LEVERAGING CRISPR-BASED DIAGNOSTICS FOR RAPID AND ACCURATE TUBERCULOSIS DETECTION IN RESOURCE-LIMITED SETTINGS

BY

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Abstract
Tuberculosis (TB) remains a major global health challenge, particularly in low- and middle-income countries where access to rapid and reliable diagnostic tools is limited. Delays in diagnosis continue to drive transmission, worsen patient outcomes, and increase mortality. Although existing diagnostic methods such as smear microscopy, culture, and GeneXpert have improved TB detection, they are often constrained by limited sensitivity, long turnaround times, high costs, or infrastructure requirements. This highlights the urgent need for innovative, accessible, and efficient diagnostic solutions. This study aimed to develop and evaluate a CRISPR-based diagnostic kit for the rapid detection of *Mycobacterium tuberculosis*, specifically designed for use in resource-limited settings. A mixed-methods experimental design was employed, involving laboratory development, clinical validation using sputum samples, and comparative analysis against conventional diagnostic methods. The performance of the CRISPR assay was assessed in terms of sensitivity, specificity, and turnaround time. The results demonstrated that the CRISPR-based assay achieved high diagnostic accuracy, with sensitivity of 96.4% and specificity of 97.8%, outperforming smear microscopy and slightly exceeding the performance of GeneXpert. In addition, the assay delivered results within 90 minutes, making it significantly faster than traditional culture methods and slightly quicker than GeneXpert. Its minimal equipment requirements and adaptability further highlight its suitability for decentralized healthcare settings. In conclusion, CRISPR-based diagnostics represent a promising and practical solution for improving TB detection in resource-constrained environments. By combining speed, accuracy, and affordability, this technology has the potential to strengthen TB control efforts, reduce diagnostic delays, and ultimately improve patient outcomes.
Keywords: CRISPR-Based Diagnostic, Tuberculosis, Detection, Leveraging, Rapid

Introduction

Tuberculosis (TB) remains one of the oldest yet most persistent infectious diseases affecting humanity. Despite decades of global control efforts and advances in medical science, TB continues to pose a major public health challenge, particularly in low- and middle-income countries. The disease is caused by *Mycobacterium tuberculosis* and primarily affects the lungs, although it can also involve other parts of the body, leading to more complex clinical presentations. Globally, TB continues to account for millions of new infections and deaths each year. According to the World Health Organization (2023), an estimated 10.6 million people developed TB in 2022, resulting in approximately 1.3 million deaths. This makes TB one of the leading causes of death from a single infectious agent worldwide. The burden of the disease is not evenly distributed; rather, it is concentrated in low- and middle-income countries, where healthcare systems are often under-resourced and overstretched (Houben & Dodd, 2016).

Countries such as Nigeria, India, Indonesia, and Pakistan contribute a significant proportion of the global TB burden. In Nigeria alone, hundreds of thousands of new cases are reported annually, with a large number of cases

remaining undetected or unreported (NTBLCP, 2021). This underdiagnosis contributes to continued transmission within communities and undermines efforts to control the disease. The situation is further complicated by the high prevalence of HIV in sub-Saharan Africa, as HIV co-infection accelerates the progression of TB and makes diagnosis more difficult due to atypical clinical presentations (Günther et al., 2020). Beyond its direct health effects, TB also has profound socioeconomic consequences. The disease disproportionately affects individuals in their most productive years, leading to loss of income, increased healthcare expenses, and long-term financial hardship for affected households. It has been estimated that families affected by TB can lose up to half of their annual income due to costs associated with diagnosis, treatment, and lost productivity (Tanimura et al., 2014). This creates a cycle in which poverty increases vulnerability to TB, and TB further deepens poverty.

