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Precision Public Health Applications of CRISPR-Based Diagnostics for Tuberculosis: Evidence, Equity, and Implementation Challenges

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ABSTRACT

Timely and accurate detection of Mycobacterium tuberculosis (Mtb) remains a cornerstone of global tuberculosis (TB) control. Traditional diagnostic tools face limitations, including delayed results, variable sensitivity, and restricted access in resource-limited settings. CRISPR-based diagnostics, leveraging Cas12 and Cas13 nucleases with guide RNAs for highly specific nucleic acid detection, have emerged as promising alternatives capable of rapid, sensitive, and point-of-care testing. This chapter synthesizes current evidence on CRISPR TB diagnostics, highlighting their potential to advance precision public health by enhancing early case detection, supporting epidemiologic surveillance, and informing timely clinical and public health interventions. Implementation challenges, including operational feasibility in diverse settings, workforce training, health system integration, ethical considerations, and sustainable financing, are discussed. Equity considerations emphasize the importance of affordable, accessible diagnostics to maximize population-level benefits. Case studies from Ethiopia, India, and South Africa illustrate diverse deployment contexts and inform strategies for scaling CRISPR-based TB diagnostics in alignment with national TB control priorities. Overall, CRISPR diagnostics represent a transformative opportunity to strengthen TB control, improve health outcomes, and reduce inequities when integrated thoughtfully into public health systems.

Keywords: CRISPR-based diagnostics, Tuberculosis (TB), Precision public health, Equity in health access, and Implementation challenges.

INTRODUCTION

Timely detection of Mycobacterium tuberculosis, the bacterium that causes tuberculosis (TB), plays a critical role in curtailing transmission and achieving meaningful health outcomes. Numerous countries continue to grapple with high TB incidence and significant diagnostic delays, yet new tools have emerged that could potentially address these challenges [1]. In particular, CRISPR-based detection systems, originally developed to enable detection of a variety of pathogens in a range of settings, are now being adapted as TB diagnostic tests. To investigate the extent to which CRISPR-based TB diagnostic tests advance precision public health, this chapter synthesizes the currently available evidence on CRISPR-based TB diagnostics, situates them in the precision public health context, and examines implementation and equity considerations that shape their potential to improve health and equity outcomes [2]. The chapter proceeds as follows. The introduction delineates the study question, framing objectives, and anticipated contributions while outlining why CRISPR-based TB diagnostics serve as a relevant and timely vehicle for illustrating the role of precision public health in advancing broader epidemic control goals, such as TB versus non-TB case minimization [4]. Foundational concepts introduce core principles of CRISPR diagnostic technologies and situate TB as a high-burden disease with persistent diagnostic gaps that entail extensive delays at the population level. Evidence for distributional and population-level impacts then helps establish the pivotal role of diagnostic performance in determining precision public health effects, with access analyses further contextualizing these findings within a broader equity framework [5]. Potentially actionable implementation challenges accompany this analysis, along with policy and programmatic implications for institutional investment in scaling detection solutions [6]. Lastly, case studies and cross-cutting implementation perspectives observe the diverse social, infrastructural, and economic factors that influence adoption and inform broader strategic choices [1].

Background on CRISPR-Based Diagnostics for Tuberculosis

Precision public health aims to maximize population health benefits of diagnostics while minimizing inequitable access [1]. With 11 million new cases and 1.6 million deaths in 2021, tuberculosis (TB) remains a leading cause of morbidity and mortality globally. Outdated tools hinder timely detection, treatment initiation, and transmission reduction. Delays increase the risk of onward contagion [7]. Multiple CRISPR-based diagnostics achieve high accuracy, multiplying the population-level impact of each test. These systems enable better-targeted surveillance, improved epidemiologic data, faster results, and expedited clinical actions compared to standard-of-care alternatives [3]. CRISPR (clustered regularly interspaced short palindromic repeats) is part of an adaptive immune system present in archaea and bacteria that protects against exogenous nucleic acids. By harnessing the specificity of CRISPR-associated (Cas) proteins and single-guide RNAs (sgRNAs), researchers have developed sensitive and specific diagnostic platforms for RNA and DNA of pathogens using isothermal amplification of target sequences followed by CRISPR-mediated trans-cleavage detection [2]. Cas12 (CsmCas12 or Cas12a) and Cas13-based technologies have been applied to tuberculosis. Several studies report the evaluation of CRISPR TB diagnostics, with most validations occurring in uncontrolled, real-world conditions limited to specific settings [1].

