTS panels.

ii. That DTS panels can be stored at temperate between -80^{o}Cand 40^{o}C for a maximum of twelve (12).

iii. Policies to have service contracts for Xpert MTB/RIF in all health facilities to mitigate errors due to equipment mal-function; thus, curbing service delivery interruptions.

iv. Up-scaling the production of the DTS panels at the National Tuberculosis Reference Laboratory (NTRL) for proficiency testing in all Xpert MTB/RIF testing sites in Nairobi City County and beyond.

5.3.1 Recommendations for Further Studies

i. Studies to evaluate the effect of temperature on stability of DTS panels for a period beyond 12 weeks to inform on the optimum storage conditions, especially in facilities without reliable electricity supplies.

ii. Studies evaluate performance of the currently developed DTS panels in Xpert MTB/RIF testing sites from multiple counties across Kenya for more generalizable data.

iii. Studies to develop a web-based platform for transmitting quality assurance (QA) results directly from the Xpert MTB/RIF testing sites to the National Tuberculosis Reference Laboratory to avoid transcriptional errors at the reporting phase.

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APPENDICES

Appendix I: Standard Bacterial Reference Strains

Mycobacterium Tuberculosis	ATCC 27294 or H37rv
Mycobacterium fortuitum	NTM

Appendix II: Introductory Letter

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: P150/CTY/PT/26853/2018 DATE: 10th February, 2020

Director General,
National Commission for Science, Technology and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MS. MARGARET WAIRIMU NGANGA – REG. NO. P150/CTY/PT/26853/18

I write to introduce Ms. Margaret Wairimu Nganga who is a Postgraduate Student of this University. She is registered for M.Sc. degree programme in the Department of Medical Laboratory Science.

Ms. Nganga intends to conduct research for a M.Sc. thesis Proposal entitled, "Development, Stability and Piloting of In-Country Dried Tube Specimen Panel for Xpert Mtb/Rif Proficiency Testing at National Tuberculosis Reference Laboratory, Kenya."

Any assistance given will be highly appreciated.

Yours faithfully,

for PROF. ELISHIBA KIMANI
DEAN, GRADUATE SCHOOL

Appendix III: Ethical Permit Exemption

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: chairman.kuerc@ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Tel: 8710901/12

Website: www.ku.ac.ke

Our Ref: **KU/ERC/ EXEMPTION/VOL.1 (001)** Date: 12th September 2023

Margaret Wairimu Nganga
P.0 Box 43844-00100
Nairobi.

Dear Ms. Nganga,

APPLICATION NUMBER: PKU/2810/I1934 - DEVELOPMENT, STABILITY AND PILOTING OF IN-COUNTRY DRIED TUBE SPECIMEN PANEL FOR XPERT MTB/RIF PROFICIENCY TESTING AT NATIONAL TUBERCULOSIS REFERENCE LABORATORY, KENYA"

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic **" Development, stability and piloting of in-country dried tube specimen panel for Xpert mtb/rif proficiency testing at National Tuberculosis reference laboratory, Kenya"**

2. APPLICANT

MARGARET WAIRIMU NGANGA

3. SITE

NATIONAL TUBERCULOSIS REGERENCE LABORATORY , KENYA

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines and **EXEMPTED from having an Informed Consent for research participants.**

5. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE P.O. Box 43844 - 00100 NAIROBI
12 SEP 2023