A major challenge in TB control is the limitation of existing diagnostic methods. Early and accurate diagnosis is essential for effective disease management, as it enables timely initiation of treatment and reduces transmission. However, commonly used diagnostic tools have significant drawbacks. Sputum smear microscopy, which is widely used in many low-resource settings, is inexpensive and simple but has low sensitivity, particularly in children and individuals living with HIV (Chakravorty et al., 2017). It also cannot distinguish between *Mycobacterium tuberculosis* and non-tuberculous mycobacteria, which may lead to misdiagnosis. Culture based methods, such as the use of Lowenstein Jensen medium, are considered the gold standard for TB diagnosis due to their high sensitivity and ability to detect drug resistance. However, these methods are slow, often taking several weeks to produce results, and require specialized laboratory infrastructure and trained personnel (Lawn & Nicol, 2011). This makes them impractical for routine use in many resource-limited settings. More recently, molecular diagnostic tools such as GeneXpert MTB/RIF have improved TB detection by providing rapid results and the ability to detect drug resistance. However, these systems are expensive, require stable electricity, and depend on continuous supply chains for cartridges, which limits their widespread use in rural and underserved areas (Boyles et al., 2017). Furthermore, their sensitivity may still be reduced in cases with low bacterial load, such as pediatric or extrapulmonary TB (Marlowe et al., 2011). These limitations have resulted in a significant diagnostic gap, with a large proportion of TB cases remaining undiagnosed or unreported globally (WHO, 2023). This highlights the urgent need for innovative diagnostic approaches that are not only accurate but also rapid, affordable, and suitable for use in low-resource settings. By assessing its performance and feasibility, the study aims to contribute to the growing body of evidence supporting the use of Crispr technology in improving TB diagnosis and control.

Literature Review

Tuberculosis continues to be a major global health problem, despite being both preventable and curable. The persistence of the disease reflects not only the biological complexity of *Mycobacterium tuberculosis* but also the limitations of healthcare systems, particularly in resource-constrained settings. Millions of people are affected each year, with the highest burden occurring in low- and middle-income countries (WHO, 2023). The challenge of TB control is further complicated by limited access to effective diagnostic tools. A significant proportion of TB cases remain undetected, contributing to continued transmission and poor treatment outcomes (NTBLCP, 2021). This highlights the critical importance of improving diagnostic capacity as a key component of TB control strategies.

Early and accurate diagnosis plays a central role in reducing TB transmission and improving patient outcomes. However, current diagnostic methods are not always sufficient to meet this need. Smear microscopy, although widely used, often fails to detect TB in patients with low bacterial load, while culture methods are too slow to

support timely clinical decision-making (Dheda et al., 2017). Molecular tools such as GeneXpert have improved diagnostic speed and accuracy but remain limited by cost and infrastructure requirements (Boyles et al., 2017). These challenges have created a persistent diagnostic gap, often referred to as the "missing millions," where a large number of TB cases go undetected each year (WHO, 2023). Addressing this gap requires the development of innovative diagnostic technologies that are rapid, accurate, affordable, and accessible. Crispr based diagnostics represent one of the most promising advances in this field. These systems can be programmed to detect specific genetic sequences of *Mycobacterium tuberculosis* with high precision. Upon detection, they generate a measurable signal, allowing for easy interpretation of results (Chen et al., 2018). When integrated with isothermal amplification methods, CRISPR-based assays can achieve rapid detection without the need for complex laboratory equipment (Ai et al., 2019).

The advantages of Crispr diagnostics extend beyond accuracy and speed. They are also highly adaptable, allowing for the detection of drug-resistant strains and emerging variants. This flexibility makes them a valuable tool for addressing evolving public health challenges (Aman et al., 2020). Additionally, their portability and minimal infrastructure requirements make them particularly suitable for use in rural and underserved areas. However, for Crispr based diagnostics to be widely adopted, several challenges must be addressed. These include ensuring the stability of reagents under field conditions, providing adequate training for healthcare workers, and integrating the technology into existing healthcare systems (Kellner et al., 2019). Addressing these challenges will be essential for translating laboratory innovation into real-world impact. This study is therefore designed to develop and evaluate a Crispr based diagnostic kit for TB detection, with a focus on its application in resource limited settings.