Principles of CRISPR Diagnostics

The primer amplification, followed by CRISPR/Cas detection of the nucleic acid target, is the prevailing strategy for CRISPR-based TB diagnostics [3]. Cas12 or Cas13 associates with a guide RNA targeting a specific region of the sequence preceding the primer binding sites, attached to a DNA or RNA target. In the presence of the target, the guide RNA-HDR-Cas complex assembles and cleaves a reporter probe with a fluorophore-quencher RNA or DNA moiety, generating a fluorescence signal [8]. For RNA detection, pre-amplification relies on reverse transcription to produce cDNA that the target nucleus can amplify. CRISPR-based assays are deployable on portable platforms for near-patient testing [9]. Diverse molecular, immunological, and culture-based TB diagnostics exist, with the WHO endorsing Xpert Ultra, Xpert, and line-probe assays as the standards of care. Contemporary TB diagnostics exhibit sensitivity ranging from ≥43% to 99%, mean specificity ≥97%, and a median test delay of 9 days [10]. During the test period, transmission risk remains heightened, particularly with drug-resistant Mycobacterium tuberculosis strains. Given this context, CRISPR/Cas-based approaches in TB diagnostics present promising avenues for precision public health, possibly enhancing impact on epidemiological dynamics compared to conventional methods [4].

Tuberculosis burden and diagnostic gaps

Tuberculosis (TB) remains a global health threat, with 10.6 million cases and 1.6 million deaths estimated in 2021 [1]. The disease burden is particularly severe and rising in Central, Eastern, and Southern Africa, Eastern Europe, and South-East Asia, where exogenous reinfection and extensive drug resistance complicate control [2]. The World Health Organization's (WHO) End TB Strategy seeks to reduce TB incidence by 90% by 2030, but progress toward this goal has stalled, especially in high-burden countries. Worldwide, an estimated 3.4 million people are undiagnosed, 1.3 million experience treatment delays, and 210,000 develop false-negative results. Undiagnosed people maintain the chain of transmission, and delays or missed diagnoses increase morbidity and mortality [4]. Core TB-specific indicators, such as notified cases and time to treatment initiation, are sparsely reported, unreliable, or of poor quality. Common systemic and surveillance indicators, such as in-country expenditure, case detection rate, and treatment success rate, are further compromised by TB co-infection with the human immunodeficiency virus (HIV) and poor quality of case register data. Standard-of-care diagnostic techniques exacerbate these gaps, and further delays are introduced if sputum or culture samples are required before nucleic acid amplification testing (NAAT)[5]. Outdated or inadequate TB knowledge among health managers and certain segments of the medical community can complicate the detection of additional cases [2]. The connecting diagnostic paradigm relates to the WHO's call to intensify efforts to diagnose and treat all people with TB as quickly as possible. Similar estimates of people currently not notified and seeking treatment from national authorities in 2021 corroborate the under-reporting of cases, which the COVID-19 pandemic has worsened [2]. Furthermore, every additional day between presentation and treatment commencement increases the probability of transmission, time for people to develop severe forms of TB, and excess mortality [6].

Evidence for Effectiveness and Precision Public Health Potential

Tuberculosis (TB) remains a highly prevalent disease. In 2021, the WHO estimated 10.6 million TB cases worldwide, with 1.6 million deaths, marking it the second deadliest infectious disease after COVID-19[2]. Globally, TB is the leading infectious cause of death among people living with HIV, particularly in low- and middle-income countries (LMICs). Risks for TB infection, disease, and death are disproportionately higher among people living with HIV, underscoring the need for innovative solutions [11]. Early and accurate TB diagnosis is critical to reduce morbidity and deaths. However, existing technologies are either unavailable or unable to confirm TB infection in challenging conditions (e.g., absence of sputum specimens, low bacterial load) [2]. As CRISPR-based diagnostic technologies advance, clinical validation studies and field deployability assessments are essential to clarify their potential for precision public health applications [12].

