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Diagnostic Accuracy of CRISPR-Based Rapid Assays for Detection of Multi-Drug-Resistant Tuberculosis

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Abstract

Multidrug-resistant tuberculosis (MDR-TB) remains a major global public health threat, driven by delayed diagnosis, limited drug susceptibility testing capacity, and the slow turnaround time of conventional culture-based methods. This study reviews and synthesizes current evidence on the diagnostic accuracy and clinical utility of CRISPR-based rapid molecular assays for the detection of *Mycobacterium tuberculosis* and associated drug resistance mutations. Traditional diagnostic platforms such as smear microscopy, culture, and GeneXpert MTB/RIF Ultra, while widely used, are constrained by either low sensitivity in paucibacillary disease or limited resistance profiling and infrastructural dependency. CRISPR-Cas–based diagnostics, particularly those utilizing Cas12, Cas13, and Cas14 effectors integrated with isothermal amplification techniques such as RPA and LAMP, demonstrate significant improvements in turnaround time, sensitivity, and point-of-care applicability. Meta-analytic evidence indicates pooled sensitivities of approximately 91–93% and specificities of 97–98% for tuberculosis detection, with even higher accuracy reported for rifampicin and isoniazid resistance-associated mutations such as *rpoB* S531L and *katG* S315T. The diagnostic workflow enables rapid detection within one hour, with the added advantage of single nucleotide polymorphism discrimination critical for MDR-TB identification. Furthermore, CRISPR-based platforms show strong performance in extrapulmonary and paucibacillary samples, where conventional diagnostics often underperform. Their compatibility with portable, lyophilized, and low-cost formats enhances feasibility for decentralized and resource-limited healthcare settings. Despite these advantages, challenges remain in large-scale enzyme production, standardization, sample preparation, and regulatory harmonization.

Introduction

The Global Resurgence of Tuberculosis and the Crisis of Drug Resistance

The landscape of global health in 2025 and 2026 remains dominated by the persistent challenge of tuberculosis (TB), an infectious disease that has likely regained its position as the leading cause of death from a single infectious pathogen globally. Despite decades of public health interventions that have saved an estimated 83 million lives since the turn of the millennium, the trajectory of the epidemic is increasingly defined by the rise of drug-resistant strains (WHO, 2026). Recent data from the World Health Organization indicates that in 2024, approximately 10.7 million people fell ill with TB, and the disease claimed over 1.2 million lives (WHO, 2025a). Within this context, multidrug-resistant TB (MDR-TB) defined as resistance to at least isoniazid and rifampicin represents a critical failure in the global control effort, primarily due to the diagnostic and therapeutic complexities it introduces (Pai et al., 2025).

The epidemiological burden of MDR-TB is characterized by profound regional disparities. In 2024, the estimated number of incident MDR/RR-TB (rifampicin-resistant) cases was 390,000, with a significant concentration in high-burden nations. Notably, four countries India, China, the Philippines, and the Russian Federation accounted for over half of the global number of people estimated to have developed MDR/RR-TB in 2024 (Global Fund, 2025). While the global proportion of new cases with MDR/RR-TB has shown a slight downward trend from 4.7% in 2015 to 3.2% in 2024, the situation remains dire for previously treated cases, where the proportion of resistance is estimated at 16% (Unitaid, 2025). Furthermore, the emergence of pre-extensively drug-resistant TB (pre-XDR-TB), which exhibits resistance to any fluoroquinolone, now accounts for 18% of the MDR/RR-TB caseload, creating an urgent demand for diagnostics that can provide comprehensive resistance profiling at the point of care (WHO, 2025b).