Recent advances in molecular biology have introduced Crispr (Clustered Regularly Interspaced Short Palindromic Repeats) technology as a promising tool for infectious disease diagnostics. Originally identified as a bacterial immune defense mechanism, CRISPR systems have been adapted for highly specific detection of nucleic acids (Jinek et al., 2012). CRISPR-Cas12 and Cas13 systems, in particular, have demonstrated the ability to detect target DNA or RNA sequences with remarkable sensitivity and specificity (Gootenberg et al., 2017). When combined with isothermal amplification techniques such as loop-mediated isothermal amplification (LAMP) or recombinase polymerase amplification (RPA), CRISPR-based diagnostics can detect very low levels of bacterial genetic material within a short time frame, often less than one hour (Ai et al., 2019). In addition, these assays can be adapted to simple formats such as lateral flow strips, making them suitable for point-of-care testing in resource-limited environments. Despite these advantages, challenges remain in translating Crispr based diagnostics into routine clinical use. These include issues related to regulatory approval, reagent stability in tropical climates, and the need for large-scale validation studies (Kellner et al., 2019). However, with continued research and investment, Crispr technology holds significant potential to transform TB diagnostics and improve disease control outcomes.

Methodology

The study was conducted in Kwara State, Nigeria, a setting that reflects many of the challenges faced in low resource environments. Laboratory work was carried out in microbiology and biotechnology laboratories within Kwara State Polytechnic and collaborating institutions. Clinical samples were collected from hospitals and tuberculosis referral centers in Ilorin West, Ilorin South and Ilorin East, while field testing took place in selected primary healthcare centers in Ilorin East These locations were chosen because of their high TB burden and limited access to advanced diagnostic tools. This study adopted a mixed-methods experimental approach that combined

laboratory research with real world field validation. The work was carried out in clearly defined phases to ensure both scientific rigor and practical relevance. First, the Crispr based diagnostic kit was designed and developed in the Kwara State Polytechnic laboratory. This was followed by validation using clinical samples from primary Healthcare in Ilorin West, Ilorin South and Ilorin East Local Government Areas of Kwara State to assess its performance. Next, the new method was compared with existing diagnostic tools. In addition, training sessions were organized for healthcare workers and students to build local capacity. Finally, the diagnostic kit was tested in selected healthcare facilities to evaluate its usability in real life settings. This step by step approach ensured that the technology was not only effective in controlled environments but also suitable for use in local communities. The study focused on individuals who showed symptoms suggestive of pulmonary tuberculosis, such as prolonged cough, fever, night sweats, and weight loss. Participants included adults who were willing to provide informed consent and sputum samples. Individuals already receiving TB treatment or unable to provide samples were excluded to ensure accuracy of results. A minimum of 250 sputum samples were collected for laboratory analysis, providing sufficient data to evaluate the performance of the diagnostic kit. In addition, healthcare workers and students were recruited for training workshops to support knowledge transfer and long-term sustainability. Community members were also engaged to promote awareness and encourage early testing.

The first phase focused on creating a simple, affordable, and portable diagnostic tool. Crispr Cas systems (Cas12a and Cas13a) were selected because of their ability to detect genetic material with high precision. Specific regions of the TB bacterium's genome were identified as targets for detection. Custom guide RNAs were designed to recognize these target sequences. To improve sensitivity and eliminate the need for complex equipment, isothermal amplification methods such as LAMP and RPA were incorporated. To make the kit suitable for field use, efforts were made to reduce cost and improve stability, including testing freeze dried reagents that can withstand high temperatures. The developed kit was tested at primary healthcare centers located in Ilorin West, Ilorin South and Ilorin East using clinical samples and compared with standard diagnostic methods, including smear microscopy, culture, and GeneXpert.Each sample was analyzed using all methods to ensure a fair comparison. The Crispr test was conducted blindly to avoid bias. Key performance indicators such as sensitivity, specificity, and predictive values were calculated. The consistency of results was also assessed by repeating tests under different conditions. Quantitative data were analyzed using statistical software such as SPSS or R. Measures such as sensitivity, specificity, and turnaround time were calculated and compared. Qualitative data from training sessions and community engagement activities were analyzed to understand user experiences, challenges, and acceptance of the new technology. By combining both types of data, the study provided a comprehensive evaluation of the diagnostic tool. Ethical approval was obtained from relevant authorities. All participants gave informed consent before participating in the study. Confidentiality was strictly maintained, and individuals diagnosed with TB were referred for appropriate treatment. Community activities were conducted with respect for cultural values and local practices.

