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SPECIMEN REFERRAL SYSTEM-RELATED FACTORS ASSOCIATED WITH LABORATORY TB CASE DETECTION: A CROSS-SECTIONAL STUDY

By

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DECLARATION

I, Robertson Chibumbya, hereby declare that the work to be presented is my own original work undertaken in partial fulfilment of a **Master of Science degree in Epidemiology and Biostatistics** and that it has not been submitted before for any degree in any other university or college and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Signed: ______________________________ Date: 2026/01/08

CERTIFICATION

The undersigned certifies that the document has been read through and hereby recommends acceptance of the dissertation entitled: Specimen Referral System related factors associated with TB Case Detection in Kafue District.

Supervisor: Dr Fredrick Chitangala

Signed: ______________________________ Date: 8/01/2026

DEDICATION

I dedicate this research to my wife Christine Musonda Chibumbya, and my entire family for the sacrifice they made during the period I was studying. Further, I dedicate it to Professor Mutemwa for encouraging me to undertake this program. I also wish to dedicate this research to my supervisor Dr Chitangala, for the fatherly guidance provided through the research process. God bless you all.

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ABSTRACT

Background: Tuberculosis (TB) remains a public health concern globally. To effectively fight and control TB, enabling timely access to rapid diagnostic tools is key for the detection of bacteriologically confirmed TB. A weak TB diagnostic network could lead to an increased number of undiagnosed TB cases due to limited access to improved rapid diagnostic tools.
Aim: To determine the association between sample referral factors and the detection of bacteriologically confirmed TB cases.
Method: The researcher used an analytical cross-sectional design to evaluate the impact of specimen referral system factors on TB case detection in routine healthcare settings. The data on bacteriologically confirmed TB cases and number of presumptive TB were collected from the facility-based presumptive register from January to December 2024. The electronically generated questionnaire was used to capture information on factors such as availability of the refrigerator, type of facility, type of rider, frequency of sample pickup, distance between referring site and the diagnostic. The adjusted multivariable negative binomial regression was fitted to determine the association of outcome and predictors.
Results: The adjusted negative binomial regression revealed that frequency of sample picks up ($p = 0.048$) and number of samples submitted ($p < 0.0001$) were highly associated with case detection. Other variables such as presence of the refrigerator, trained rider, type of rider, facility attendance and presumptive TB clients that submitted samples were not associated with TB case detection.
Conclusion: This study was conducted to determine the association of bacteriologically confirmed TB and referral related factors. It has been noted that frequency of sample pickup and number of samples submitted had an impact on case detection. These findings suggest that investment specimen referral logistics may remarkably improve TB detection in the health facilities.

Keywords: *Specimen Referral, System-Related Factors, Tuberculosis Case Detection*

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LIST OF ABBREVIATIONS AND ACRONYMS

ASLM	African Society for Laboratory Medicine
DHL	Dalsey, Hillblom and Lynn
DNA	Deoxyribonucleic acid
EID	Early Infant Diagnosis
HIV	Human Immunodeficiency Virus
IEC	Information Education & Communication
LF-LAM	Lateral Flow Urine Lipoarabinomannan assay
MoH	Ministry of Health
NTP	National Tuberculosis Program
PLHIV	People Living with HIV
SRS	Sample Referral System
TAT	Turnaround Time
TB	Tuberculosis
UNICEF	United Nations Children's Fund.
USAID	United States Agency for International Development
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Tuberculosis (TB) remains the leading cause of death from a single infectious agent globally, surpassing other major communicable diseases. In 2023, more people were infected with TB than with HIV/AIDS, highlighting the persistent global burden of the disease despite decades of control efforts (WHO, 2024). TB continues to pose a major public health challenge, particularly in low- and middle-income countries where health system constraints limit timely diagnosis and treatment.

In Zambia, despite substantial national efforts to end TB, the disease burden remains high. The estimated TB incidence rate in 2023 was 271.8 per 100,000 population, while the notification rate for new and relapse TB cases was 220.5 per 100,000 population (WHO, 2025a). This gap between estimated incidence and notified cases suggests ongoing challenges that limit the detection of many more TB cases in both facilities and communities. Delayed diagnoses contribute to continued community transmission, an increased number of deaths and mortality, and poor treatment outcomes. To address these challenges, the End TB Strategy emphasises improving access to World Health Organisation (WHO)-recommended rapid diagnostic (WRD) tests (WHO, 2025a). Rapid diagnostic technologies play a critical role in early TB detection by reducing diagnostic delays, enabling prompt initiation of treatment, and limiting further transmission within communities.

Globally, however, access to WRD remains limited. In 2022, only 3.5 million of the estimated 7.5 million newly diagnosed TB cases worldwide were tested using WRD platforms, with the African region accounting for about 48% of this coverage (WHO, 2022). In Zambia, around 70% of newly diagnosed TB patients were tested using WRD methods, indicating notable progress but also highlighting that a significant proportion of presumptive TB cases still lacked access to rapid diagnostic testing. This gap emphasises ongoing disparities in diagnostic access, especially in resource-limited settings. The most widely used WHO-recommended rapid diagnostic test for TB is the Xpert MTB/RIF Ultra assay. This molecular test detects both *Mycobacterium tuberculosis* complex (MTBC) and rifampicin resistance, allowing for rapid diagnosis and early detection of drug-resistant TB. Due to limited scalability of the test, the impact of Xpert MTB/RIF Ultra heavily depends on the functionality of specimen referral systems, timely sample transportation, and efficient laboratory networks. If the specimen transport system is weak, it can impair access to these highly sensitive tools, ultimately affecting TB control efforts.

1.1.1 GeneXpert Technology and Scale-up

According to the CDC, the Xpert MTB/RIF assay is a cartridge-based assay that is used with GeneXpert platforms. Once the sputum sample is collected from the presumed TB patient, it is thoroughly mixed with the reagent buffer to liquify the sample before processing. The cartridge is inoculated with the sample reagent mix before running the tests. Sample processing is done with a limited number of manual procedures to prevent errors and cross-contamination. The assay is also designed to detect multidrug-resistant TB (MDR TB), another form of TB which takes longer to treat. MDR TB is TB that is resistant to both isoniazid (INH) and RIF. RIF resistance is a predictor of MDR TB because resistance to RIF frequently occurs alongside resistance to INH. Therefore, timely detection of RIF resistance using these newer rapid tests that are cartridge-based allows TB patients to be initiated on effective TB treatment compared to the use of drug susceptibility testing (DST) methods with longer testing time, such as mycobacterium growth indicator tube (MGIT) or Lowenstein Jensen Medium (LJ) (CDC, 2025). Globally, the cost of cartridges is not cheaper. The cost of one cartridge is $9.98, which is offered as a discounted price. As of 2017, 34 million cartridges were sold to 130 of the 145 eligible countries. The discounted priced is not applicable in private sites (Ponnudurai et al., 2018). The high cost of cartridges poses a significant barrier to large-scale implementation of the rapid test. The most reasonable solution to the challenge is to implement a robust and effective sample referral system where non-diagnostic facilities are linked to the diagnostic sites in a network.

1.1.2 Specimen Transport System

A specimen referral network is a range of collaborative actions where health facilities without testing capacity refer samples to the TB diagnostic sites. Two types of referral systems exist; vertical or horizontal. A vertical referral is when a sample is referred to the higher-level facility, while a horizontal referral is sending samples to a facility that is at the same level to the referring sites (ASLM, 2018). To implement an effective courier system, the health system should ensure that management, transportation and logistics, human resources, infrastructure, equipment, and supplies and monitoring are put into consideration. According to Challenge TB guidelines, transport systems such as motorcycles are utilised to transport TB specimens from the referring site to the nearest GeneXpert diagnostic facility.

While transporting these samples, riders should be provided with rider's gear and other materials such as cool boxes and triple-package containers. When vehicles are used, these must have a cold chain with specific temperature requirement such as room temperature, 2-8 °C and -20 °C. Prior to the implementation of the transport system, sample referral maps must be developed to provide efficient routes between peripheral health facilities and TB diagnostic site. These facilities must be organized in distinct groups. These defined groups of facilities should be allocated to either a driver or rider with a schedule stipulating the time and sample pick up frequency. Triple packaging materials such as sputum cups, transportation boxes, Standard Operating Procedures (SOPs), guidelines, and recording/reporting tools must be made available. The SOPs and guidelines must be developed for specimen collection, triple packaging procedures, sample transport, specimen tracking, biosafety, and the return of results. Other documents such as referral forms and registers, tracking slips, transportation manuals, logbooks, and data collection tools for monitoring and evaluation must be available. In addition, health facility staff must be trained in the collection and triple packaging. To ensure stability of sputum samples, fridges must be provided for the storage of collected specimens. Communication systems must be established regarding sample collection and return of results. Lastly, mentoring, supervision and corrective action should be part of the implementation process (challenge TB, n.d.).

Prior to 2020, the specimen transport systems in Zambia had huge challenges that included poor integrated system, limited access to readily transport to allow smooth movement of samples, long turnaround time and delayed result feedback especially in rural settings. All these issues led to inefficient patient management across the full range of health services. In response to this challenge, MoH in collaboration with stakeholders developed the sample integrated referral guidelines in 2020 to facilitate the establishment of an efficient and sustainable integrated specimen transport and referral system to support different types of samples such as TB and HIV (MoH, 2020). A hub and spoke model were established and implemented across the country to provide equal access to newly deployed rapid methods. This model consisted of central hub where samples were collected from multiple sites and delivered to the hub for either immediate testing or for further transportation to other labs with testing capacity. This allowed prudent use of resources as cost of transportation was drastically reduced (DHL, 2024). To improve oversight and better resource allocation, the referral must be segmented into different levels such inter-sections; Inter-provincial, inter-district and intra district courier system. The inter-provincial and inter-district was supported by postal courier and using MoH vehicles. Intra-district courier is supported using, mainly, motorbikes procured by both MoH and partners.

1.1.2.1 Laboratory Information System

Effective sample tracking and result feedback using a laboratory information system (LIS) should be an integral part of the referral system. The MoH implemented eLABs and DISA*LAB, digital health solutions, aimed at strengthening collaboration across clinical, laboratory, and patient pathways across the specimen value chain. The main purpose of this implementation was to provide automated tracking and tracing of specimens in the diagnostic cascade. (RightToCare, 2022). According to Mezzanine, eLABS was implemented to improve patient management especially in low-resource settings where paper-based processes are prevalent and laboratory information systems are fragmented. eLABS digitised visibility, and accountability to improve turnaround time and lower sample rejection rates. The application system provided for immediate notifications of patient results to the referring facility and real-time monitoring of specimen testing status (Mezzanine, 2024). DisaLab was developed to facilitate sample registration, monitor workloads, manage test requisitions, access patient records, manage doctors' profiles, track samples, and create test summaries. The system also allowed workstations management at multiple locations, create customized worksheets, view test results, conduct audits, and distribute electronic reports in multiple formats. Additionally, it allowed medical personnel to access central data repositories, set up custom lab equipment interfaces, view patient demographics, manage electronic health records (EHR), and create barcode labels for request forms (Software Advice, 2025).

This study is founded on the health system strengthening theory which clearly emphasize developing initiatives that improve access, coverage, quality and efficiency with a well-maintained infrastructure (WHO, 2024b). Specimen referral system is one of the core strategies that ultimately improve access of non diagnostic sites to high quality and sensitive diagnostic services. Drawing from this theory, the study conceptualized TB case detection as a function of specimen referral factors such as frequency of sample pick up, distance to the diagnostic sites, presence of the refrigerators, type of the rider transporting the TB samples and facility workload. Hence, this research was designed to assess specimen referral system factors that could affect TB case detection in Kafue district.