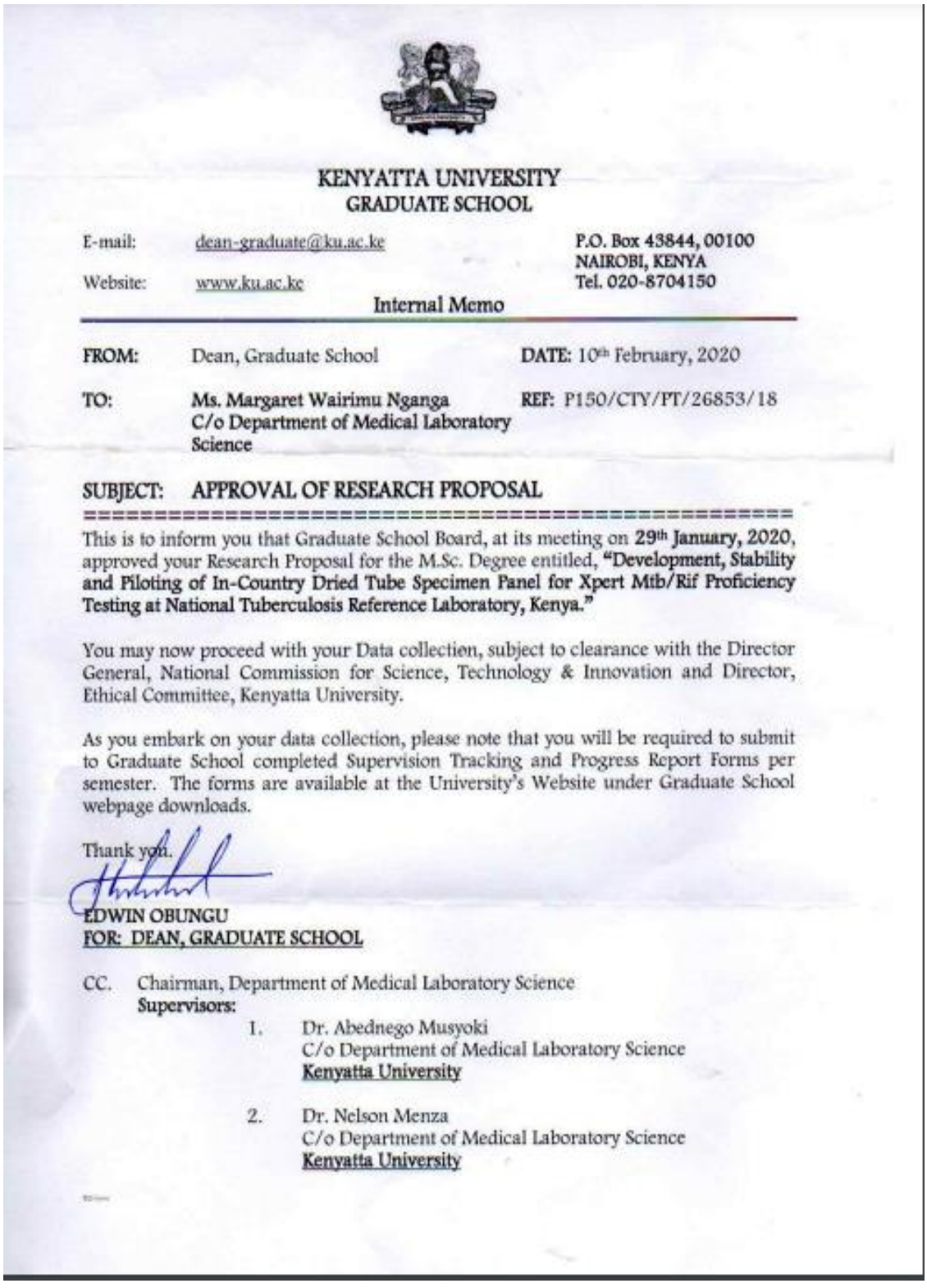
PROF. JUDITH KIMIYWE
CHAIRMAN ETHICS REVIEW COMMITTEE

I MARGARET W. NGANGA accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of 12TH SEP 2023.

cc.
DVC-Research Innovation and Outreach

Appendix IV: Research Authorization



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke
P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Dean, Graduate School **DATE:** 10th February, 2020

TO: **Ms. Margaret Wairimu Nganga**
C/o Department of Medical Laboratory Science
REF: P150/CTY/PT/26853/18

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on **29th January, 2020**, approved your Research Proposal for the M.Sc. Degree entitled, **"Development, Stability and Piloting of In-Country Dried Tube Specimen Panel for Xpert Mtb/Rif Proficiency Testing at National Tuberculosis Reference Laboratory, Kenya."**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation and Director, Ethical Committee, Kenyatta University.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

EDWIN OBUNGU
FOR: DEAN, GRADUATE SCHOOL

CC. Chairman, Department of Medical Laboratory Science
Supervisors:

1. Dr. Abednego Musyoki
   C/o Department of Medical Laboratory Science
   Kenyatta University
2. Dr. Nelson Menza
   C/o Department of Medical Laboratory Science
   Kenyatta University

AppendixV: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **435825**

Date of Issue: **02/April/2020**

RESEARCH LICENSE

This is to Certify that Ms.. MARGARET WAIRIMU NGANGA of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: DEVELOPMENT, STABILITY AND PILOTING OF IN-COUNTRY DRIED TUBE SPECIMEN PANEL FOR XPERT MTB/RIF PROFICIENCY TESTING AT NATIONAL TUBERCULOSIS REFERENCE LABORATORY, KENYA for the period ending : 02/April/2021.

License No: **NACOSTI/P/20/4470**

435825

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

AppendixVI: Map of XPERT MTB /RIF testing sites piloted in Nairobi City County