Table 1. Global Epidemiological Burden of Tuberculosis and MDR-TB (2024/2025 Estimates)

Epidemiological Parameter (2024/2025 Estimates)	Value	95% Uncertainty Interval
Global TB Incidence	10.7 Million	10.0–11.4 Million
Global TB Deaths	1.2 Million	1.1–1.3 Million
Incident MDR/RR-TB Cases	390,000	360,000–430,000
Deaths from MDR/RR-TB	150,000	93,000–210,000
Proportion of New Cases with MDR/RR-TB	3.2%	2.5–3.9%
Proportion of Previously Treated with MDR/RR-TB	16%	8.3–23%
MDR/RR-TB Cases with Pre-XDR-TB	18%	15–20%

The economic and social implications of this crisis are staggering. Over 81% of households affected by drug-resistant TB experience catastrophic healthcare costs, which further exacerbates the cycle of poverty and disease in low- and middle-income countries. The ongoing recovery of essential health services following the COVID-19 pandemic has seen a stabilization in incidence, yet progress remains insufficient to meet the ambitious targets set by the WHO End TB Strategy (TB Partnership, 2025). The gap between those who fall ill and those who are bacteriologically confirmed remains one of the largest obstacles; in 2024, only about 78% of people who developed TB were newly diagnosed and accessed treatment, leaving millions of undetected cases to continue the cycle of transmission (Dheda et al., 2026).

The Evolution and Limitations of Current Diagnostic Modalities

The historical gold standard for tuberculosis diagnosis and antibiotic susceptibility testing has been pathogen cultivation. While culture-based methods provide definitive profiles of drug resistance, they are plagued by prolonged turnaround times, often requiring weeks or even months to produce a result due to the slow doubling time of Mycobacterium tuberculosis. This delay is a primary driver of the MDR-TB epidemic, as patients may be placed on ineffective first-line regimens while waiting for results, facilitating the selection of further resistance and continued transmission within communities (WHO, 2025b).

Molecular diagnostics, most notably the GeneXpert MTB/RIF and its successor, the GeneXpert Ultra, have revolutionized the field by reducing the time to detection to less than two hours. These assays utilize semi-quantitative real-time PCR to detect both the presence of the MTB complex and mutations in the 81-bp Rifampicin Resistance Determining Region (RRDR) of the rpoB gene. Despite their widespread adoption, these platforms face significant operational and economic constraints. In many resource-limited settings, the high capital cost of the equipment, the need for stable electricity, and the requirement for consistent air conditioning to maintain the sensitive modules have limited their deployment to centralized laboratories (Chakaya et al., 2025).

A longitudinal study in the Amhara Region of Ethiopia between 2019 and 2024 highlights these real-world challenges. Out of 587,128 samples examined, the rate of unsuccessful results encompassing errors, invalid tests, and "no result" outcomes remained consistently above the national target of 5%, with some years seeing rates as high as 7.66% (Allué-Guardia et al., 2026). These technical failures are often linked to sample quality, power fluctuations, and mechanical wear in high-volume settings. Furthermore, while GeneXpert is highly effective at identifying rifampicin resistance, its ability to detect isoniazid resistance and other second-line drug resistance markers requires additional, more expensive modules or separate line probe assays (LPAs) (Global TB Caucus, 2025).

Table 2. Comparative Analysis of Tuberculosis Diagnostic Modalities

Diagnostic Platform	Sensitivity	Specificity	Turnaround Time	Primary Limitations
Smear Microscopy	48.5%	High	Hours	Low sensitivity in paucibacillary
Sputum Culture	71.6%	100%	Weeks	Extremely slow TAT, biosafety
GeneXpert Ultra	78–90%	99.1%	2 Hours	High cost, infrastructure needs
Line Probe Assay	High	High	1–2 Days	Complex workflow, lab-based
CRISPR-Based Assays	91–97%	97–100%	<1 Hour	Early stage, sample prep needs

In response to these gaps, the World Health Organization has increasingly emphasized the need for near-point-of-care (NPOC) and low-complexity molecular tests. These technologies must be battery-operable, robust to environmental fluctuations, and significantly more affordable than current cartridge-based systems. This is the context into which Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

technology has emerged, offering a programmable, ultra-sensitive, and rapid alternative to traditional PCR-based systems (Guo et al., 2025).