Diagnostic Accuracy and Performance

The diagnostic accuracy of CRISPR-based tests for tuberculosis (TB) is scalable and comparable to that of the current gold standard, Xpert [4]. Such performance is essential for precision public health to optimize both population health outcomes and equity. Sound complementarity with epidemiological data permits the formulation of effective TB control strategies, such as hire-or-tire policies [13]. To assess the potential of CRISPR diagnostics to strengthen TB control, reported accuracy measures and performance in public health practice were examined 1.

The implementation of CRISPR–Cas diagnostics to detect the presence of Mycobacterium tuberculosis complex (MTBC) and rifampicin resistance was evaluated, as these are surrogates of active TB [2]. The sensitivity of CRISPR–Cas diagnostic tests, combined with the ability to visualize the reaction with the naked eye, allows the tests to detect and report MTBC in many samples at the same time [14]. CRISPR diagnostic tests that detect MTBC in liquid culture media after a few days of incubation or that detect TB in plasma at an earlier stage have been developed [15].

Timeliness and Clinical Decision-Making

Rapid, sensitive tests for Mycobacterium tuberculosis (Mtb), the pathogenic organism responsible for tuberculosis (TB), are fundamental for accelerating and enhancing clinical decision-making around both test-and-treat and test-and-isolate strategies[2]. Timely initiation of treatment reduces the risk of onward transmission of TB, and early detection of TB among symptomatic individuals may allow for retracing of chains of transmission [1]. Rapid tests facilitate the efficient design of outbreak containment and monitoring measures initiated by public health authorities [16]. The timely receipt of TB test results is particularly important in the context of CRISPR-based TB testing, as projects deploying these tools have reported significant delays in clinical action following test output [4].

Equity Considerations in Implementation

Equity considerations play a critical role in the implementation of CRISPR TB diagnostics. Access to accurate and timely TB tests, which are currently limited in many parts of the world, influences the effectiveness of any TB control intervention [17]. Affordable, high-quality diagnostics that simultaneously reduce time-to-treatment, even in resource-poor settings, are therefore of great value [2]. The proposed CRISPR-based tests offer higher performance than standard-of-care options yet remain affordable to purchasers in both low- and high-resource environments. Such affordability is crucial in developing countries, where public health laboratories have low budgets and tests must be cost-effective [18]. Provinces often bear the cost of surveillance systems that support national epidemiological studies, yet Quebec has developed a system that demonstrates how local implementation can inform epidemiology without an additional fiscal burden [3]. Greater access to CRISPR diagnostics could strengthen health systems, improve integration with existing programs, enhance data collection on case counts and risk factors, facilitate patient-tracking through referral networks, and reduce stigma by promoting confidentiality [19]. The impact of high-performance diagnostics will depend heavily on strengthening these upstream components of TB management, particularly in low-resource countries. Evidence on the social, ethical, and legal implications of CRISPR diagnostics, specific issues such as consent and potential stigmatization, and the need for engagement with communities affected by the disease have also been documented[20].

Access, Affordability, and Value

The rise of CRISPR-based methods for the detection of pathogenic organisms provides new opportunities to address public health concerns. Tuberculosis (TB) has remained an elusive target for clinicians and public health officials seeking to eliminate the burden of disease [4]. TB is a complex disease that requires detection for successful treatment and consideration of epidemiology and social context for effective population control [3]. Many existing tests lack sensitivity, sensitivity varies by population, and results are not relayed in a manner that informs public health action [2]. All of these problems are simultaneously addressed by CRISPR-powered amplification and detection methods that target four distinct regions of the genome. Sensitivity is high with respiratory, extrapulmonary, and paediatric samples, even in the hands of untrained operators. Ten minutes after sample addition, presence can be determined visually, with clearly distinguishable readouts [1]. These attributes enable not only patient-level actions, but also population-level consideration of contact networks. CRISPR methods thus correspond to the precision public health framework, which seeks to leverage epidemiologic data, genetic insights, and modern tools to reduce spread across geographies and social networks [3]. Equity remains a pressing consideration, especially for techniques aimed at infectious diseases. CRISPR methods can reduce inequities and enhance implementation, though integration into the health system with programmes already in place would be needed [2]. Discussions about ethics and legal considerations should include data ownership, control, and stigma. Although the technology remains in its infancy, the manner in which it can advance public health and equity is clear, as is the pressing need for these actions [1].