1.2 Statement of the Problem

Despite Kafue's well-established TB program, they continue facing a reduction on the number of bacteriologically confirmed cases. For example, bacteriologically confirmed TB cases declined over the past two years despite the availability of highly sensitive rapid TB molecular methods at two facilities. The proportion of bacteriologically confirmed TB cases dropped from 86% in 2022 to 63% in 2024; indicating a reduction of 27% in laboratory confirmed cases. During the same period in 2024, active case finding (ACF) activities were implemented at all levels of health care system in the district with support from implementing partners, but the situation remained the same. Some of the ACF activities were screening of household contacts to confirmed TB patients, People living with HIV (PLHIV), relapse cases, those who were treated visiting the outpatient and those admitted in the facilities (WHO, 2015a). In addition, outreach programs in the communities with the use of the computer aided detection TB (CAD4TB) were also part of the ACF activities. All these strategies have not yielded remarkable improvement in the number of TB cases identified in the district. Some have attributed poor performance to limited access to healthcare, rural residence, gender, financial insecurities, long distance to the health facilities, poor knowledge and awareness regarding TB, poor referral mechanisms and inadequate resources, untrained staff and lack of sensitive diagnostic tools have been attributed as reasons for low number of confirmed TB cases (Kuo et al., 2023b). Other specimen transport system factors such as inadequate specimen packaging materials, poor transport system, lack of sample storage spaces and failure to submit samples by patients have the potential to affect TB patient identification. Though inadequate specimen referral system has been cited as one of the contributing factors, no studies have been systematically conducted to provide empirical evidence. Therefore, it is critical to understand the gap in the specimen transport system and identify areas for improvement. If the decline of TB cases is not addressed, the district will continue missing TB cases and may lead to worsening TB burden in the district and thereby reversing the gains the TB program has achieved for a long time.

1.3 Study Objectives and Hypotheses

1.3.1 General Objective

To assess the impact of specimen referral factors on TB case detection in health facilities.

1.3.2 Specific Objectives

1.3.2.1 To determine if the frequency of sample pick up is associated with TB case detection.

1.3.2.2 To assess if the distance to the diagnostic site is associated with TB case detection.

1.3.2.3 To determine if the availability of a refrigerator to support cold chain system is associated with TB case detection.

1.3.2.4 To assess whether the use of dedicated riders contributes to TB case detection.

1.3.2.5 To examine the association between facility attendance, number of presumed TB cases, number of samples submitted and TB case detection.

1.3.2.6 To assess whether type of health facility based on setting is associated with TB case detection.

1.3.3 Research Hypotheses

Null Hypothesis:

H_0: Specimen referral system factors (TB sample pick up frequency, distance to diagnostic site, presence of the refrigerator, geographical setting of health facility, facility workload, type of rider and trained riders) have no significant effect on TB case detection rates.

Alternative Hypothesis:

H_1: Specimen specific factors (TB sample pick up frequency, distance to diagnostic site, presence of the refrigerator, geographical setting of health facility, facility workload, type of rider and trained riders) have significant effect on TB case detection rates.

1.4 Study Rationale

The sample transportation system is a critical component of the health care delivery continuum, particularly in the diagnosis and management of tuberculosis (TB). An efficient and reliable specimen referral system ensures timely movement of patient samples from peripheral health facilities to diagnostic laboratories, thereby supporting early detection, timely access to treatment, and improved health outcomes among TB patients. The failure to effectively implement this system may result into delayed transportation, loss of specimen quality which can ultimately lead to submission of poor-quality samples. All these can compromise the accuracy and timeliness of TB diagnosis if not addressed. This research is therefore necessary to identify sample referral–related factors that contribute to low TB case detection. These factors may include sample pick up schedules, rider availability and competency; logistical coordination, specimen handling procedures, and challenges related to distance or infrastructure. Understanding these gaps can enable the development of interventions supported by empirical evidence aimed at strengthening the integrated sample referral system. By addressing specific barriers within the sample courier system, this study seeks to enhance the functional, efficient and stable specimen transportation systems, improved laboratory turnaround times, and ultimately support early TB detection and better patient outcomes. Strengthening this component of the health system will also contribute to broader TB control efforts and robust health system, particularly in resource-limited settings.

1.5 Scope of the Study

This study focused on examining key specimen referral system-related factors and their influence on tuberculosis (TB) case detection in Kafue District, Zambia. The study included all health posts and health centres in rural and urban setting with limited sample testing capacity and refer TB samples to the nearby GeneXpert diagnostic facility. Specifically, the study investigated the distance to the diagnostic facility, availability of refrigeration for specimen storage, frequency of sample pickups, and the type of riders responsible for transporting TB specimens. These factors were systematically selected based on their potential to affect the timeliness, integrity, and reliability of sample transport, which are key components of an efficient diagnostic cascade for prompt TB diagnosis. The research covered the entire calendar year of 2024, allowing for a comprehensive analysis of trends, patterns, and challenges in TB case detection at different time intervals attributable to potential inefficiencies in the specimen referral system. Hence, the study captured periodic seasonal changes, fluctuations in sample workloads, and operational obstacles that could influence diagnostic outcomes. It is important to note that this study did not explore other determinants of TB case detection, such as socio-economic barriers, patient-related factors, or broader health system gaps unrelated to specimen referral, such as stock-outs of reagents or equipment failures. Therefore, the findings specifically reflect the influence of referral system factors rather than the full spectrum of variables that may affect TB diagnosis and management. Additionally, the study’s results are specific to Kafue District. Therefore, caution should be exercised when attempting to generalize the findings to other regions of Zambia. The small sample size of the health facilities included and the focus on a single district mean that contextual differences in infrastructure, workforce, and logistical capacity in other districts may lead to varying results of the research. Despite these limitations, the study aimed to generate evidence-based recommendations for the Ministry of Health (MoH) and other stakeholders to enhance TB case detection rates in Kafue District. By identifying service delivery gaps and areas for improvement within the specimen referral system, the study provides actionable insights that can provide guidance for policy making, program planning, and targeted actions, ultimately contributing to more efficient TB diagnostic processes and improved patient wellbeing in the district.

CHAPTER TWO
LITERATURE REVIEW

2.1. Overview

An effective referral system remains key in improving TB case finding in the health care system. This literature review explored current body of knowledge on the specimen referral system and TB case detection at global and regional level. In addition, the current bottlenecks to implementation were reviewed. The literature search was conducted using PubMed, Google Scholar and WHO library. We used Boolean operators and advanced search filters during the research process. References and citations were generated using mybib.com application system.

2.2. Global Perspective

According to WHO (2025), a total of 10.8 million were reported as having been infected with TB in 2023 of which 56% were men while 28% were women and the remaining being children. In 2014, WHO proposed the End TB strategy that advocated for reduction of TB incidence by 80% and TB deaths by 90%. It further aimed to reduce TB related catastrophic costs for the patients by 2030 (WHO, 2015). To meet these targets, it requires improved TB diagnostic capacity and access to highly sensitive technologies (Mohammed et al., 2020c). Hence, the effective control of TB cases should rely not only on effective treatment of patients but also on the rapid diagnosis of TB, hence, requiring quick access to fast, accurate and rapid diagnostic platforms (WHO, 2024). For accurate and rapid detection of TB, the WHO recommends molecular methods such as MTB/RIF Ultra and Truenat assays. Though molecular methods have improved case detection, studies have shown that there are still challenges associated with the implementation of rapid methods. For instance, several studies conducted in low- and middle-income countries found that material resources and service implementation challenges affected implementation at all levels (Brown, Leavy and Jancey, 2021).

Globally, finding TB is not easy. The National Library of Medicine (2008) reported that TB case detection is a complex task and requires that patients have access to health care infrastructure and high-quality diagnostic laboratory procedures. According to WHO (2016), all national TB programmes (NTP) should focus on establishing a network of TB laboratories that have a strong and efficient referral system to improve case detection. This strategy improves access to high-quality patient care while reducing catastrophic costs for TB patients (WHO, 2022b). International organizations such as World Health Organization (WHO), the U.S. Centres for Disease Control and Prevention (US-CDC), the United Nations Dangerous Goods Programme (UN DGP), and the International Organization for Standardization (ISO) have all provided guidelines on how to implement and roll out specimen transport systems. However, implementing the system is still a challenge in most TB programs. The gaps include poor coordination mechanisms, minimal national financial support, poor implementation of laboratory policies, poor transport services, and insecurity (Dama et al., 2024).

Dowdy (2025) stated that lack of specimen referral contributes to high mortality of TB patients in the health systems. Despite the importance of the referral system, in most settings, sample transport is not planned from the beginning of the program implementation (Riders for Health, 2024). Specimen transportation should be optimal. Riders for Health (2024) clearly stated that if transportation of biological samples is delayed, they tend to degrade quickly, and this may not allow for quality assured results. That is the reason why the International Health Regulation (2005) requires that biological samples are transported in a safe manner to ensure reliable and accurate results (WHO, 2005). Specimen transportation has major components.

2.3. Regional Perspective

In Sub Saharan Africa, specimen referral remains a challenge in the health systems programs. A study conducted by Kebede et al (2016) revealed that poor specimen-transport logistics, lack of riders and specimen containers including extended turnaround time (TAT) affected transportation system. Another study conducted in Guinea showed that the specimen referral challenges were associated with coordination and lack of trained staff to safely transport the samples (Standley et al, 2019). A study in Ethiopia also found that diagnosis of TB was being affected by passive screening, inadequate financial, material and human resources (Mohammed et al., 2020). All these studies mainly identified gaps relating to health system inputs and none highlighted gaps associated with sample referral. In contrast, a study conducted in Uganda found that out of all clients that were eligible for testing, only 26% of the patients had their sputum samples referred for testing (Nalugwa et al., 2020b). This was attributed to poor linkages to diagnostic sites and failure of the hub & spoke model to improve uptake of Xpert testing. Most studies have not highlighted the importance of decentralization in the diagnostic cascade when implementing the specimen referral system. This finding shows that reducing the distance to the diagnostic facility by placement of additional machines can facilitate and improve sample movement in a timely manner.

2.4. Local Perspective

According to the Ministry of Health (2022), Zambia has a total of 537 laboratories located in the ten provinces. For efficient laboratory support, the labs are organised in a four-tiered structure, namely, health centre, district, provincial and tertiary level structure serving a population of 19.7 million (ZSA, 2024). Due to an increase in demand, the country has expanded the use of the primary test for TB using GeneXpert devices from 69 in 2016 to 291 in 2020 (Girdwood et al, 2023). Despite the rapid scale-up of the GeneXpert technology, access remains a huge challenge. Lungu (2022) reported that only 44% of the total population had access to TB diagnostic services, implying that 66% were underserved due to limited access. In a diagnostic network optimisation (DNO) study conducted in Zambia, Girdwood et al (2023) found that the proportion of TB tests performed onsite reduced from 73% to 69% while the total Xpert MTB/RIF tests examined remained the same. In addition, a TB specimen was transported within a distance of 11km to the testing site on average but later reduced to 7km after conducting DNO. In line with this strategy, the research conducted in Mpongwe and Mpulungu found that long distances to the testing sites affected the smooth operation of the specimen referral, leading to sample losses (Lyson Nkhoma et al., 2023b; Goma et al., 2022). This finding only emphasises the need to reduce distance to the diagnostic sites to improve sample management during transportation.

2.5. Gaps Identified in The Literature

Several studies have examined factors affecting the referral system in healthcare, including logistical, infrastructural, and operational components. However, there is a paucity of research specifically investigating specimen-related factors that influence TB case detection across all levels of healthcare delivery. While existing studies have often described challenges within the referral system, few have assessed the strength of association between specimen referral factors and TB case detection, leaving a critical gap in the evidence base. Recognising this gap, the current study was designed to systematically assess courier-related and process-related factors that could potentially influence the detection of TB cases. Although multiple studies have focused on referral systems in general, none have specifically explored the significance of specimen referral system factors on TB case detection in sub-Saharan Africa. Most prior research has highlighted challenges affecting the safe transportation of TB samples, such as delays, loss of samples, or compromised temperature-controlled conditions, rather than evaluating their direct impact on the outcome of diagnostic testing.