Core Mechanisms of CRISPR-Cas Molecular Diagnostics

The transition of CRISPR-Cas systems from bacterial immune mechanisms to diagnostic platforms represents one of the most significant technological shifts in molecular biology. Unlike the Cas9 system commonly used for genome editing, which primarily utilizes cis-cleavage to create double-stranded breaks, the diagnostic utility of CRISPR relies heavily on Class 2 Type V and Type VI effectors, such as Cas12 and Cas13 (Wang et al., 2025).

Biophysics of Trans-Cleavage Activity

The breakthrough in CRISPR diagnostics was the discovery of "trans-cleavage" or "collateral" activity. When a Cas protein (such as Cas12a or Cas13a) binds to its specific target sequence guided by a CRISPR RNA (crRNA), it undergoes a conformational change that activates its nuclease domain. For Cas12 and Cas13, this activation triggers a non-specific cleavage of surrounding single-stranded nucleic acids (ssDNA for Cas12 and ssRNA for Cas13) (Basit et al., 2025).

By introducing a quenched fluorescent reporter molecule, a short nucleic acid linker with a fluorophore at one end and a quencher at the other researchers can convert target recognition into a visible signal. Upon activation, the Cas enzyme cleaves the reporter, separating the fluorophore from the quencher and producing a measurable fluorescent signal. This signal amplification occurs at a high catalytic turnover rate, allowing for the detection of nucleic acids at attomolar (10^{-18} M) concentrations (Singh et al., 2025).

Classification of Cas Effectors in TB Detection

The selection of a specific Cas protein dictates the type of target nucleic acid and the readout mechanism. The programmable nature of these proteins allows for the design of assays targeting various conserved regions of the MTB genome or specific resistance-conferring mutations (Feng et al., 2021).

Table 3. Characterization of Cas Effectors for Molecular Diagnostics

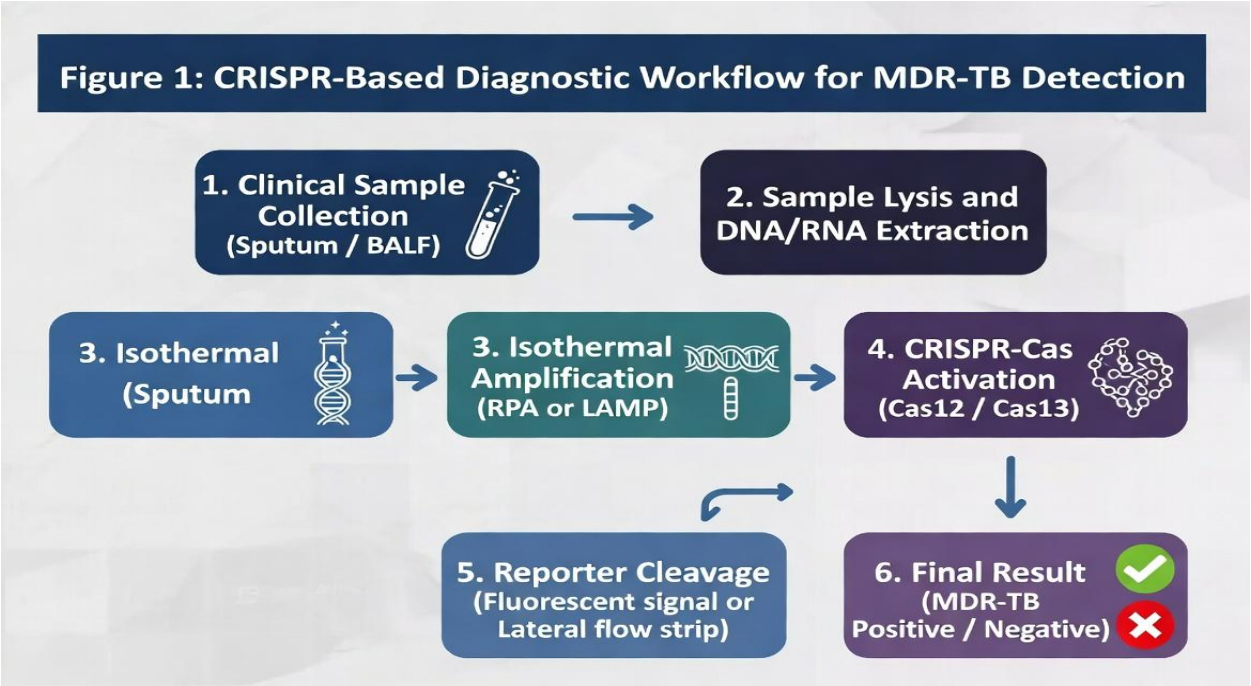
Cas Variant	Signature Mechanism	Diagnostic Suitability for TB
Cas9	Cis-cleavage of dsDNA	Targeted identification of MTB sequences
Cas12a/b	Trans-cleavage of ssDNA	Highly sensitive DNA detection (DETECTR)
Cas13a	Trans-cleavage of ssRNA	Ultra-sensitive RNA detection (SHERLOCK)
Cas14a (Cas12f)	PAM-independent ssDNA	High-fidelity SNP discrimination in rpoB