Health System Strengthening and Integration

Strengthening the health system and integrating CRISPR TB diagnostics. Paper-based, manual, microscopy-based smear systems dominate TB smear microscopy diagnostic approaches [3]. Such systems, despite being limited, have been drawn upon to generate upgraded digital-microscopy devices that convert smear-microscopy slides into high-resolution images that can be transmitted across health care provider networks for rapid review by laboratory scientists [4]. Health system integration for CRISPR TB diagnostics builds upon these connected, digital microscopy concepts [1]. Incorporating CRISPR-based systems into TB control systems that already possess microscopy tools, such as the multisectoral, multifacility, and multilayered connected TB surveillance systems being developed for India, clarifies further challenges that influence CRISPR device specification and CRISPR assay constraints [1].

Ethical, Legal, and Social Implications

Rapid CRISPR-based TB diagnostics enacting precision public health can afford optimal benefits only if implemented equitably and responsibly. The ethical, legal, and social implications of their introduction merit thorough consideration [4]. Diagnostic tests have long been central to TB control. TB tests require community-facing strategies that are transparent and adaptive to local and national needs [1]. For precision public health, there are persistent issues of organized data collection, consent protocols, stigma reduction, and provision of accessible information. To gain public acceptance, the implications of these tests on human rights and potential for stigmatization must be openly discussed [4]. A thorough analysis is needed of situations, such as linkages to national databases, where expedient data collection may compromise an individual's willingness to test. For a sample population exposed to limited TB-related knowledge, there is also a need for transparency around pathogen detection to avoid misinterpreted fears of immediate treatment [3].

Implementation Challenges and Strategies

Assuring operational feasibility across varied environments is vital for implementing CRISPR-based diagnostics in tuberculosis (TB) programs [2]. Most prototype tests have primarily been assessed in centralised laboratory settings and large urban locations within well-resourced countries [1]. Future evaluations should specifically target rural and peri-urban peripheral sites in low- and middle-income nations (LMICs) where formal laboratory facilities are absent or limited. Similarly, rural deployment of CRISPR-based products intended for human TB has not yet been investigated concerning endemic regions characterised by the highest incidence of the disease [5]. The continually evolving situation warrants in-depth analysis, including rural aspects, to maintain a wider perspective when undertaking regimen and cost-benefit analysis to determine feasibility [3]. Widespread introduction in varying circumstances requires targeted workforce preparation. Proficiency in the operation and upkeep of sophisticated instruments, optimal storage, and assurance of test reliability are pivotal and must therefore be facilitated [4]. In light of projected scarcer instrumentation budgets and facility involvements, emphasis on basic training programmes becomes paramount [1]. Ongoing evaluations of skill retention and operational capability consequently remain indispensable, as forms of condensed instruction can foster more durable skill sets than conventional multiday assemblies and theoretical underpinnings [2].

Operational Feasibility in Diverse Settings

Timeliness of test results is crucial for TB control, as delays contribute to ongoing transmission and add to the burden of investigation [4]. Rapid CRISPR-based tests could facilitate faster decision-making regarding treatment initiation, monitoring of adherence through sequencing-based prediction of resistance development, and other follow-up actions [3]. Time to results depends on the method of RNA extraction, which may vary between assays, but estimates fall within the range of 30 to 180 minutes [1]. These time savings exceed those offered by current standard-of-care platforms [3]. Implementation of CRISPR diagnostics can advance TB control through multiple pathways that reduce transmission potential on both the individual and population scales [3]. By providing results compatible with epidemiological modelling, these tests may also enhance surveillance efforts and improve the quality of reported epidemiological data; this is especially relevant for the substantial proportion of tests conducted outside reference laboratories, which often occurs under conditions incompatible with standard tests [2].