In Zambia, only two local studies have addressed aspects of the referral system; however, both primarily focused on sample losses within the system, and neither considered the direct implications for TB case detection. As such, there remains a lack of empirical evidence linking operational features of the specimen referral system—such as frequency of sample pickups, availability of refrigeration, or type of courier to actual TB diagnostic outcomes. This study, therefore, represents the first of its kind to evaluate the strength of association between specimen referral factors and TB case detection. By systematically investigating how specific, distinct features of the referral system influence testing performance outcomes, the study provides critical insights that could inform strategies to optimise TB sample transport, improve case detection rates, and ultimately strengthen TB control programs in settings with high TB burden.

2.6. Conclusion

The reviewed studies emphasised the critical role that specimen referral systems play in the health sector, particularly in the control and management of tuberculosis (TB). Effective referral systems ensure the timely and safe transport of patient samples from peripheral health facilities to diagnostic laboratories, which is essential for accurate diagnosis, prompt treatment initiation, and overall disease control. The literature highlights that well-functioning referral mechanisms are essential to maintaining seamless patient care and improving health outcomes, especially in resource-limited settings. Globally, reports indicate that significant progress has been made in strengthening sample transportation systems, including the adoption of standardised procedures, training of personnel, and implementation of logistical innovations. Despite these advancements, challenges persist, particularly in terms of effects that have not been studied regarding the transport system on actual TB case detection rates. Many studies focus on operational and logistical challenges such as delays, sample losses, or cold-chain failures but few perform quantitative analysis of how these factors influence the identification of TB cases within the broader health system. This literature review therefore provided a well-established basis for investigating the relationship between specimen referral systems and TB case identification in a health system with multitiered structures especially in peripheral facilities. By understanding how core operational features such as the frequency of sample pickups, type of courier, availability of refrigeration, and facility setting affect TB case detection. This study aims to generate evidence that can inform strategies for streamlining referral systems, improving timeliness of TB diagnosis, and ultimately improving TB control at both facility and population levels.

2.7. Theoretical Framework

This study adopted a Health Systems Strengthening (HSS) approach, emphasizing the distinct and comparative advantage of harnessing resources across all levels of the health system to improve service delivery and health outcomes. The HSS framework points out that the performance of health systems depends on the effective integration of finances, workforce, medical products, service delivery mechanisms, vaccines, technologies, and health information systems (WHO, 2024c). Each of these components plays a critical role in ensuring that primary, secondary and tertiary health care services are accessible, efficient, and of high quality. In the context of TB patient care and management, service delivery improvements may include enhancing transport systems, optimize specimen logistics, and ensure timely and safe movement of patient samples from collection points to diagnostic facilities. Meanwhile, robust health information systems facilitate effective communication, facilitate real-time monitoring, and provide timely feedback on test results, which are essential for tracking patient care and improving overall system responsiveness. The effectiveness of the specimen referral system, health infrastructure, communication channels, and logistics is directly dependent on adequate financial resources. Sufficient funding enables the procurement and maintenance of equipment, the recruitment and training of skilled personnel, and the establishment of well functioning processes. Strengthening these elements collectively can enhance overall health system performance and increase TB case detection by ensuring that patients' samples are collected, transported, and tested in a consistent and timely manner. Conversely, failure to address weaknesses in the specimen referral system, long distances to testing sites, inadequate infrastructure, and poor communication can significantly limit access to diagnostic services, delaying TB detection and limiting treatment initiation. This emphasizes the linked structure of the health system components, where the performance of one element directly influences other components. When these elements are strengthened and coordinated, they work complimentarily to produce the intended result which is improved TB case detection.

Therefore, the specimen referral system is a critical component within the HSS approach, serving as a key mechanism to enhance the quality, efficiency, and accessibility of TB diagnostic services. By reinforcing this system, health facilities can ensure timely sample transport, reliable testing, and rapid feedback of results, ultimately contributing to better patient management and overall public health outcomes.

2.8. Conceptual Framework

The conceptual framework for this study suggests that the effectiveness of the specimen referral system is determined by a combination of various factors such as logistical, infrastructural, and operational factors. The key determinants include transportation mechanisms, logistics management, communication systems, the type of rider responsible for sample delivery, the availability and functionality of cold-chain equipment such as refrigerators, and the geographical location of health facilities. These elements are related and collectively influence the timeliness and reliability of specimen transport from collection points to diagnostic laboratories. In addition, the framework recognizes that communication such as the availability of trained personnel and diagnostic capacity, play a significant role in shaping the efficiency of the specimen referral system. Effective coordination of these factors ensures that specimens are transported under appropriate conditions, reaching testing sites in a timely manner, thereby enabling accurate and prompt TB diagnosis. On the contrary, operational gaps in any of these components can result in delays, compromised sample integrity, and suboptimal diagnostic outcomes. Ultimately, the framework suggests that the interaction of these logistical, infrastructural, and operational factors has a direct impact on TB case detection and, by extension, on the management of TB patients. Poorly functioning specimen referral systems can lead to delayed diagnosis, missed cases, and substandard patient care, which may perpetuate transmission within communities. Based on this conceptual understanding, the study hypothesizes that specific characteristics of the specimen referral system such as frequency of sample pickups, the type of rider employed for specimen transport, the presence and functionality of refrigeration equipment, and the geographical setting of the health facility significantly influence TB case detection. Investigating these relationships provides a guiding structure for identifying operational bottlenecks and informing strategies to strengthen the specimen referral system, ultimately contributing to improved TB control (Figure 1).

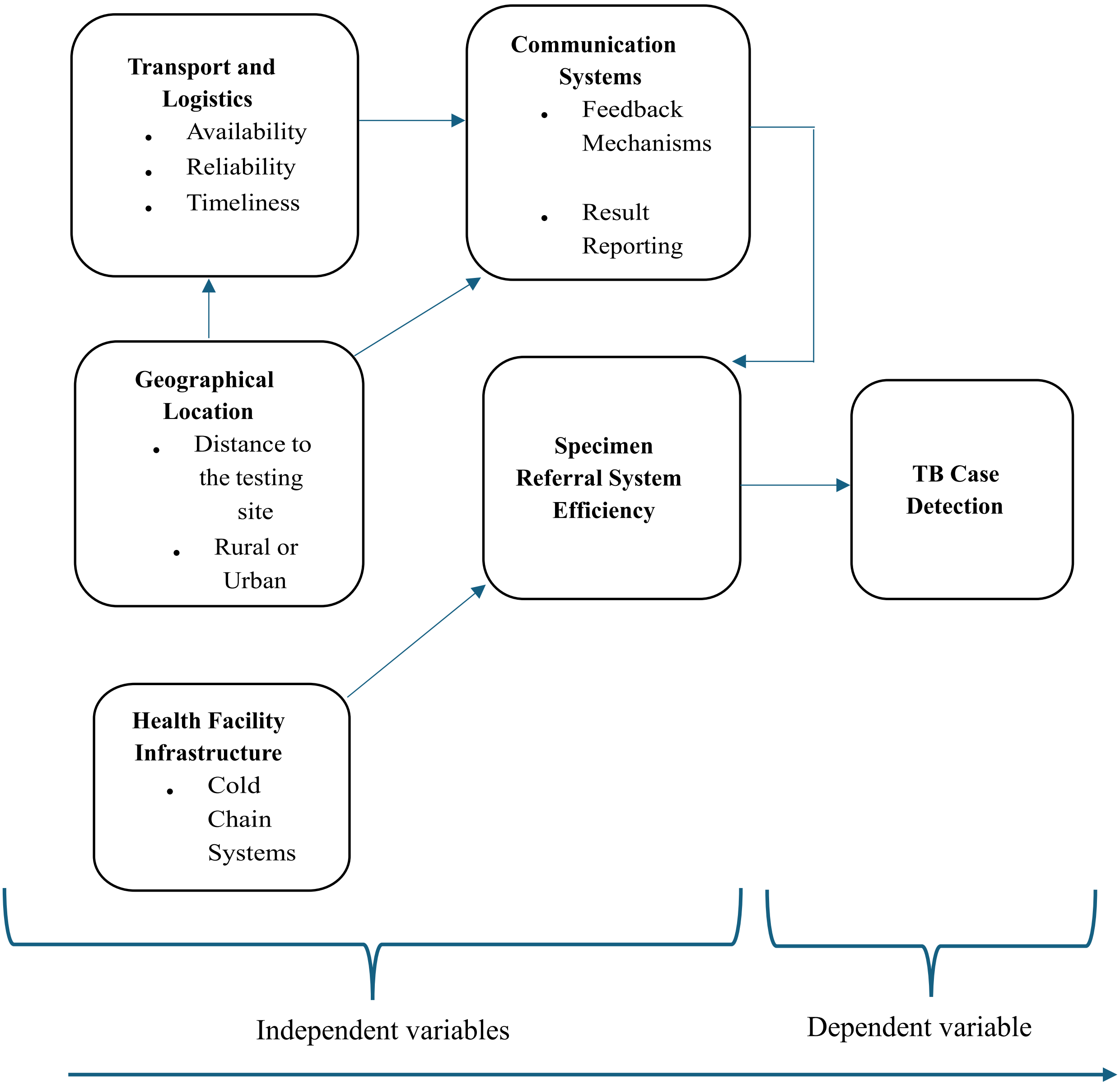

Figure 1: Conceptual Framework on Sample Referral System

CHAPTER THREE
METHODOLOGY

3.1 Introduction

This chapter presents a detailed outline of the methodology employed in this study. It provides a comprehensive description of the research approach and design adopted to address the eight study objectives, ensuring that the methods are suitable and scientifically robust. The chapter further explains the study population, the sampling approaches used, and the rationale behind the determination of the sample size. Furthermore, the methodology section highlights the characteristics of the study participants, which are crucial for understanding the context and generalizability of the findings. By clearly detailing the data collection and analysis procedures, this chapter ensures transparency and reproducibility and establishes the foundation for interpreting the study results.

3.2 Research Approach

This study employed a quantitative research approach to systematically investigate the characteristics and operational factors of the specimen delivery system for TB diagnosis. The analysis using a quantitative method was considered relevant because it allows for the collection of numerical data, which can be analysed statistically to identify patterns and trends within the system. By using this approach, the study was able to generate objective and measurable findings that provide insights into how different factors influence TB sample referral and case detection. The selection of a quantitative methodology was motivated by its ability to test hypotheses and draw evidence-based conclusions based on well-arranged and structured data. The quantitative method emphasises precision, reproducibility, and the ability to link relationships between variables. This makes it particularly suitable for investigating system performance, such as the frequency of sample pickups, transport logistics, availability of cold-chain equipment, and other operational aspects of the specimen referral system. The research design incorporated structured data collection tools, including standardized questionnaires to gather empirical evidence from the study population. These instruments were carefully developed to ensure reliability and validity, allowing for the consistent measurement of variables across multiple health facilities. The structured nature of the data collection facilitated the analysis of large datasets, enabling statistical comparisons and the identification of key factors associated with effective TB sample transport and case detection.

Overall, the quantitative research approach provided a rigorous structure for examining the relationships of the specific components of the specimen referral system and TB case detection. Because of its analytical nature, the study was able to generate evidence that can inform improvements in health system performance and support evidence-based interventions for TB management, especially at the primary health care level.