A critical development in this field is the engineering of Cas variants to improve their efficiency. For instance, the engineered Cas12f1_ge4.1 dual-mode system utilizes a miniaturized Cas protein that has been optimized for significantly higher detection efficiency compared to wild-type versions. These miniaturized nucleases are particularly attractive for point-of-care devices due to their reduced molecular mass and high stability (Javaid & Choi, 2021).

Synergy with Isothermal Amplification

While the CRISPR-Cas system is inherently specific, its sensitivity in clinical samples is maximized when integrated with pre-amplification steps. Traditional thermal

cycling required for PCR is often replaced in CRISPR assays by isothermal amplification methods, which operate at a constant temperature (Wang et al., 2025). The combination of these techniques creates a rapid workflow: DNA is extracted from a sample, amplified isothermally, and then detected by the CRISPR-Cas complex. This synergy allows for "one-pot" reactions where amplification and detection happen in a single tube, minimizing the risk of cross-contamination and simplifying the user's operational requirements (Yee et al., 2024). Figure 1 demonstrates how isothermal amplification and CRISPR-based detection are combined into a rapid near-point-of-care testing system.



Diagnostic Accuracy and Sensitivity: A Systematic Review

The diagnostic performance of CRISPR-based assays has been extensively validated against the gold standards of liquid culture and GeneXpert MTB/RIF Ultra. Meta-analyses of clinical studies provide a clear picture of the technology's maturity and its ability to detect M. tuberculosis across diverse patient populations (Ali-Hassanzadeh et al., 2025).

Meta-Analysis Findings and Clinical Validation

A comprehensive meta-analysis of 13–14 studies involving over 2,100 MTB strains confirms that CRISPR-based methods exhibit substantial diagnostic sensitivity and specificity. The pooled results indicate an overall sensitivity of approximately 91%–93% and a specificity of 97%–98%. The Diagnostic Odds Ratio (DOR), a single measure of diagnostic effectiveness, was reported as high as 498.67, indicating excellent discriminatory power (Abavisani et al., 2024).

Table 4. Meta-Analysis of Pooled Diagnostic Accuracy for CRISPR-Based Assays

Study Cohort/Metric	Pooled Sensitivity	Pooled Specificity	Area Under Curve (SROC)
Overall MTB Detection	91–93%	97%	0.97–0.99
Sputum Samples	92%	97%	0.97
Extrapulmonary Samples	89%	98%	High

CRISPR-Cas12 Specific	93%	98%	0.97
Resistant MTB Detection	96%	100%	High

One of the most promising aspects of CRISPR diagnostics is their performance in paucibacillary samples, such as those from patients with extrapulmonary TB (e.g., cerebrospinal fluid, pus, or pleural fluid) or those co-infected with HIV. In a clinical evaluation using bronchoalveolar lavage fluid (BALF), a CRISPR-Cas12a-based assay achieved a sensitivity of 94%, significantly surpassing culture (67%) and GeneXpert (78%) in the same cohort while maintaining 100% specificity. This suggests that CRISPR's attomolar sensitivity allows it to detect infections that are below the limit of detection for current state-of-the-art molecular tests (Huang et al., 2022).