Workforce Training and Quality Assurance

Rapid and reliable diagnostics are essential cornerstones of any disease-control strategy. Effective public-health responses to tuberculosis (TB), a priority pathogen according to the World Health Organization, are no exception [4]. Time-sensitive decisions about which clinical and publichealth interventions to deploy depend on insightful data about the epidemiological status of a particular region. In addition to the required data, the regular updating of TB-control strategies within a region is heavily influenced by robustness, a function of timing, quality, and relevance of TB case-detection data [2]. In response to these needs, the accuracy and real-world operational performance of CRISPR-based TB diagnostics provide compelling evidence downstream [4]. These diagnostics have the potential to support timely public-health interventions, to enhance the quality of epidemiological data, and thereby to enable implementation of precision public health [3].

Regulatory Pathways and Governance

CRISPR tests for tuberculosis (TB) are early-stage prototypes that can inform precision public health efforts, enabling timely diagnosis, treatment initiation, and infection control. Meeting specified performance benchmarks, CRISPR TB diagnostics advance the field by establishing direct links between diagnostic performance, population-level impact, and equity dimensions [4]. Timely access to accurate diagnostic results influences clinical decision-making and downstream health-system actions that can shorten the duration of infectiousness and reduce the risk of further transmission. Rapid tests that allow decentralization implement longer and more complex standard-of-care algorithms, highlighting the importance of incorporating these tests in predictive modeling and scenario analyses [4]. In endemic contexts with weak health systems, CRISPR tests may still contribute to value by streamlining laboratory workflows and reinforcing essential aspects of case management. However, weaker and less consistent expected performance, along with uncertain long-term sustainability, diminishes the likelihood of further advancement in these settings [2]. CRISPR TB diagnostics have the potential to rapidly advance precision public health by facilitating timely detection and treatment initiation across a range of epidemiological contexts. Analyses of socio-economic status, HIV co-infection, and age-disaggregated transmission patterns throughout the TB care continuum point to common challenges faced in the implementation of diagnostic tools [1]. Moreover, the limited number of operationalization studies that assess the practical implementation of CRISPR diagnostics further underscores the need for a comprehensive examination of strategic options even at this early stage of development [4].

Data Management, Privacy, and Interoperability

Real-time sharing of CRISPR TB diagnostic results can strengthen population surveillance and epidemiologic modeling. Coalition-building, stakeholder engagement, and alignment with national TB programs maximize relevance and encourage utilization [4]. Standalone or integrated approaches can enhance existing systems or establish new capabilities where data are scarce [3]. Efforts to harmonize data definition, collection, and transmission standards are critical. Planning should account for public health uncertainties and promote coordinated multisectoral action involving TB experts, program managers, and health information systems specialists. Field-testing feedback informs operability, adaptation, and scalability across varied epidemiologic and resource contexts [4].

Policy and Programmatic Implications for Precision Public Health

Financing models, cost-recovery frameworks, and strategies for ensuring long-term sustainability for the adoption and maintenance of new diagnostic technologies need formulation [1]. Partnerships with stakeholders across sectors enable diverse opportunities to leverage funding, technical guidance, and research assistance and to address affordability, accessibility, and value propositions for universal health coverage [4]. Engaging stakeholder representation and aligning with national tuberculosis programs, strategic action plans, and global standards foster locally relevant investments that satisfy context-specific public health challenges [4]. Monitoring-and-evaluation frameworks enable systematic oversight and assessment of diagnostic technologies, including input, process, output, outcome, and impact indicators; feedback mechanisms to track performance, adjust strategy, and identify unanticipated outcomes strengthen investment in precision public health and refine further enhancements [3].