3.3 Research Design

The researcher employed an analytical cross-sectional study design to evaluate the influence of specimen referral system factors on tuberculosis (TB) case detection within routine healthcare settings. This design was chosen because it allows for the concurrent assessment of multiple variables at a specific point in time, providing a current status of existing practices and their association with TB diagnostic outcomes. By capturing data on both system characteristics and TB case detection, the study was able to identify key system-level factors that may facilitate or hinder effective sample referral and timely diagnosis. Data collection focused on bacteriologically confirmed TB cases and the scope of specimen delivery system features that are critical to the efficiency and reliability of the referral process. These included the availability and functionality of refrigerators for maintaining proper storage conditions, the number of staff involved in specimen handling, the frequency of sample pickups, the distance between the referring site and the diagnostic facility, and the presence of dedicated riders responsible for sample transportation. By collecting detailed data on these operational characteristics, the study aimed to uncover patterns and relationships that could inform improvements in the specimen referral system. Data pertaining to presumed TB cases, samples submitted and TB cases identified were gathered for the period from January to December 2024, providing a full year of routine TB programming. Development of the electronic questionnaire and sending to the eligible sites including receiving and analysis of data was done between August and October 2025. This timeline allowed the researchers to clarify any missing information and ensure completeness of the submitted data in collaboration with the district TB coordinator. The cross-sectional design provided multiple benefits for this investigation. It enabled the assessment of current practices and their immediate effects on TB case detection without requiring longitudinal follow-up, making it cost-effective and feasible within the study's timeframe. Additionally, this design facilitated the identification of associations between specific referral system factors and diagnostic performance, which could provide policy relevant insights for strengthening specimen referral systems and improving TB management in routine healthcare settings.

3.4 Study Site And Population

The study was conducted in Kafue District, one of the six districts in the Lusaka Province of Zambia. Geographically, Kafue shares borders with Chilanga, Chongwe, Rufunsa, Luangwa, and Lusaka districts within Lusaka Province, and borders Mazabuka, Chirundu, and Chikankata districts in Southern Province. The district has a total population of 219,574 inhabitants, with an approximately equal distribution of males and females. Kafue is economically significant and well known to produce fertiliser, clothing, boats, beer, and concrete (Kafue Council, 2025). Kafue is located within the Kafue Catchment, one of Zambia's six major catchments, which covers approximately 20% of the country's total land area. The catchment's land cover is predominantly cropland, although soil quality varies across the region. While much of the cropland has relatively poor soil quality, the lower Kafue Catchment exhibits a high density of fertile land suitable for agriculture. The district benefits from good access to water resources, primarily due to the presence of the Kafue River, which contributes to its high agricultural potential. The main economic activities in the district include hydroelectricity generation, agriculture, municipal water supply, and domestic services (Warma, 2022). Within the district, there are a total of 30 public health facilities providing general health services. Two facilities, namely Kafue Estate Clinic and Kafue General Hospital, were excluded from the study as they offered molecular-based rapid TB diagnostic services, which operate under different referral system requirements. Of the remaining 28 primary health facilities, 20 facilities participated in the study, comprising 14 rural, 4 urban, and 2 peri-urban sites. The target population for the study included all health facilities that transported TB specimens within Kafue for bacteriological TB confirmation.

The study focused on bacteriologically confirmed TB patients aged 15 years and above, as diagnosis in adults relies heavily on the specimen referral system. Children under 15 years were excluded because paediatric TB often presents as paucibacillary disease, characterized by a low bacterial load, which makes diagnosis more challenging and less dependent on the efficiency of specimen transport. Kafue District was selected as the study site due to its high TB prevalence and the availability of routine, free-of-charge TB care services. Conducting the study in this setting allowed for a comprehensive assessment of specimen referral system practices in a real-world context where TB case detection and management are critical public health priorities. By including a diverse mix of rural, peri-urban, and urban facilities, the study captured variations in operational and logistical practices that may influence TB diagnosis and patient outcomes across different settings within the district.

3.5 Sample Size and Sampling Methods

Due to the relatively small number of health facilities in the district, a conventional sample size calculation was deemed unnecessary and impractical. Traditional sample size formulas are designed for larger populations and may not be appropriate when the total number of units is limited, as is the case in this study. To ensure comprehensive coverage and maximise the representativeness of the data, the study therefore adopted a census approach, whereby all available facilities were included in the research. As a result, the study encompassed all 20 referring health facilities within the district, allowing for the collection of complete and detailed information on the specimen referral system across the entire population of interest. By including every facility, the study was able to capture the full spectrum of operational practices, logistical challenges, and infrastructural differences that may influence TB case detection. This census-based strategy not only provided a complete set of data but also strengthened the study's ability to identify patterns and relationships across the entire system, rather than relying on a potentially biased or incomplete sample. Consequently, the findings obtained from this approach offer a robust foundation for informing interventions aimed at improving specimen referral systems and enhancing TB diagnostic outcomes.

3.5.1 Inclusion Criteria

- Health facilities that relied on sample transport
- Patients aged >14 with suspected TB who had their specimens referred for diagnostic testing.

3.5.2 Exclusion Criteria

- All diagnostic sites that perform GeneXpert testing for routine TB diagnostic services.
- Patients whose specimens were not processed due to incomplete data or insufficient sample.

3.6 Data Collection

A Google Form was developed and used to administer the survey questionnaire to the selected respondents. The use of an online platform facilitated efficient data collection, allowing respondents to submit their answers in real time and ensuring timely submission.

The completed responses were then exported to Microsoft Excel for initial cleaning and validation before being imported into STATA software for detailed statistical analysis. This process enabled the research team to monitor the progress of data collection, verify completeness, and identify any inconsistencies promptly, ensuring high-quality data. Notably, the entire dataset was submitted within one week, demonstrating the efficiency of the electronic data collection method. The questionnaire was designed to include both close-ended and open-ended questions, allowing the capture of both quantitative numerical data and qualitative explanatory information where necessary. This structure enabled the study to collect standardized information for statistical analysis while still accommodating additional context or clarifications from respondents. Data were collected from multiple routine sources, including the TB treatment register, laboratory register, presumptive TB register, and specimen referral schedules and records. These sources provided comprehensive documentation of TB diagnostic processes, specimen handling, and referral activities. The primary data collected included respondents' demographic information, specimen referral history, types of specimens (e.g., sputum, blood), collection dates, referral dates, and diagnostic outcomes. In addition, information was extracted on the documentation of referral processes, specimen tracking logs, and TB case detection outcomes, which enabled an assessment of the efficiency and reliability of the specimen referral system. In some instances, follow-up questions were directed to riders or facility staff to obtain additional information or clarify specific records, ensuring accuracy and completeness of the dataset. Overall, the combination of electronic survey tools, routine registers, and direct follow-up allowed for a robust, multi-source data collection process that captured both operational and clinical aspects of TB specimen referral within the study setting.

3.7 Study Variables

Bhandari stated that there are basically two types of variables used in research; dependent and independent variables. An independent variable is the variable that can be manipulated or changed in an experimental study to explore its effects. It is called independent because it cannot be affected by another variable in the study. Independent variables are also called predictor or explanatory. A dependent variable is the variable that can be changed by the independent variable. It is also called response or outcome variable (Bhandari, 2020). The study utilized number of bacteriologically confirmed TB cases as dependant (outcome) variable. This variable was believed to influence the predictor variables. The independent variables were frequency of sample pick up, availability of the functional refrigerator, distance to the diagnostic site and type of rider picking the TB specimens. Other predictor variables included rider trained in specimen referral services, facility attendance, Total TB patients presumed, Presumptives submitting samples and geographical setting. The data set had count, categorical and discrete variables. The scale of measurement was composed of interval, ordinal and nominal as shown in **Table 1**.

Table 1: Types of Variables

Variable Type	Variable Name	Variable Description	Variable Categories/Measurement Outcome	Measurement Category	Scale of Measurement
Dependent variable	Confirmed TB Cases	Number of bacteriologically confirmed TB cases	Discrete value	Count data	Interval
Independent variable	Sample Pickup	Frequency of sample pick up	No/Yes	Categorical	Ordinal
	Functional refrigerator(s)	Availability of the functional refrigerator,	No/Yes	Categorical	Nominal
	Distance (Km)	Distance to the diagnostic site	Discrete value	Categorical	Nominal
	Type of the rider	Type of the rider picking the TB specimens	Discrete value	Categorical	Nominal
	Rider Trained	Rider trained in specimen referral services	No/Yes	Categorical	Nominal
	Facility attendance	Total number of attendees at a facility	Discrete value	Discrete	Interval
	Presumptive TB	Total TB patients presumed	Discrete value	Discrete	Interval
	Presumptives submitting samples	Number of presumptives submitting samples	Discrete value	Discrete	Interval
	Location	Type of geographical setting	Discrete value	Categorical	Ordinal

3.8 Data Analysis

The first step in the data management process involved data cleaning, which is a critical procedure to ensure the accuracy and reliability of the dataset before analysis. Data cleaning was performed using STATA version 14.2, a statistical software package that allows for efficient handling and verification of large datasets. The cleaning process involved several key steps, including the identification and management of missing values, the detection of data inconsistencies, and the verification of data completeness. A range of STATA commands was employed to facilitate this process. The browse command was used to inspect individual records for anomalies, while summarise provided descriptive statistics that helped identify unusual values or outliers. The tabulate and codebook commands were utilised to examine the distribution of categorical variables and review variable attributes. Duplicate entries were identified using the duplicates list command, and irrelevant or erroneous records were removed using drop. Additionally, variable names and labels were standardised and renamed where necessary to ensure clarity and consistency across the dataset. By systematically performing these data cleaning procedures, the study ensured that the dataset was accurate, consistent, and complete, thereby reducing the risk of errors in subsequent analyses. This process enhanced the reliability and reproducibility of the research findings, as well as the transparency of the analytical workflow. Ensuring a clean and well-structured dataset is a critical step in quantitative research, as it provides a solid foundation for performing robust statistical analyses and drawing valid conclusions.

3.8.1 Poisson Regression

Since the dependent variable in this study comprised count data, a Poisson regression model was employed for analysis. Poisson regression is a widely used statistical method for modelling count-based outcomes, particularly when the data represent the number of events occurring within a fixed time or spatial unit (Leung, 2025). In the context of this study, the model was appropriate because the outcome variable represented the frequency of TB cases detected over a defined period. Poisson regression is specifically designed for non-negative integer values and allows for the estimation of the relationship between explanatory variables and the expected count of events. The model assumes that the logarithm of the expected count is a linear function of the predictor variables, enabling interpretation of results in terms of incidence rate ratios (IRRs). This makes Poisson regression particularly useful in public health research, where disease occurrence and event counts are commonly analysed. The application of the Poisson regression model requires that certain assumptions be satisfied to ensure valid and reliable estimates. These assumptions are discussed below.

3.8.2 Assumptions of the Poisson Distribution

According to Zach (2021), the Poisson distribution is based on four key assumptions:

3.8.1.1 The number of events can be counted

This assumption states that the outcome of interest must be measurable as a count. That is, the number of events occurring within a defined time or geographical area can take only non-negative integer values. In this study, TB case counts meet this requirement.

3.8.1.2 The occurrence of events is independent

This assumption requires that the occurrence of one event does not influence the probability of another event occurring. In other words, TB case detection events are assumed to be independent of one another within the specified time frame and setting.

3.8.1.3 The average rate at which events occur is constant

This assumption states that events occur at a constant average rate over the observed interval. While individual counts may vary, the underlying rate of occurrence is assumed to remain stable during the study period.

3.8.1.4 Two events cannot occur at the same instant in time

This assumption implies that events occur discretely and not simultaneously. Within extremely small time intervals, either one event occurs, or none occurs, ensuring that events are temporally distinct. The Poisson distribution for a random variable Y has this equation for a given value $Y = y$:

$$\mathrm{P}(Y = y|\lambda) = \frac{e^{-\lambda}\lambda^{y}}{y!},$$

The single parameter λ is the mean rate of occurrence for the event being measured. The rate is determined by a set of predictors. The fundamental Poisson regression model for observation i is given by;

$$\mathrm{P}(Y_i = y_i|\mathbf{X}_i, \beta) = \frac{e^{-\exp\{\mathbf{X}_i\beta\}}\exp\{\mathbf{X}_i\beta\}^{y_i}}{y_i!}.$$

3.8.1.2 Types of Poisson Model

The are four types of Poisson models, namely: Negative Binomial Regression, Quasi-Poisson Regression, Zero-Inflated Models and Generalised Poisson Regression.