SHINE-TB and the Colombian Experience

The SHINE-TB platform (Streamlined Highlighting of Infections to Navigate Epidemics for Tuberculosis) represents a major step toward a truly portable, sputum-to-result system. In a blinded clinical validation conducted in Cali, Colombia, the SHINE-TB assay demonstrated 100% sensitivity and 100% specificity when compared to liquid culture. Notably, the assay matched the sensitivity of GeneXpert Ultra but exhibited superior specificity in this small cohort, as GeneXpert produced one "low-positive" result in a sample that was subsequently found to be culture-negative (Bell et al., 2025).

The SHINE-TB architecture is particularly refined, utilizing a dual-target approach. It employs a Cas13a assay to detect two conserved, multicopy insertion sequences in the MTB genome (*IS6110* and *IS1081*) and a parallel Cas12a reaction that serves as an internal control by targeting human DNA. This design ensures that a negative result is not due to sample inhibition or failed extraction, a common problem in point-of-care testing (Dunkley et al., 2025).

Detecting Multi-Drug Resistance: Targeted Molecular Insights

The primary value proposition of CRISPR-based rapid assays for MDR-TB is their ability to perform high-fidelity Single Nucleotide Polymorphism (SNP) discrimination. Because CRISPR systems require precise crRNA-target complementarity, they can be programmed to distinguish between wild-type and mutant alleles that differ by only a single base (Pan et al., 2026).

Genetic Determinants of Resistance

To detect MDR-TB, CRISPR assays must target the specific genes associated with resistance to rifampicin and isoniazid. Research across Brazil, Iran, China, and Ethiopia has identified a consistent set of "hotspot" mutations that serve as reliable biomarkers for resistance (Mahmoud & Tan et al., 2023).

Rifampicin Resistance (*rpoB*):

Over 95% of resistance is linked to mutations in the 81-bp RRDR region. The most frequent substitutions occur at codons 531 (S531L), 526 (H526Y/D/P), and 516 (D516V) (Auma, 2025).

Isoniazid Resistance (*katG* and *inhA*):

Resistance is primarily associated with the *katG* codon 315 (S315T) mutation (70–82% of cases) and the *inhA* promoter region -15 C>T mutation (8–20% of cases) (Omoteso et al., 2025).

Table 5. Key Genetic Determinants and Mutation Hotspots for Multi-Drug Resistance

Target Gene	Locus	Common Mutation	Frequency in MDR Strains	Predicted Phenotype
rpoB	Codon 531	TCG → TTG (S531L)	77–81%	High-level Rifampicin resistance
rpoB	Codon 526	CAC → GAC (H526D)	Frequent	Rifampicin resistance
katG	Codon 315	AGC → ACC (S315T)	70–82%	High-level Isoniazid resistance
inhA	Promoter	-15 C → T	8–20%	Low-level Isoniazid resistance

Comparative Accuracy in Resistance Profiling

Clinical isolates from China and Southeast Asia were used to evaluate a CRISPR-Cas14a platform against phenotypic drug susceptibility testing. The platform accurately identified 39 out of 42 rifampicin-resistant isolates (93.3% sensitivity, 100% specificity) and 38 out of 39 isoniazid-resistant isolates (97.5% sensitivity, 100% specificity). When these results are compared to the overall predictive value of genetic markers, the CRISPR platform demonstrates a near-perfect correlation with sequencing data (Xiao et al., 2025).

Interestingly, studies in Brazil have suggested that a simplified "dual-marker" panel detecting only *katG* S315T and *rpoB* S531L may be sufficient for identifying up to 86% of MDR-TB cases in certain endemic areas. This finding has significant implications for crRNA design, as it suggests that a streamlined CRISPR assay targeting a limited number of high-frequency mutations can achieve high diagnostic utility while maintaining a simplified multiplexing workflow (Wu et al., 2025).