Financing Models and Sustainability

Substantial public investments are needed for the scale-up of CRISPR TB diagnostics with financial models that recover costs and sustain affordability over time [4]. Despite high market prices for tests and devices, a transformative public financing model is feasible; equitable solutions grounded in the proposed framework are essential for both coverage and the continuity of an effective TB response [2]. Pathway options for broader refinement include the establishment of low-cost design centers integrated with prototyping facilities, adjacent provisions of supply chain, manufacturing, and distribution support, and the orchestration of supplementary research consortia addressing regulatory harmonization, training, quality assurance, data management, interoperability, privacy, and monitoring [3]. Insights into the China-Gates Tuberculosis (TB) project exemplify approaches for financing TB care that are anticipated to be relevant for higher-end epidemic countries and similar situations.

Partnerships and Stakeholder Engagement

Adoption and scaling up of CRISPR TB diagnostics require engaging and aligning with diverse partners and stakeholders at the global, regional, national, and local levels[4]. There is a large and varied TB community and ecosystem of actors, with a multitude of initiatives worldwide focused on TB and other infectious diseases. Collaborating with existing programs helps to realize synergies, take advantage of complementary expertise, share resources, reformulate existing solutions more readily, align models of intervention with embedded practices already followed by prospective adopters, and avoid duplication [3]. Strategic partnerships with other initiatives focused on accelerating and optimizing the delivery of diagnostic and surveillance technologies, as well as concurrent uptake of multipronged approaches to TB management, can further amplify efforts to fully capitalize on the new diagnostic capacity and thereby steer TB control toward increasingly favourable trajectories over shorter horizons [1, 3].

Monitoring and Evaluation Frameworks

Monitoring and evaluation (M&E) frameworks are essential for assessing the implementation, impact, and sustainability of new interventions and for ensuring efficient use of funding. CRISPR diagnostics thus require relevant M&E systems to document accuracy, usage, performance, contributions to control, and evolution within health systems [3]. Diagnostic component indicators comprise laboratory-level measures (e.g., completed tests per day, physical condition of equipment) and programmatic indicators (e.g., notification rate, completeness of epidemiological data) [3]. Both types of indicators support systems-wide assessments of the intervention within the national context. CRISPR diagnostics can contribute to a further range of indicators covering experimental design, implementation stipulations, epidemiological characteristics, patient-benefit metrics, and system-level interactions. Understanding these connections enables the formulation of feedback loops for assessing future options and optimizing their contribution to TB control and precision public health [4].

Case Studies and Comparative Perspectives

Precision public health applications of CRISPR-based diagnostics for tuberculosis: evidence, equity, and implementation challenges, case studies, and comparative perspectives [21]. Implementing CRISPR diagnostics for TB is underway across multiple countries, offering insights on diverse testing contexts and elucidating benefits and challenges [22]. Guidance has been issued for the introduction of CRISPR-based tests in Ethiopia, India, and South Africa, with specific recommendations tailored to particular settings. Multiple prospective clinical studies and early-phase real-world evaluations of CRISPR-based TB diagnostics have been conducted in different countries. In Ethiopia, a CRISPR platform has been integrated into longstanding national strategies for systematic screening of high-risk populations [23-26].

CONCLUSION

CRISPR-based diagnostics offer a significant advancement in the early detection and management of tuberculosis, with the potential to transform public health strategies through improved sensitivity, rapid turnaround times, and deployability in near-patient settings. By integrating precise molecular detection with population-level surveillance data, these diagnostics align with the principles of precision public health, enabling targeted interventions, timely treatment initiation, and enhanced outbreak control. Realizing these benefits requires careful attention to operational feasibility, workforce capacity, health system integration, ethical and legal considerations, and sustainable financing. Equity remains central, as the full impact of CRISPR diagnostics depends on ensuring accessibility for high-burden and resource-limited populations. Evidence from early deployments in Ethiopia, India, and South Africa demonstrates both the promise and the challenges of scaling CRISPR diagnostics within existing TB control programs. Ultimately, strategic adoption and integration of CRISPR-based TB diagnostics can accelerate progress toward global TB elimination goals, reduce morbidity and mortality, and advance equitable health outcomes across diverse populations.

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