3.8.1.2.1 Negative Binomial Regression

Negative binomial regression can be used for over-dispersed count data when variance exceeds the conditional mean. It has the same mean structure as Poisson regression, and it has an extra parameter to model the over-dispersion. The confidence intervals for the Negative binomial regression are likely to be narrower as compared to those from a Poisson regression model (UCLA, 2024).

3.8.1.2.2 Quasi-Poisson Regression

The Quasi-Poisson Regression is used when there is overdispersion in the data set (RSTUDIO, 2023). According to Nguyen (2025), the Quasi Poisson has some notable limitations. The model does not use the full likelihood function. The AIC, BIC, and Likelihood Ratio Tests do not apply when the model is considered in the analysis. The model is not a strict generalised linear model (GLM). Quasi-Poisson is also used if the Negative Binomial Regression is not preferred. However, Negative Binomial Regression is always a better model than quasi-Poisson because it is a true GLM with a full likelihood function, whereas Quasi-Poisson just offers a quasi-likelihood approach.

3.8.1.2.3 Zero-Inflated Models

This model has been used in research when there are excess zeros. Hence, Zero-inflated models are useful for the count model and when zeros are in excess (UCLA, 2024).

3.8.1.2.4 Generalised Poisson Regression

The generalised Poisson regression is used both for over-dispersed and under-dispersed count data. This is also useful in predicting a response variable affected by one or more covariates. (Consul and Famoye, 1992). The most problematic issue in Poisson regression is overdispersion and underdispersion, which should be put into consideration when using the models. Overdispersion takes place when the variance of the count data is greater than the mean. Both negative binomial regression and Quasi-Poisson can be used when addressing overdispersion. Negative Binomial regression includes an additional parameter to account for overdispersion. It allows the variance to be greater than the mean, while Quasi-Poisson regression adjusts the standard errors of the estimates to account for overdispersion. Unlike the Negative Binomial regression model, the Quasi model adjusts the variance to be proportional to the mean. Zero-inflated Poisson (ZIP) or zero-inflated negative binomial (ZINB) models are used when data has an excess number of zeros, which can cause overdispersion (Leung, 2025). Underdispersion takes place when the variance of the count data is less than the mean. We can use Generalised Poisson Regression and Quasi-Poisson Regression Models. The generalised Poisson regression model allows for underdispersion by including a dispersion parameter that can be less than one. Quasi-Poisson regression is very useful when adjusting the standard errors to account for underdispersion (Leung, 2025).

3.8.1.3 Multivariable Negative Binomial Regression

Due to the existence of overdispersion, we used a multivariable negative binomial (NB) regression analysis (Figure 2). The NB model operates under the assumption that the response variable (dependent variable) follows NB distribution. The NB model allows the Poisson parameter u_i to vary randomly following a gamma distribution. The NB probability density can be depicted using the following formula:

$$f(y_i|\boldsymbol{x}_i) = \frac{\Gamma(y_i + \tau)}{y_i!\,\Gamma(\tau)}\left(\frac{\tau}{\tau + u_i}\right)^{\tau}\left(\frac{u_i}{\tau + u_i}\right)^{y_i}$$

where $\tau > 0$ is a dispersion parameter, and $\Gamma(\cdot)$ is a gamma function. The conditional mean and the conditional variance are given by

$$\mathrm{E}(y_i|\boldsymbol{x}_i) = u_i = \exp(\boldsymbol{x}_i^T\boldsymbol{\beta}), \mathrm{Var}(y_i|\boldsymbol{x}_i) = u_i\left(1 + \frac{1}{\tau}u_i\right).$$

In this model, the variance is always greater than the mean. Furthermore, as $\tau \to \infty$, the NB distribution converges to the Poisson distribution. The maximum likelihood is utilised to calculate the parameters of NB regression (Zhang et al., 2018). To determine overdispersion, we used the formula to display Pearson Chi2 / df. At a significance level of p-value ≤ 0.05, the model established a relationship between the mean response and its predictors through a logarithmic link function, creating a linear relationship with the parameters. The following covariates as determinants of bacteriologically confirmed cases were evaluated: frequency of sample pick up (frequency of referral times, presence of the refrigerator, type of rider, distance to the diagnostic sites, trained rider, facility attendance, number of presumptive TB cases and presumptive with submitted TB samples.

3.8.2 Multicollinearity

This is a statistical phenomenon that takes place when two or more predictors are highly correlated. It accurately determines the individual effects of each independent variable on the outcome variable. If not addressed, it can also lead to unstable and unreliable coefficient estimates. Result interpretation becomes difficult including making meaningful inferences and reduce the statistical power of the model. One of the diagnostic tools of multicollinearity is variance inflation factor (VIF). Considering the range of R^2 ($0 \leq R^2 \leq 1$), $R^2 = 0$ (complete absence of multicollinearity) minimizes the variance of the regression coefficient of interest, while $R^2 = 1$ (exact multicollinearity) makes this variance infinite. The reciprocal of the variance inflation factor ($1 - R^2$) is known as the tolerance. If the variance inflation factor is greater than 5 to 10, multicollinearity exists. Strong multicollinearity increases the variance of a regression coefficient. The increase in the variance also increases the standard error of the regression coefficient (because the standard error is the square root of the variance). The increase in the standard error leads to a wide 95% confidence interval of the regression coefficient. The inflated variance also results in a reduction in the t-statistic to determine whether the regression coefficient is 0. With a low t-statistic value, the regression coefficient becomes insignificant. The wide confidence interval and insignificant regression coefficient make the final predictive regression model unreliable (Kim, 2019).

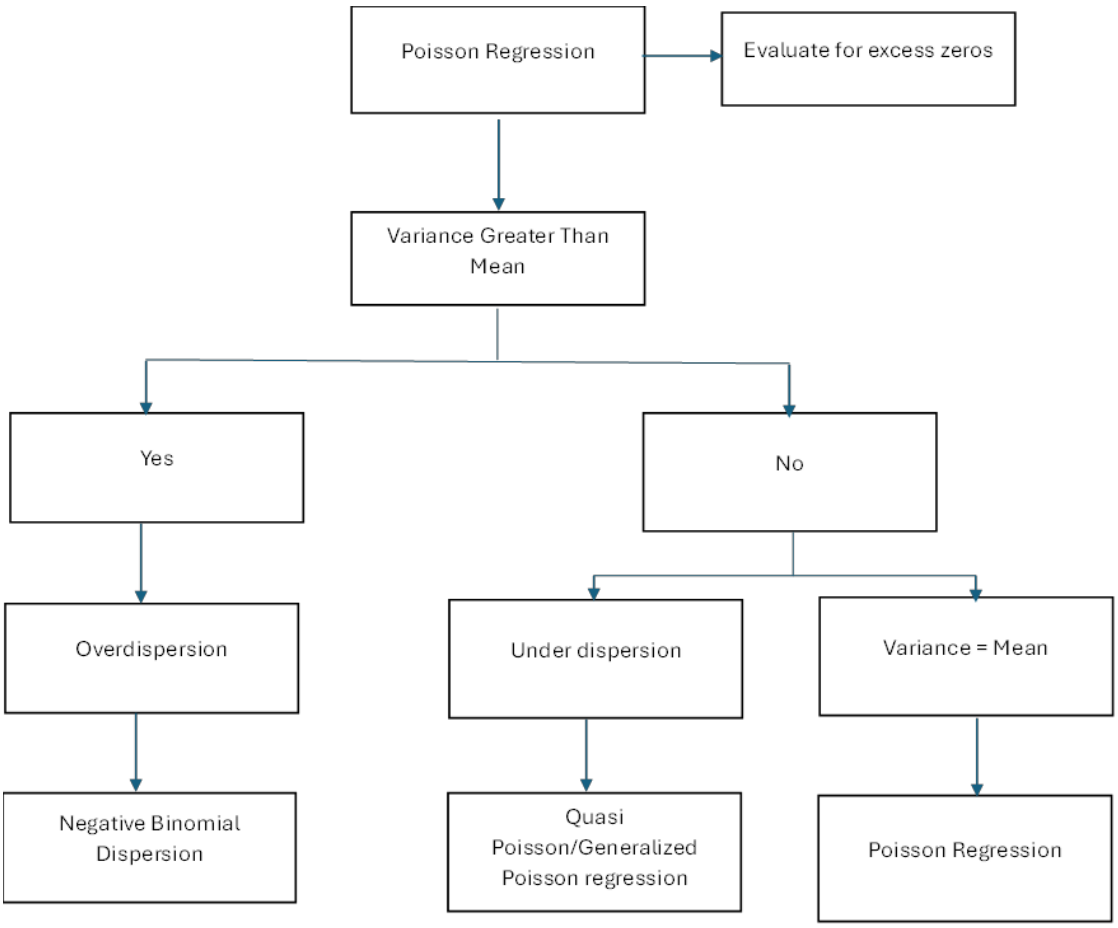

Figure 2: Flow Chart for Selection of Statistical Model

To determine the most appropriate statistical model for analysing the factors influencing TB case detection, we compared three count regression models: the Poisson regression, Quasi-Poisson regression, and Negative Binomial regression. Model selection was guided by commonly used information criteria, including the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), which assess model fit while penalising for complexity. The calculated AIC and BIC values were 141 and 152, respectively, and were similar across the Poisson, Quasi-Poisson, and Negative Binomial models, indicating that all three models had comparable goodness-of-fit in general terms. Despite the comparable information criterion values, the choice of the most appropriate model was further informed by the distribution of the outcome variable. Inspection of the data revealed severe overdispersion ($\alpha > 0$), where the variance of the outcome variable exceeded the mean—a violation of the Equi dispersion assumption required by the Poisson model. The Quasi-Poisson model adjusts for overdispersion by modifying the standard errors but does not explicitly model the variance structure, whereas the Negative Binomial model includes an additional parameter (α) to directly account for overdispersion, making it more suitable for the observed data. Consequently, the Negative Binomial regression model was selected as the most appropriate analytical approach for this study. This model allowed for accurate estimation of the associations between specimen referral system factors and TB case detection, while properly accounting for the extra-Poisson variation inherent in the dataset. By choosing a model that addressed overdispersion, we improved the validity, reliability, and interpretability of the statistical results.

3.9 Validity and Reliability

The validity of the study was ensured using complete and verified data obtained from National TB Program-approved reporting and recording tools. By relying on standardised, nationally recognised instruments, the study minimised the risk of systematic errors and ensured that the data accurately reflected TB case detection and specimen referral practices across the facilities. Furthermore, robust and appropriate statistical methods were applied to the dataset to identify and account for potential issues such as multicollinearity and other sources of confounding, thereby strengthening the credibility of the analytical results. To enhance the reliability of the study, nationally standardised primary data collection tools, including TB treatment registers, laboratory registers, and presumptive TB registers, were employed uniformly across all participating facilities. These instruments provided a consistent framework for capturing key variables, such as patient demographics, specimen collection details, referral timelines, and diagnostic outcomes. The standardized approach ensured that measurements were comparable across sites, improving the reproducibility of the findings. In addition, cross-validation of data was conducted in certain cases by comparing records from diagnostic facilities with those from the referring sites. This step helped to identify and resolve any discrepancies in reporting and reinforced the accuracy and completeness of the dataset. By combining standardised tools with cross-checking procedures, the study was able to achieve high data integrity, thereby providing reliable evidence on the factors influencing TB case detection and the performance of the specimen referral system. Overall, these measures ensured that the study's findings were both valid and reliable, supporting evidence-based conclusions and strengthening confidence in the recommendations derived from the research.