Overcoming the PAM Restriction with Cas14a

A traditional limitation of Cas12a is the requirement for a Protospacer Adjacent Motif (PAM) typically TTTN near the target site. This constraint can prevent the placement of a gRNA exactly over a critical mutation site. The emergence of Cas14a (a Type V effector) has resolved this issue, as it is PAM-independent for ssDNA targets. This allows for the precise positioning of the gRNA over any part of the *rpoB* RRDR, enabling the detection of all eight predominant mutation types associated with rifampicin resistance without the geographical "blind spots" of earlier Cas systems (Liu et al., 2024).

Operational Viability and Implementation Logistics

The potential for CRISPR-based assays to reach decentralized clinics depends not only on their accuracy but also on their "robustness" in the field. Key factors include sample pretreatment, reagent stability, and ease of use (Chakraborty, 2024).

Sample Pretreatment and Sputum Handling

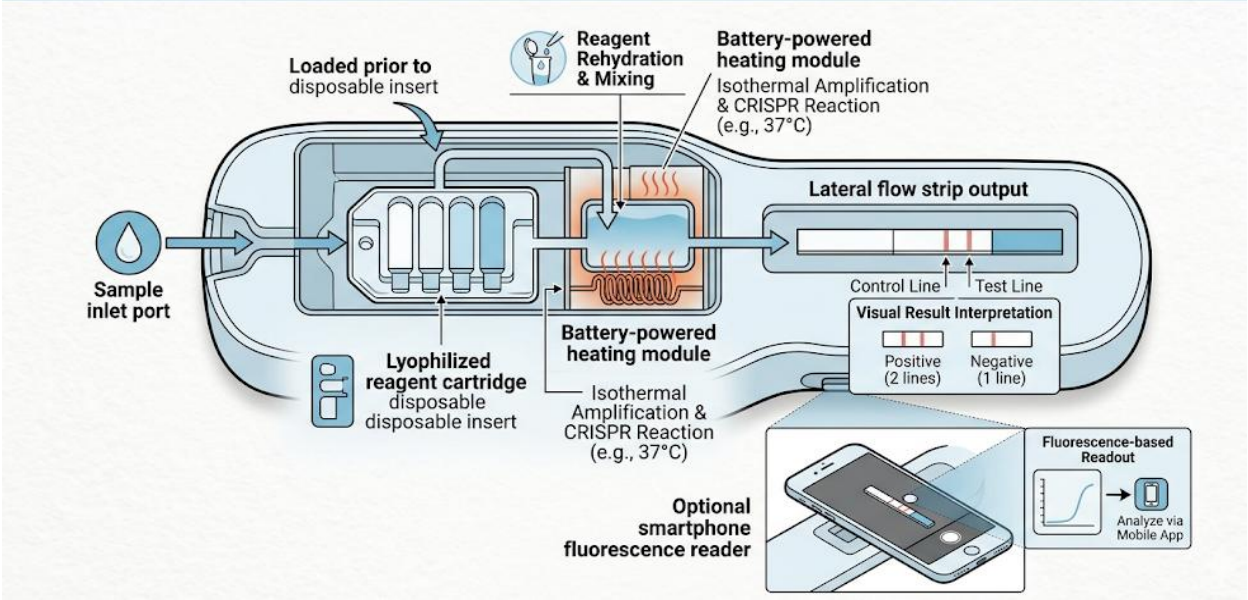
The extraction of high-quality DNA from sputum remains a technical hurdle. The viscous nature of sputum and the presence of inhibitors such as mucin and hemoglobin can impair isothermal amplification. Most CRISPR protocols currently utilize rapid lysis buffers and simple heat blocks to extract DNA in under 15 minutes. Some platforms, however, are exploring blood-based tests that detect circulating MTB

DNA, offering a non-invasive alternative that could be particularly valuable for patients who cannot produce sputum, such as children or those with HIV-associated paucibacillary TB (Kimwomi, 2024).

Lyophilization: The Key to "Warm Chain" Storage

For CRISPR diagnostics to be truly portable, reagents must be stable at ambient temperatures. Lyophilization, or freeze-drying, removes water through sublimation, leaving behind shelf-stable powders that do not require refrigeration. Reagents for both the RPA and CRISPR stages of the SHINE-TB and DETECTR platforms have been successfully lyophilized, maintaining their sensitivity and catalytic activity for months at room temperature. This eliminates the need for expensive cold-chain logistics, which is a major barrier to the deployment of molecular tests in tropical climates (Arizti Sanz, 2024). To enable field deployment in resource-limited settings, CRISPR diagnostics are being integrated into portable device architectures. A conceptual model of a near-point-of-care CRISPR testing device is shown in Figure 2.