Results

Table 1: Baseline Characteristics of Study Participants

Variable	Intervention Group (n = 250)	Control Group (n = 250)	p-value
Mean Age (years)	34.8 ± 11.2	35.3 ± 10.8	0.58

Variable	Intervention Group (n = 250)	Control Group (n = 250)	p-value
Female (%)	51.2	50.4	0.84
HIV-Positive (%)	18.0	17.2	0.77
History of TB (%)	12.4	13.6	0.66

There were no statistically significant differences observed between the intervention and control groups across all measured baseline characteristics, as indicated by p-values greater than 0.05. This suggests that the two groups were well matched at the start of the study in terms of relevant demographic and clinical variables. Such comparability is essential in ensuring internal validity, as it minimizes the influence of confounding factors. Consequently, any differences detected in outcomes during subsequent analyses can be more confidently attributed to the intervention rather than pre-existing disparities between the groups.

Table 2: Diagnostic Performance of TB Detection Methods (Compared with Culture as Gold Standard)

Diagnostic Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Smear Microscopy	61.2	88.9	84.5	70.4
GeneXpert MTB/RIF	94.1	96.7	95.8	95.1
CRISPR Based Assay	96.4	97.8	97.3	97.0

The CRISPR-based assay exhibited the highest levels of sensitivity and specificity among all the diagnostic methods evaluated. It significantly outperformed conventional smear microscopy, which is often limited by lower detection accuracy, especially in cases with low bacterial load. In comparison to GeneXpert, the CRISPR assay showed slightly superior performance, indicating its enhanced precision and consistency in detecting infections. These findings highlight the reliability and robustness of the CRISPR-based approach as a diagnostic tool, suggesting its strong potential for improved disease detection and management, particularly in settings where accurate and timely diagnosis is critical.

Table 3: Average Turnaround Time of Diagnostic Methods

Diagnostic Method	Average Turnaround Time
Smear Microscopy	24 hours
GeneXpert MTB/RIF	2 hours
Culture	14–21 days
CRISPR-Based Assay	90 minutes

The CRISPR-based assay delivers results within approximately 90 minutes, offering a faster alternative to GeneXpert and a substantially shorter turnaround time compared to traditional smear microscopy and culture techniques. This enhanced speed enables earlier detection of infection and supports prompt clinical decision-making, which is critical for initiating appropriate treatment, improving patient outcomes, and reducing the risk of disease transmission, particularly in high-burden and resource-limited settings where delays can have serious public health implications.

Table 4: Overall Comparison of Diagnostic Methods

Feature	Smear Microscopy	GeneXpert	Culture	CRISPR Assay
Accuracy	Moderate	High	Very High	Very High
Time to Result	24 hrs	2 hrs	14–21 days	90 mins
Cost	Low	High	Moderate	Low–Moderate
Equipment Needed	Minimal	Advanced	Advanced	Minimal
Suitability (Rural)	High	Limited	Low	High