3.10 Ethical Considerations

This study was conducted in accordance with internationally recognised ethical principles for research involving human participants, ensuring the protection of rights, privacy, and confidentiality throughout the research process. Ethical clearance was obtained from the University of Lusaka Research Ethics Committee (UNILUS-REC) as well as the National Health Research Authority (NHRA), demonstrating compliance with national and institutional guidelines for conducting research in Zambia. Before data collection, formal permission was also obtained from the Lusaka Provincial Health Office and the Kafue District Health Office, ensuring that the study was conducted with the approval and support of relevant local health authorities. This step was critical to facilitate smooth access to health facilities and maintain transparency with stakeholders at all levels. The study primarily utilised facility-level data obtained from routine registers and records and did not involve direct interaction with TB patients. Therefore, informed consent was obtained from the facility staff who completed the questionnaires on behalf of their respective facilities. Staff were provided with clear information regarding the purpose of the study, the nature of their participation, and their right to decline or withdraw without any consequences. To ensure confidentiality and data security, access to all collected data was strictly limited to the research team. Overall, adherence to these ethical principles ensured that the study was conducted responsibly, with respect for participants' rights, and in a manner that promotes trust and accountability in the research process.

CHAPTER FOUR
RESULTS

4.1 Introduction

This section presents the results of the cross-sectional study examining the factors influencing the specimen referral system and their impact on TB case detection in Kafue District. The findings have been systematically organised and presented to provide a clear and comprehensive understanding of the data collected. Both graphical and tabular representations have been used to illustrate key patterns, distributions, and relationships among variables, allowing for easier interpretation and comparison of results. The presentation of results follows a logical sequence, starting with descriptive statistics to summarise the demographic and operational characteristics of the study population and facilities. This is followed by analytical findings that explore associations between specimen referral system factors—such as frequency of sample pickups, type of rider, availability of refrigeration, and facility location—and TB case detection outcomes. By using multiple forms of data visualisation, including tables, charts, and graphs, this section ensures that the results are accessible, interpretable, and reflective of the study objectives. The use of both numerical and graphical formats also facilitates the identification of trends, variations, and potential gaps in the specimen referral system, providing a foundation for subsequent discussion and interpretation in relation to the conceptual framework and existing literature. Overall, this section aims to present the study findings in a clear, systematic, and meaningful way that supports evidence-based conclusions and recommendations.

4.2 Sociodemographic Characteristics

A total of 20 participants from health facilities across Kafue District were included in the study. Of these, 9 (45%) were males, and 11 (55%) were females, indicating a slightly higher representation of female respondents. The participants' ages were categorised as follows: 25–29 years (n = 4, 20%), 30–34 years (n = 6, 30%), and ≥35 years (n = 10, 50%). The median age was 33 years, with an interquartile range (IQR) of 29–38.5 years, reflecting a relatively mature and experienced workforce. In terms of educational qualifications, most participants held certificates (n = 11, 55%), followed by degree holders (n = 7, 35%), and diploma holders (n = 1, 5%). Regarding professional cadre, Clinical Officers numbered 5 (25%), Laboratory Staff 9 (45%), Nurses 10 (50%), Public Health Technologists 5 (25%), Nutrition Technologists 1 (5%), and Community Health Assistants 1 (5%). This diverse composition ensured representation across key personnel involved in TB specimen collection, referral, and management. The health facility settings included 14 (70%) rural, 2 (10%) peri-urban, and 4 (20%) urban facilities, highlighting a predominance of rural sites within the study sample. Sample pick-up frequency varied across facilities: one site (5%) reported zero pick-ups, four facilities (20%) conducted weekly pick-ups, eleven facilities (55%) conducted pick-ups twice per week, three facilities (15%) had three weekly pick-ups, and one facility (5%) conducted pick-ups four times per week. The availability of refrigerators for cold-chain maintenance was reported in 13 facilities (65%), whereas seven facilities (35%) lacked refrigeration.

Regarding distance to diagnostic facilities, one facility (5%) was located 1–2 km away, two facilities (10%) were 3–5 km away, and the majority, 17 facilities (85%), were located more than 5 km from the diagnostic site. Most specimen transport was conducted by dedicated riders (90%), with only two facilities using non-dedicated riders. Additionally, 16 riders (80%) had received formal training on specimen handling, while 4 riders (20%) were untrained.

The overall facility attendance during the study period was 174,699 patients. Of these, 24,183 (14%) were presumptive TB cases, and 15,188 (63%) of presumptive clients submitted TB samples for diagnostic testing (Table 1). A total of 301 bacteriologically confirmed TB cases (2%) were recorded during the study period. Statistical analysis indicated that certain operational factors were significantly associated with TB case detection. While the frequency of sample pick-ups was not statistically significant ($p = 0.48$), the number of sputum samples submitted showed a strong positive association with TB case detection ($p < 0.001$), suggesting that higher sample throughput improves the likelihood of identifying TB cases (Table 2).

Table 2: Respondent and Referral Characteristics

Variables	Frequency (n)	(%)
Demographic characteristics		
Age group (years)		
25-29	4	20
30-34	6	30
>35	10	50
Sex		
Male	9	45
Female	11	55
Level of Education		
Certificate	1	5
Diploma	17	85
Degree	2	10
Designation		
Clinical Officer	7	35
Laboratory Staff	1	5
Nurse	9	45
Public Health Technologist	1	5
Nutrition Technologist	1	5
Community Health Assistants	1	5
Sample Referral characteristics		
Setting		
Rural	14	70
Peri-urban	2	10
Urban	4	20
Sample Pick up Frequency		
Zero	1	5
Once per week	4	20
Twice per week	11	55
Three times per week	3	15
Four times per week	1	5
Refrigerator available		
No	7	35
Yes	13	65
Distance to Diagnostic site		
1-2 km	1	5
3-5km	2	10
>5km	17	85
Type of Rider		
Non dedicated	2	10
Dedicated	18	90
Riders trained		
No	4	20
Yes	16	80

The boxplot demonstrates differences in facility attendance, presumptive TB cases, and bacteriologically confirmed TB cases among rural, peri-urban and urban settings (Figure 3). As expected, facility attendance and number of presumptive TB cases were highest in urban settings indicating greater service utilization. In contrast, peri-urban facilities showed that facility attendance compared with rural setting had the lowest median. Peri urban showed the lowest number of presumed TB cases. Generally, bacteriologically confirmed TB cases were low across all settings though urban setting had slightly higher number of confirmed TB cases.

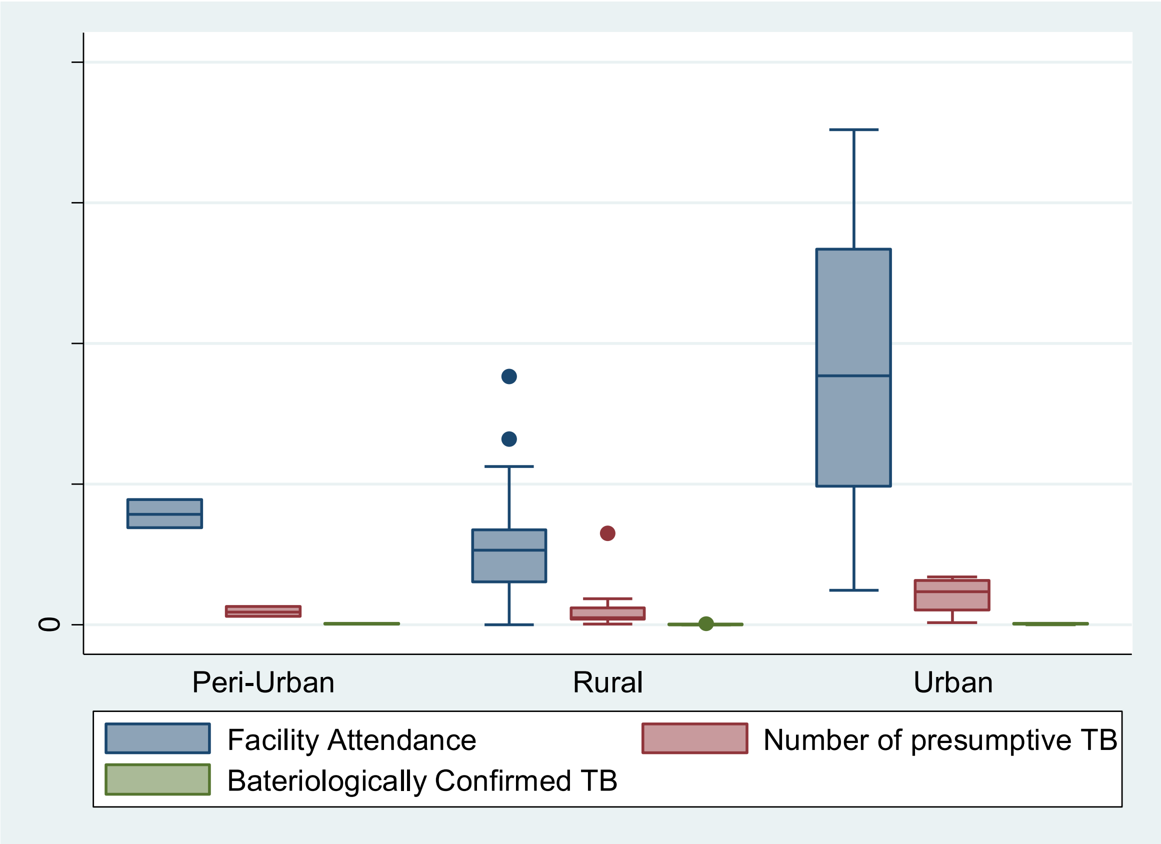

Figure 3: Facility Attendance by Geographical Setting.

4.3 Model Diagnostics and Multicollinearity

Model diagnostics, including assessment of overdispersion and goodness-of-fit, were conducted to evaluate model assumptions. Competing count models were compared using information criteria to select the most appropriate model. Firstly, we ran Poisson and performed model diagnostics using the Pearson goodness-of-fit test. It follows a *Poisson distribution*. The Pearson goodness-of-fit was 98.75 with 13 degrees of freedom. This yielded a dispersion of 7.6, suggesting overdispersion. To assess excess zeros, predicted counts ($\hat{\mu}$) were calculated for each site to check the model's expected TB cases. The predicted probability of zero cases was calculated using the formula $P(Y = 0) = e^{-\hat{\mu}}$. The data set contained 20 sites, of which only one site had no cases. When expected and observed zeros were examined, there was little evidence that there were excess zeros. Hence, a zero-inflated model was not appropriate.

The kernel density plot illustrates the distribution of the predicted number of events obtained from the multivariable negative binomial regression model (Figure 4). The distribution is highly right-skewed, with a strong concentration of predicted values at the lower end of the scale (approximately 0–15 events). This indicates that most facilities are predicted to have relatively low event counts. The absence of symmetry and the presence of the right-skewed shape justify the use of the negative binomial model, as it accounts for overdispersion and heterogeneity in the outcome variable. The smooth density curve, coupled with a lack of extreme irregularities, indicates that the model produces stable and reliable predictions.