Figure 2: CRISPR Diagnostic System Architecture (Point-of-Care Device Model)



Readout Modalities and Dual-Mode Systems

CRISPR assays support multiple readout formats, allowing the same technology to be used in high-throughput laboratories and remote field sites (Bock et al., 2022).

Fluorescence Readout:

Utilizes portable fluorometers or microplate readers to provide a quantitative signal. It offers the highest sensitivity and is ideal for laboratory confirmation.

Lateral Flow Readout (LFC):

Uses simple paper strips, similar to a lateral flow immuno-chromatography test. The test line appears when the reporter molecules are cleaved by the Cas enzyme. This provides a visual "yes/no" result within 10–15 minutes and requires no electronic equipment for interpretation (Santinha et al., 2025).

Health Economics and the Cost of Innovation

The successful scale-up of any TB diagnostic is ultimately driven by unit cost. The current dominant platform, GeneXpert, has been criticized for its high cartridge prices, which have historically been a barrier to universal access (Elbehiry et al., 2025).

Comparative Costs of Molecular Diagnostics

A significant advocacy movement, the "Time for 5" campaign, has pressured major manufacturers like Danaher and Cepheid to reduce cartridge prices. In late 2023, the price of the standard GeneXpert MTB/RIF Ultra cartridge was reduced from 9.98 to 7.97 for the Global Fund and other eligible groups. However, the more complex GeneXpert XDR cartridge, which detects resistance to second-line drugs, remains priced at 14.90, and targeted next-generation sequencing (tNGS) costs remain high at approximately 150 per test (Pai et al., 2025).

Table 6. Comparative Economic Metrics and Capital Costs of Molecular Diagnostics

Diagnostic Strategy	Consumable Cost (USD)	Capital Cost (USD)	Cost per Case Detected
Smear Microscopy	3.1	Minimal	55.8
GeneXpert MTB/RIF Ultra	7.97	17,000+	77.9
GeneXpert XDR	14.90	High	Higher
Targeted NGS	150.00	Very High	Expensive
CRISPR-Based Assay (Est.)	~ 5.00	Low (Portable)	Projected Cost-Saving

The economic advantage of CRISPR assays lies in their low capital requirements. Because they do not require complex instrumentation or high-maintenance modules, the overall budget impact of switching to near-point-of-care (NPOC) molecular tests can be significant. In hypothetical modeling of high-burden countries, the use of NPOCs was shown to provide cost savings of 38% to 55% compared to current automated nucleic acid amplification systems. This allows national TB programs to significantly expand their testing volume within existing budgets (Unitaid, 2025).

Cost-Effectiveness and the Impact on Transmission

The cost-effectiveness of rapid diagnostics is measured not just in per-test cost, but in the health benefits achieved through early treatment. Rapidly identifying resistance to rifampicin and isoniazid allows patients to be placed on the correct treatment regimen months earlier than traditional methods. This reduces the period of infectiousness and prevents secondary cases of MDR-TB (Feng et al., 2021). In South Africa, an optimized strategy of "Xpert followed by Xpert XDR" was found to be the most cost-effective approach, yielding an Incremental Cost-Effectiveness Ratio (ICER) of 6,554 per DALY averted. CRISPR-based assays, with their even lower infrastructure costs and rapid TAT, are positioned to be highly cost-effective alternatives as they move toward commercialization (Singh et al., 2025).

Regulatory Pathways and the Future of the CRISPR Market

As of 2026, the CRISPR diagnostic landscape is rapidly maturing from research-only tools to clinically validated products (Guo et al., 2025).