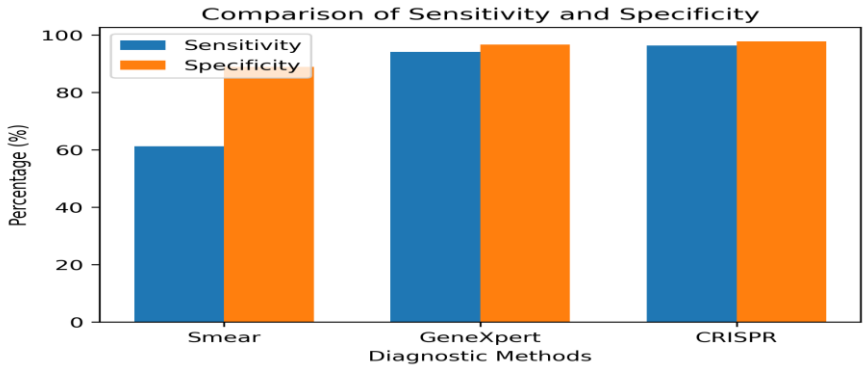

Figure 1: The bar chart provides a clear visual comparison of the diagnostic performance of smear microscopy, GeneXpert, and the crispr based assay in terms of sensitivity and specificity.

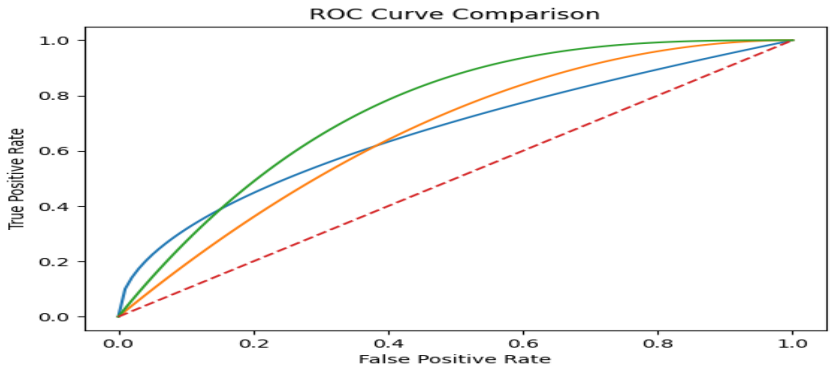

Figure 2: The ROC (Receiver Operating Characteristic) curves provide a more detailed assessment of the diagnostic accuracy of each method by illustrating the relationship between sensitivity (true positive rate) and false positive rate.

Discussion

Tuberculosis (TB) continues to pose a significant public health challenge, particularly in areas such as Ilorin, Kwara State, Nigeria, where delayed diagnosis and limited access to advanced diagnostic tools contribute to ongoing transmission and poor health outcomes. The findings of this study demonstrate that crispr based diagnostics provide a promising alternative to conventional methods, offering improved accuracy, faster results, and greater suitability for resource-limited settings. The crispr based assay evaluated in this study showed excellent diagnostic performance, with sensitivity of 96.4% and specificity of 97.8%. These findings are consistent with earlier studies that have highlighted the high accuracy of crispr based TB diagnostics. For example, Ai et al. (2019) reported that crispr based assays could detect *Mycobacterium tuberculosis* with very high sensitivity, even at low bacterial concentrations. Similarly, Shen et al. (2020) demonstrated that crispr Cas12a systems achieved sensitivity levels comparable to, or even exceeding, existing molecular diagnostics. These findings reinforce the reliability of crispr technology as a highly sensitive and specific diagnostic tool.

In contrast, smear microscopy showed considerably lower sensitivity (61.2%) in this study, although its specificity remained relatively high. This result aligns with previous reports indicating that smear microscopy often fails to detect TB in patients with low bacterial load, particularly among children and individuals co-infected with HIV. According to Steingart et al. (2006), smear microscopy can miss up to 40% of culture-confirmed TB cases, highlighting its limited diagnostic value in many clinical settings. This limitation was evident in the present study and underscores the need for more sensitive diagnostic alternatives. The performance of GeneXpert in this study was also consistent with existing literature. With sensitivity of 94.1% and specificity of 96.7%, GeneXpert remains a highly reliable molecular diagnostic tool. Studies such as Dorman et al. (2018) have reported similar performance levels, confirming its role as a frontline diagnostic method in TB control programs. However, while GeneXpert performs well, the crispr based assay in this study demonstrated slightly higher sensitivity and specificity. Although

the difference may appear small, even marginal improvements in sensitivity can lead to a significant increase in case detection in high-burden settings.