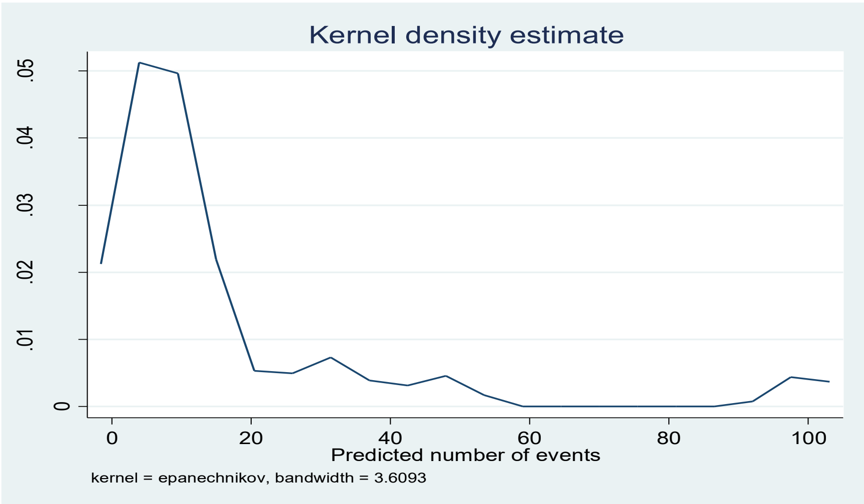

Figure 4: Kernel Density Distribution of Predicted Event Counts

We tested for multicollinearity to check if the predictors had a perfect link using variance inflation factor (VIF). The results of the multicollinearity have been presented (Table 3). The overall VIF results were between 1.22 and 5.56, indicating that multicollinearity was not very severe among the variables included in the model. The highest VIF values were observed for samples submitted (VIF = 5.56) and facility attendance (VIF = 4.42), suggesting moderate correlation between these two variables, though still within acceptable limits for regression analysis. Predictors such as sample pickup frequency, presumptive TB, facility setting, presence of a refrigerator, type of rider, and distance to the testing site had VIF values below 2, indicating minimal multicollinearity. These results confirm that all the predictors can be included in the regression model without significantly inflating standard errors or compromising model stability.

Table 3: Multicollinearity Testing

Variable	VIF
Samples submitted	5.56
Facility Attendance	4.42
Sample Pickup Frequency	1.75
Presumptive TB	1.46
Setting	1.34
Presence of a Refrigerator	1.28
Type of Rider	1.25
Distance to the testing site	1.22

VIF = Variance inflation factor

We conducted pairwise correlation analysis of predictor variables. The results showed that there was no strong multicollinearity among most of the variables (Table 4). However, we observed a strong positive correlation between facility attendance and the number of samples submitted ($r = 0.86$). This indicates that facility attendance was strongly associated with increased sample submission. In this analysis, facility attendance was dropped from the analysis since the number of samples submitted was directly related to TB case detection. Although there was moderate multicollinearity observed between setting and attendance ($r = 0.46$), and presumed TB clients and the number of samples submitted ($r = 0.45$), all the variables were dropped. Negative correlations were observed between sample pickup and both attendance ($r = -0.49$) and number of samples submitted ($r = -0.53$), suggesting that pickup services may be associated with decreased facility interaction. On the other hand, distance showed minimal and statistically non-significant inverse associations with attendance and sample submission, implying a modest effect on service uptake. Overall, the findings suggest that facility attendance plays a critical role in affecting sample submission, while most other predictors show low level of association. These results support the inclusion of multiple variables in subsequent regression analyses without increased risk of multicollinearity but excluding the facility attendance.

Table 4: Pairwise Correlation Matrix for TB-related Predictor Variables

	Setting	Pickup	Refrigerator	Distance	Attendance	Rider	TB_Pres	#Submitted
Setting	1.0000							
Pickup	-0.1557	1.0000						
Refrigerator	-0.0584	-0.2637	1.0000					
Distance	-0.2237	0.1387	0.2105	1.0000				
Attendance	0.4591	-0.4880	0.0579	-0.2383	1.0000			
Type of rider	-0.0619	0.3782	-0.1048	0.1339	-0.2915	1.0000		
TB_Pres	0.2462	-0.1907	0.2745	-0.0893	0.2916	-0.1710	1.0000	
#Submitted	0.4438	-0.5341	0.1691	-0.2915	0.8619	-0.1314	0.4547	1.0000

4.4. Adjusted Multivariable Negative Binomial Regression

We investigated the association between specimen referral factors and bacteriological TB detection using adjusted multivariable negative binomial regression by analysing the incidence rate ratios (IRR), confidence intervals and p values. Sample pickup frequency was significantly associated with the confirmed TB cases (IRR = 1.54; 95% CI: 1.00–2.35; p = 0.048). Comparably, the number of samples submitted also showed a statistically significant association with confirmed TB cases (IRR = 1.001; 95% CI: 1.001–1.002; $p < 0.0001$). On the other hand, setting (IRR = 0.76; 95% CI: 0.42–1.36; p = 0.358), availability of a refrigerator (IRR = 1.41; 95% CI: 0.67–2.95; p = 0.361), distance to the diagnostic site (IRR = 0.78; 95% CI: 0.50–1.22; p = 0.279), type of rider (IRR = 1.05; 95% CI: 0.37–1.00; p = 0.928), and number of presumptive TB cases (IRR = 0.9998; 95% CI: 0.9995–1.0001; p = 0.325) were not statistically significantly associated with the confirmed TB cases (Table 5).

Table 5:Adjusted Multivariable Negative Binomial Regression

	IRR	95% CI	p-value
Setting	0.7602	0.4237 – 1.3638	0.358
Sample Pickup Frequency	1.5351	1.0033-2.3487	0.048*
Presence of Refrigerator	1.4107	0.6746-2.9497	0.361
Distance to the testing site	0.7823	0.5015-1.2203	0.279
Type of the rider	1.0490	0.3692-1.0001	0.928
Presumptive TB clients	0.9998	0.9995-1.1.0001	0.325
Number of samples submitted	1.0014	1.0008-1.0019	<0.0001*

IRR: Incidence Rate Ratios, CI: Confidence Interval, *significant at α = 5%

CHAPTER FIVE
DISCUSSION

This study examined the impact of specimen referral system factors on TB case detection in different settings such as urban, peri-urban and rural areas at district level. We found that number of samples submitted and frequency of sample pick up were significantly associated with bacteriologically TB case detection, with the association being stronger for frequency of sample pickup than for number of submitted samples. These findings suggest that operational factors related to sample transportation between the GeneXpert sites and the referring sites particularly pickup frequency and sample submission remain very key in determining the outcome of interest.

These findings suggest that non-submission of sputum samples among presumed TB clients remain a challenge in the health facilities. A study in Mpongwe revealed that 5.2% of the patients failed to submit the sputum samples (Lyson Nkhoma et al., 2023c). (Papadopoulou et al., 2024) found that younger population, individuals that live in urban areas and those with high socioeconomic status were less likely to expectorate a sputum sample. The reason for not submitting sputum samples was difficulty in producing the sample, refusing to produce the sample, transcription errors and the client's unavailability to serve the sample. Carratala-Castro et al., (2024) similarly noted difficulty in collecting sputum samples from children and people living with HIV. Other studies have identified gender related barriers to sputum submission. A study conducted in Pakistani found that women were unwilling to expectorate sputum than men (Khan et al., 2007); though the instructional video on sputum sample submission improved the submission rate. Another study conducted in Malawi also observed lower submission rate of sputum among women (J et al., 2025). A study in Nicaragua (Macq, 2025) found that longer waiting times, travel expenses, social stigma and health workers personnel behaviour affected sample submission from presumed TB clients. Collectively, these studies provide evidence that individual related factors exist which can affect sputum sample submission and ultimately delay or prevent TB case detection. It was beyond the scope of our study to provide patient level data.

Up to now, no large-scale study has been conducted to evaluate the impact of referral factors on TB case detection. Most existing studies focuses on socioeconomic and behavioural determinants of TB. Though Narasimhan et al., (2013) described the relationship between health system factors and TB. However, the study never evaluated the impact of specimen referral factors on TB detection. There may be no available evidence that research has been conducted to attribute TB case detection to frequency of sample pick up.

We had several limitations on this study. The sample size was small, and this may have reduced the statistical power of the study. Conducting the study in a district with few health facilities also affected the generalizability of the finding. In addition, factors such as stock out of reagents and consumables were not evaluated as this could have contributed to sample testing and ultimately to TB case detection. In addition, this research didn't collect patient-level data to identify the category of patients that were most likely to fail to submit sputum specimen.

Despite these limitations, these findings have greater policy implications. They underscore the need to promote rapid diagnostic tools that utilise non-respiratory specimens to minimise over-reliance on sputum samples that are difficult to collect, especially in the younger population. Additionally, strengthening the sample referral system should be prioritised at all levels to ensure TB samples reach the diagnostic site on time. Tailored strategies that are aligned with the burden of TB should be prioritised to optimise resource use. Overall, this research highlights the importance of implementing interventions that are evidence-based to improve case finding and reduce TB burden across TB programs.

5.1. Conclusion

We have rigorously assessed the impact of TB specimen referral factors on TB case detection in urban, peri-urban and rural health facilities. The findings demonstrate that frequency of sample pickup and number of samples submitted were significantly associated with TB case detection.

To improve case detection, targeted interventions should be made focussed on improving sample submission and ensuring efficient sample courier services. Achieving this will require involvement of facility staff and all stakeholders to ensure operations gap are identified and addressed. Such improvement will ensure timely submission of specimens and linkage to the nearest diagnostic site to ensure no cases are missed at the facility.

Additionally, expanding the use of non-respiratory samples may improve access to TB diagnostic tools and ensure early sample submission especially among populations unable to produce good quality specimens. Highly sensitive true point-of-care (POC) platforms should be developed and adopted to improve access and rapid detection of TB cases at all levels. There is a need to conduct research with a large sample size to accurately determine the impact of the specimen transport system on case detection.

5.2. Recommendation

In view of the findings from this study, key recommendations are:

- Consider introducing true point-of-care rapid diagnostic methods in remote areas to ensure timely case detection.
- Develop a tailored intervention to enhance the identification of TB presumptive based on the geographical setting.
- Review the scheduled sample transport plan to optimise coverage and efficiency.
- Proactively monitor and track courier system performance, including pick-up frequency to identify gaps for immediate corrective action.
- Implement a deliberate program to sensitise communities about TB and the importance of timely testing during outreach programs to increase community awareness on TB diagnosis procedures.
- Encourage health facilities to collect and submit all suspected TB samples routinely to prevent missing TB cases.
- Through the use of job aids and other Information, Education, and Communication (IEC) materials, provide clear guidelines to health care workers on proper sample collection and storage to ensure good quality sample submission.
- Allocate more resources towards specimen referral to ensure regular pick up of TB samples in the facilities.
- Conduct a robust facility-based monitoring system to review case identification procedures in all the facilities.
- Research to determine the impact referral referral-related factors on drug-resistant TB detection, considering the strict sample collection and transportation requirements.

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APPENDICES

Appendix 1: Study Questionnaire

Title: *Effect of Specimen-Related Factors on TB Case Detection in Kafue, Zambia*

Instructions:
This questionnaire is part of an academic study. Your participation is voluntary, and all information will be treated confidentially. The survey takes only a few minutes. For more information, contact: **0963747745**.

SECTION A: Informed Consent

1. Do you agree to participate in this study?
☐ Yes ☐ No

SECTION B: Sociodemographic Information

1. Age Group
☐ 15–17 ☐ 18–28 ☐ 29–38 ☐ 39–48 ☐ 49–58 ☐ 59+

2. Gender
☐ Male ☐ Female

3. Level of Education
☐ College ☐ University

4. Occupation
☐ Nurse ☐ Clinical Officer ☐ Lab Staff ☐ Doctor ☐ Other (specify): ______________

5. Facility Name: ___________________________

____

SECTION C: Objective 1 – To determine if the frequency of sample pick up contributes to TB case detection

1. Frequency of sample pick-up per week:
☐ Once ☐ Twice ☐ Three times ☐ Four times ☐ Daily

....

SECTION D: Objective 2 – To assess if the distance between the referring facility and the diagnostic site affects case detection.

