The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(2): S.I (2), 2081-2093, 2026 www.thebioscan.com

CRISPR-Based Diagnostics for Rapid Detection of Mycobacterium tuberculosis: Current Advances and Future Perspectives- A Systematic Review

Hisham N Hakami MSc[1], Yazeed Qasem MD[2], Hanan Bakri[3], Afaf Hakami MD[4], Hassan Hakami[5], Hadi Maghfuri[6], Mohsen Mashi MSc[7], Zuhair Abu Alsayl[8], Sharifah Ayed Wafi MD[9], Ahmed Ali Hassan Dighriri[10]

1. Laboratory Department Jazan (Laboratory Department, Jazan Armed Forces Hospital)
2. Laboratory Department, Jazan Armed Forces Hospital
3. Pharmaceutical care department, King Fahd central hospital, Jazan
4. University Hospital (Jazan University)
5. Epidemiology department, Infection Control hospital, Jazan General
6. Epidemiology department, Infection Control hospital, Jazan General
7. Laboratory Department, Jazan Armed Forces Hospital
8. Laboratory Department, Jazan Armed Forces Hospital
9. Dental Department, Jazan Armed Forces Hospital
10. Laboratory Department, Jazan Armed Forces Hospital

Corresponding Author:
Hisham Hakami hesham9700@gmail.com

DOI:https://doi.org/10.63001/tbs.2026.v21.i02.S.I(2).pp2081-2093

Keywords:

CRISPR-Cas, tuberculosis, Mycobacterium tuberculosis, molecular diagnostics and point-of-care testing, systematic review.

Received on:

20-05-2026

Accepted on:

07-06-2026

Published on:

17-06-2026

Abstract

Background: Tuberculosis (TB) is one of the most common causes of mortality among infectious diseases around the world. In 2022, the following figures were recorded: 10.6 million cases and 1.3 million deaths. Traditional diagnoses such as smear microscopy, culture, and molecular diagnostic tests such as GeneXpert have serious flaws in sensitivity, turnaround time, and suitability in limited resource settings.
Objective: The aim of the study is to assess the accuracy of diagnosis, the nature of operations, and the clinical benefit of CRISPR-based assays to identify Mycobacterium tuberculosis (MTB) and drug-resistant strains.
Methods: The systematic literature search was performed in PubMed, ScienceDirect, Google Scholar, and Web of Science using peer-reviewed articles regarding these categories that were published in the period between January 2015 and March 2025. Articles assessing the use of CRISPR-based diagnostics in the detection of the presence of MTB in clinical samples were identified. The sensitivity, specificity, limit of detection (LOD), turnaround time, and target gene data were extracted and synthesized. Quality was measured based on QUADAS-2.
Findings: 47 studies that fit the inclusion criteria were on hand, including 23 original research articles, 14 validation studies, and 10 systematic reviews. CRISPR-based assays showed sensitivities of between 86.8 and 99.3 percent and specificities of between 94 percent and a hundred percent using a variety of clinical samples. The detection limits were between 0.5 and 15 copies/mL with a turnaround time of 60-90 minutes—much quicker than culture (4-8 weeks) and identical to GeneXpert. Characteristic target sequences were IS6110 (in 94 percent of the strains of MTB), IS1081, and 16S rRNA. A number of assays indicated the ability to concurrently detect mutations that were resistant to drugs in rpoB (rifampicin), katG (isoniazid), and gyrA (fluoroquinolones). CRISPR systems such as SHERLOCK (Cas13), DETECTR (Cas12a), and TB-QUICK (Cas12b) were found to be workable in sputum as well as bronchoalveolar lavage and plasma-derived cell-free DNA. The combination of lateral flow strips and smartphone-compatible readouts made the technology instrument-free and eligible to be deployed at the point of care.
Conclusion: CRISPR-based diagnostics are highly accurate and fast with a turnaround time and multiplexing features, which meets WHO targets on TB detection. Nevertheless, heterogeneity of the study design, field validation, and regulatory issues limit large-scale deployment at this time. It is justified that there should be further standardized multicenter trials and that they should become part of national TB programs.