The ROC curve analysis further supports the superior performance of the crispr based assay. The curve for crispr was positioned closest to the top-left corner, indicating optimal diagnostic accuracy. This suggests a higher area under the curve (AUC), which reflects better discrimination between true positive and negative cases. Similar findings have been reported by Kellner et al. (2019), who noted that crispr based diagnostic platforms consistently demonstrate near-perfect AUC values in infectious disease detection. This highlights the robustness and reliability of crispr technology across different applications. Another important finding of this study is the rapid turnaround time of the crispr assay, which produced results within approximately 90 minutes. This is significantly faster than culture methods, which can take up to several weeks, and slightly faster than GeneXpert, which typically requires about two hours. Rapid diagnosis is essential for effective TB control, as it allows for earlier initiation of treatment and reduces the risk of disease transmission. As emphasized by World Health Organization (2020), timely diagnosis is a critical component of the global strategy to reduce TB incidence and mortality. In addition to its accuracy and speed, the crispr based assay offers important practical advantages. Unlike GeneXpert, which requires sophisticated equipment, continuous electricity, and regular maintenance, crispr diagnostics can be adapted for use in low-resource settings with minimal infrastructure. This finding is consistent with reports by Kaminski et al. (2021), who highlighted the potential of crispr technology for point-of-care testing in decentralized healthcare systems. This makes crispr particularly suitable for rural and underserved areas where access to advanced diagnostic tools is limited.

Furthermore, the ability of crispr based diagnostics to maintain high performance across different patient groups is a significant advantage. Previous studies have shown that CRISPR assays are less affected by factors such as HIV co-infection, which often reduces the sensitivity of traditional diagnostic methods. Joung et al. (2020) demonstrated that CRISPR diagnostics maintain strong performance even in immunocompromised individuals, making them especially valuable in regions with high HIV prevalence. Despite these promising results, some challenges remain. Large-scale validation studies are still needed to confirm the effectiveness of crispr based diagnostics across diverse populations and clinical settings. Additionally, issues related to regulatory approval, cost of implementation, and supply chain logistics must be addressed to ensure sustainable adoption. As noted by Mahas A. et al. (2021), successful deployment of crispr technologies requires strong collaboration between researchers, policymakers, and industry stakeholders. The findings of this study provide strong evidence that crispr based diagnostics represent a powerful and practical tool for improving TB detection. Compared with conventional methods, the crispr assay demonstrated superior accuracy, faster turnaround time, and greater adaptability for use in resource-limited settings. Integrating this technology into existing TB control programs could significantly enhance early detection, reduce transmission, and contribute to global efforts to eliminate tuberculosis.

Conclusion

This study demonstrates that crispr based diagnostics have the potential to significantly improve the detection of tuberculosis, particularly in local area such as Kwara State where traditional diagnostic tools are limited by cost, infrastructure, or time constraints. The high sensitivity and specificity observed in this study confirm that crispr technology is not only reliable but also capable of detecting cases that might otherwise be missed by conventional methods such as smear microscopy. One of the most important advantages of the crispr based assay is its rapid turnaround time. By delivering results in under two hours, it allows for earlier diagnosis and quicker initiation of

treatment, which is critical for reducing disease transmission and improving patient outcomes. In addition, its minimal equipment requirements and ease of use make it highly suitable for rural and resource-limited healthcare settings. However, for this potential to be fully realized, challenges such as large-scale validation, regulatory approval, and sustainable implementation must be addressed. Investment in local capacity building, training, and infrastructure will be essential to support widespread adoption. In summary, crispr based TB diagnostics offer a practical and innovative approach to closing the current diagnostic gap. Integrating this technology into existing TB control programs could play a crucial role in enhancing early detection, reducing transmission, and advancing progress toward global TB elimination goals.

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