1. Distance to diagnostic site (km): ________________________

2. Mode of transport used for sample movement
☐ Motorbike ☐ Public Vehicle ☐ GRZ Vehicle ☐ Bicycle

3. Time taken for a specimen to reach diagnostic site
☐ Same day ☐ 1–2 days ☐ 3–5 days ☐ More than 5 days

4. Does distance contribute to low TB case yield at your facility?
☐ Agree ☐ Disagree ☐ Not Sure

5. Does distance contribute to delayed result feedback?
☐ Yes ☐ No ☐ Not Sure

....

SECTION E: Objective 3 – To assess if the availability of a TB sample refrigeration system at the referring site contributes to case detection.

1. Does your facility have a refrigerator for TB samples?
☐ Yes ☐ No

2. Is the refrigerator functional?
☐ Yes ☐ No

3. Is the refrigerator regularly serviced?
☐ Yes ☐ No

....

SECTION F: Objective 4 – To assess whether the use of dedicated riders contributes to case detection.

1. Is the rider dedicated to the Sample Referral System (SRS)?
☐ Yes ☐ No

2. How often does the rider visit the facility?
☐ Daily ☐ Twice weekly ☐ Weekly ☐ Other: _____________

3. Is the rider trained in sample transport?
☐ Yes ☐ No

4. Is the rider paid a monthly salary?
☐ Yes ☐ No

5. Does the rider also collect results when they are ready?
☐ Yes ☐ No

....

SECTION G: Objective 5 –To examine the association between facility attendance and TB case detection.

1. Annual facility attendance: ________________________

2. Annual presumptive TB cases: ______________________

3. Number of clients who submitted samples in 2024: ______________________

4. Number of bacteriologically confirmed TB cases: ______________________

....

SECTION H: Objective 6 –To assess whether type of health facility based on geographical setting is associated with TB case detection.

1. Facility Location

☐ Rural ☐ Peri-urban ☐ Urban

Thank you for your participation.

Appendix 2: NHRA Research Approval Letter

NATIONAL HEALTH RESEARCH AUTHORITY

Lot No. 18961/M, off Kasama Road, Chalala, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@nhra.org.zm | www.nhra.org.zm

NHRA-2660/04/09/2025 **12th September 2025**

The Principal Investigator,
Mr. Robertson Chibumbya,
UNILUS,

Dear Mr. Robertson Chibumbya,

Re: Request for Authority to Conduct Research

The National Health Research Authority Is in Receipt of Your Request for Authority to Conduct Research Titled **"Effects of Specimen Referral System-Related factors on Laboratory TB Case Detection in Kafue District of Zambia"**

I wish to inform you that following submission of your request to the Authority, our review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised.
2. Progress updates are provided to NHRA bi-annually from the date of commencement of the study.
3. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country.
4. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, University leadership, and all key respondents.

Yours sincerely,

National Health Research Authority

Prof Victor Chalwe,
Director and Chief Executive Officer

Appendix 3: UNILUS -REC Ethical Clearance

UNIVERSITY *of* LUSAKA

Passion for Quality Education: Our Driving Force

UNIVERSITY OF LUSAKA RESEARCH ETHICS COMMITTEE (UNILUS-REC)

Plot No. 37413, Off Alick Nkhata Mass Media. P. O Box 36711, Lusaka.
Phone: +260211258505, 258409 Fax +260211233409; Cell +260976075850,961917862,
E-mail:unilus@zamnet.zm,ictar@zamnet.zm

UNILUS-RESEARCH ETHICS COMMITTEE

Ref no: FWA00033228-1001(08)/(08)/{2024}

Date: 03 September 2025

STUDENT NAME: **Mr. Robertson Chibumbya**

Effect of Specimen Referral System on TB Case Detection

The above research was submitted to the research ethics committee for review. The study has no major ethical problems and is approved subject to the following:

1. The study cannot be changed without express permission of the UNILUS research ethics committee.
2. Approval from the necessary authority should be sought.

1 of 2

Professor Kasonde Bowa

MSc(Glasgow),M.Med(UNZA),FRCS(Glasgow),FACS,FCS,DPH(LSTMH),MPH(UCL)

Chairman- UNILUS REC

Professor of Urology and Consultant Urologist

Deputy Vice-Chancellor – Research and Innovation

Executive Dean - School of Medicine and Health Sciences

Appendix 4: GCLP Certificate

National Institute of Allergy and Infectious Diseases
Division of AIDS

for completing training on:
Good Clinical Laboratory Practice (GCLP)

Date: *May 8, 2025*
Course hours: *14 hours*

presents this certificate of attendence to:

Robertson Chibumbya

Sarah Read, MD, MHS
Deputy Director, DAIDS

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

NIH
National Institute of Allergy and Infectious Diseases

Appendix 5: Lusaka Provincial Health Office Research Approval Letter

All correspondence should be addressed to the Provincial Health Director
Telephone: +260 211 256813
Fax: +260 211 256814
Telephone: +260 211 256815
Cell: +260 956 399643
+260 963 908260

REPUBLIC OF ZAMBIA

MINISTRY OF HEALTH

In Reply please quote:

File No. LSKPHO/101/8/1

Lusaka Provincial Health Office
P.O. Box 32573
LUSAKA

15th September, 2025

Robertson Chibumbya
University of Lusaka
LUSAKA
0963747745

PERMISSION TO CONDUCT RESEARCH

My office is in receipt of your letter requesting for permission to conduct a study titled "**Effects of Specimen Referral System Related Factors on Laboratory TB Case Detection in Kafue District of Zambia**"

My office is glad to inform you that it has no objection to your request provided that;

1. The relevant Institution Director where the study is being conducted are fully appraised;
2. Progress updates are provided to Lusaka Provincial Health Office and the District Health Office biannually from the date of commencement of the study;
3. The final study report is cleared by NHRA before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted.

Kindly ensure minimum interruption in health service delivery at selected health facilities.

By copy of this letter, the District Health Office / Institution are advised to allow you undertake the above-mentioned research and provide you with the relevant support.

Yours faithfully,

Dr. Simulyamana Aspha Choonga
Provincial Health Director
LUSAKA PROVINCE

CC: District Health Director – Lusaka

Appendix 6: Kafue DHO Research Notification Letter

DHD
Proceed! Research is cleared. 30/09/25
Note to Officer to provide feedback of the research.

University of Lusaka

Pioneer Campus plot no. 37413

P.O Box 36711

Lusaka

30 September 2025

REPUBLIC OF ZAMBIA
MINISTRY OF HEALTH
KAFUE DISTRICT HEALTH OFFICE
30 SEP 2025
DISTRICT HEALTH DIRECTOR
P. O. BOX 360025, KAFUE

The District Health Director

Kafue DHO

Kafue

Dear Sir

Ref: Notification to Collect Data for My Academic Research

I would like to notify you that I have been granted permission by Lusaka PHO to collect data in health facilities in Kafue for my academic research following the ethics clearance from my school, University of Lusaka (UNILUS).

I am a final year student at UNILUS pursuing a Master of Science in Epidemiology and Biostatistics. My student number is MSCEB23119411. I am a registered researcher with National Health Research Authority with registration number NHRAR-R-732/14/07/2022. Before I complete my studies this year, I am required to conduct research as a pre-requisite. My research title is "**Effects of Specimen Referral System-Related Factors on Laboratory TB Case Detection In Kafue District of Zambia**". This research will be conducted in Kafue at health centers referring to TB sputum samples to GeneXpert testing sites (Kafue Estate and Kafue General Hospital). I plan to collect data in October 2025.

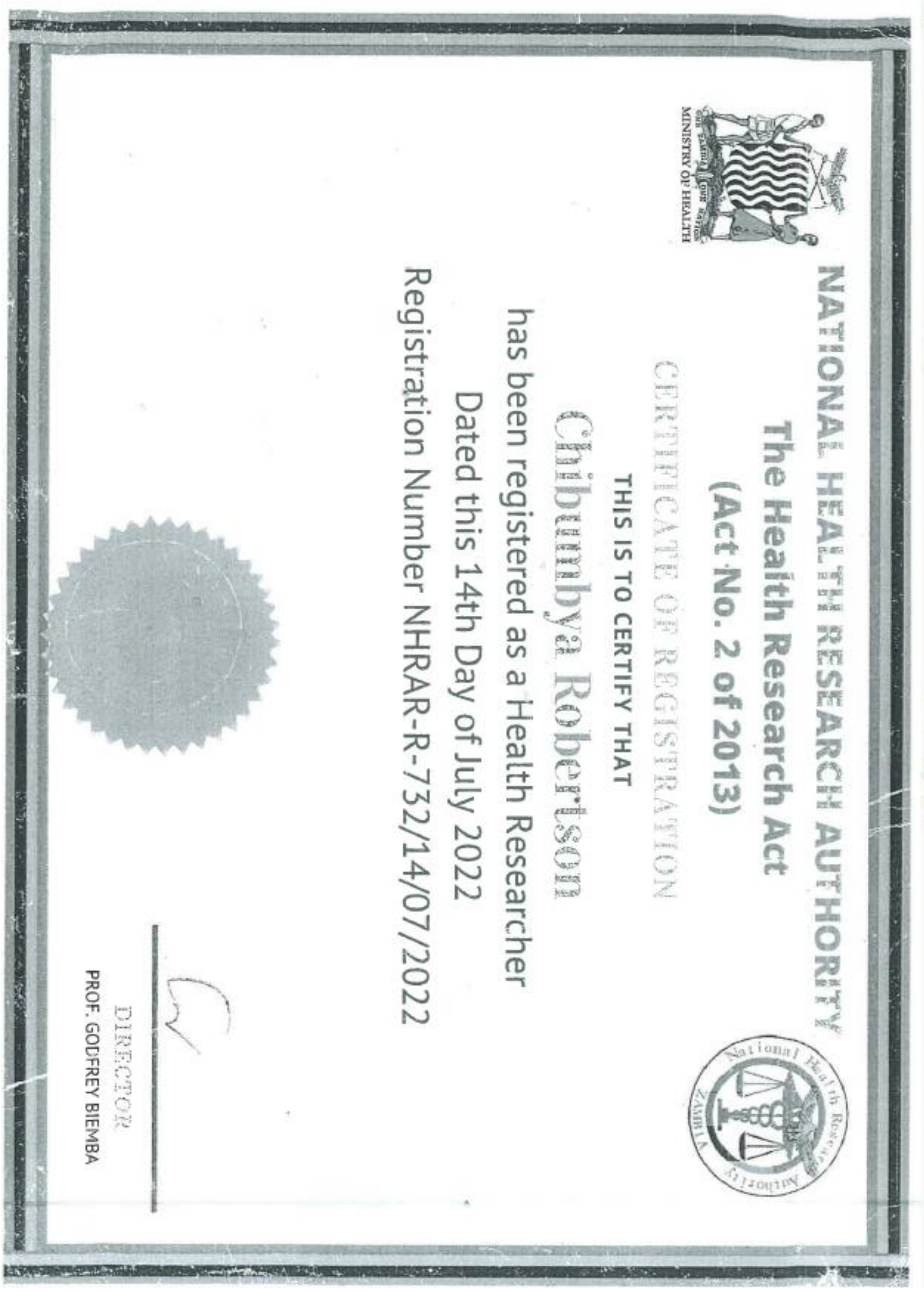
I request your support for this activity.

Sincerely Yours,

Robertson Chibumbya

0963747745

Appendix 7: NHRA Registration Certificate


MINISTRY OF HEALTH

NATIONAL HEALTH RESEARCH AUTHORITY

The Health Research Act

(Act No. 2 of 2013)

CERTIFICATE OF REGISTRATION

THIS IS TO CERTIFY THAT

Chibumbya Robertson

has been registered as a Health Researcher

Dated this 14th Day of July 2022

Registration Number NHRAR-R-732/14/07/2022

National Health Research Authority ZAMBIA

DIRECTOR

PROF. GODFREY BIEMBA