Regulatory Reclassification and FDA Breakthroughs

The U.S. Food and Drug Administration (FDA) and international bodies like the WHO are adapting their frameworks to keep pace with these technological advancements. In 2026, the FDA released draft guidance on a "plausible mechanism framework" for platform technologies, which could allow a single clinical trial to validate a CRISPR system that is then customized for various pathogens or mutations (Basit et al., 2025).

A major milestone was reached in 2025 when the FDA granted Breakthrough Device Designation to IntelliGenome's CRISPR-TB Blood Test. This designation accelerates the development and regulatory review of technologies that provide more effective diagnosis of life-threatening diseases. Similarly, the WHO has expanded its diagnostic pipeline to include nearly 100 products, with a strong preference for battery-operated devices that eliminate the need for specialized laboratory infrastructure (Allué-Guardia et al., 2026).

Market Growth and Regional Dynamics

The global market for CRISPR-based assays is projected to experience explosive growth over the next decade.

Market Size:

From a base of approximately 3.08– 3.5 billion in 2024–2025, the market is expected to reach 15.14– 17.0 billion by 2034–2035.

CAGR:

The sector is projected to grow at a Compound Annual Growth Rate (CAGR) of 16.6% to 17.1%.

Regional Trends:

Asia-Pacific currently holds a significant portion of this market and is expected to be the fastest-growing region. China, Japan, and India are leading the way through government-backed research and the rapid adoption of gene-based detection procedures in their healthcare systems (Allué-Guardia et al., 2026).

Table 7. Global CRISPR-Based Diagnostics Market Projections (2025–2035)

Market Forecast Element	2025 Estimate	2030–2035 Projection
Global Market Value (USD)	3.5 Billion	15.14– 17.0 Billion
Kits and Reagents Share	61.7%	Expected Dominance
Leading Application	Infectious Diseases	Infectious Diseases (52.9%)
Technology Leader	Cas9	Shift toward Cas12/Cas13/Cas14

Challenges and Implementation Barriers

Despite the technical prowess of CRISPR diagnostics, several hurdles remain for global scale-up.

Bioinformatics and Guide Design:

The high specificity of CRISPR means that crRNAs must be meticulously designed to account for all known circulating mutations of MTB. This requires robust bioinformatics tools and continuous genomic surveillance to ensure that new resistance variants do not lead to "false negative" results (Wang et al., 2025).

Enzyme Manufacturing and Scalability:

The current cost of producing high-purity recombinant Cas enzymes is relatively high. Achieving large-scale production through optimized protein expression and purification will be essential to bring the per-test cost down to the target 5 level (Chakaya et al., 2025).

Standardization and Quality Control:
Ensuring lot-to-lot consistency of enzymes and reagents is critical. The field urgently requires standardized reference materials such as accredited pseudoviral particles or synthetic DNA concentrations to allow for inter-laboratory comparison and regulatory alignment (Dheda et al., 2026).

Integrated Sample Preparation:
The ultimate "holy grail" is a fully integrated, closed-system device that takes a raw sputum sample and provides a digital or visual result without any manual processing steps. While platforms like SHINE-TB have made great strides, the complexity of sputum lysis remains a challenge for complete automation in a low-cost format (Unitaid, 2025).

Conclusions

The integration of CRISPR-Cas technology into the global tuberculosis diagnostic framework addresses the critical limitations of current laboratory-dependent systems by providing a programmable, ultra-sensitive, and cost-effective alternative for decentralized clinics. By achieving over 90% sensitivity and near-perfect specificity through advanced effector proteins and isothermal synergy, these assays enable rapid detection of both *M. tuberculosis* and its most prevalent drug-resistance markers, such as *rpoB* S531L and *katG* S315T. Despite ongoing challenges regarding the complete automation of sputum processing and the scale-up of enzyme manufacturing, the transition toward clinically validated, shelf-stable CRISPR platforms supported by the 2025 FDA breakthroughs and robust market growth offers a definitive pathway toward closing the diagnostic gap. Ultimately, the deployment of these rapid, near-point-of-care tools is essential for early therapeutic intervention, which not only improves individual patient outcomes but also serves as a critical public health strategy to halt the transmission of multi-drug resistant strains in high-burden regions.

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