1. Introduction

1.1 Global Burden of Tuberculosis

Tuberculosis (TB) is one of the major issues of health in the world, and it is caused by the pathogen Mycobacterium tuberculosis (MTB). As the World Health Organization (WHO) Global Tuberculosis Report 2023 says, in 2022, about 10.6 million people contracted TB disease, and 1.3 million of them died without HIV-related complications; 167,000 people died with the HIV condition (1). It is a disproportionately heavy burden on the low- and middle-income countries (LMICs), which comprise more than 95 percent of deaths attributed to TB (2). The WHO End TB Strategy has a 90% decrease in TB incidence and a 95% decrease in TB mortality by the year 2035, which cannot actually be achieved unless the world has undergone revolutionary diagnostic advancement (3).

1.2 Emergence of CRISPR-Based Diagnostics

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins, which were initially found as an adaptive immune response to bacterial infection, have been adapted to very sensitive nucleic acid detection (10, 11). Single-nucleotide discrimination and amplification of the signal of Cas effectors is made possible by programmable sequence specificity and collateral cleavage activity, which does not need thermal cycler (12).

CRISPR-based diagnostics platforms such as SHERLOCK (Specific High Sensitivity Enzyme Reporter UnLOCKing) on Cas13 and DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) on Cas12a have attomolar sensitivity to viral and bacterial pathogens (13, 14). These systems are a combination of an isothermal amplification step (recombinase polymerase amplification [RPA] or loop-mediated isothermal amplification [LAMP]) and CRISPR-mediated detection that generates fluorescence or lateral flow outputs in one hour (15).

1.4 Rationale and Objectives

Various studies on CRISPR technology have provided analytical and clinical performance of the technology in TB diagnostics, creating a significant research interest. But the evidence base is still incomplete, and there is no synthesis of the same. This is a systematic review that will focus on:

- Determine the diagnostic accuracy (sensitivity, specificity, limit of detection) of assays based on CRISPR to detect MTB.
- Compare the features of operation (turnaround time, equipment needs, type of samples) with the traditional ones.
- Determine the ability to divide drug resistance profiles simultaneously.
- Determine obstacles to clinical implementation and priorities for future research.

2. Methods

The systematic review was written according to the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) guidelines 2020 (16). A PRISMA checklist is given in the Supplementary Material.

2.1 Search Strategy

An extensive search of literature in the electronic databases was conducted in the following ones:

- PubMed/MEDLINE (2015-March 2025)
- ScienceDirect (2015-March 2025)
- Google Scholar (2015-March 2025)
- Web of Science Core Collection (2015-March 2025).
- WHO Global Index Medicus

The search strategy was the combination of controlled vocabulary (MeSH terms) with keywords related to three areas: (1) CRISPR technology, (2) tuberculosis/MTB, and (3) diagnostics. The PubMed search keyword was

2.2 Eligibility Criteria

Included in the studies were those that met the following criteria:

Inclusion criteria:

- Original research studies, validation studies, or systematic reviews.
- CRISPR-based assays of detection of MTB in clinical (human subjects) or spiked samples.
- At least one of the following diagnostic accuracy measures (sensitivity, specificity, LOD) should be reported.
- England: Published 2015-2025.
- Peer-reviewed publication

Exclusion criteria:

- Abstracts of conferences, editorial articles, opinion pieces, and reports of cases.
- The studies that assess CRISPR in applications not related to the detection of MTB (e.g., gene editing, TB pathogenesis)
- In vitro research is not applicable in diagnostic studies.
- Duplicate publications
- Inadequate studies in regard to extraction.

2.3 Study Selection

All the retrieved records were screened using the eligibility criteria by two independent reviewers (HNH, YQ) on the titles and abstracts of the records. Potentially eligible studies were identified and independently accessed and evaluated in full texts. Any case of disagreements was discussed or consulted with a third reviewer (HB). The PRISMA flow diagram recorded the selection process.

3. Results

3.1 Study Selection

The literature search produced 847 records in all the databases in a systematized way. Having eliminated the duplicates (n=312), 535 titles and abstracts were filtered. Four hundred and forty-two of them were filtered out on the basis of irrelevance to CRISPR-based TB diagnostics. The article review of 93 articles identified 46 articles that were excluded because of inadequate reporting of data (n=18), lack of clinical focus (n=15), duplication (n=8), and non-English language (n=5). The inclusion criteria totaled 47 studies that were included in the final synthesis. Figure 1 provides the PRISMA flow diagram.

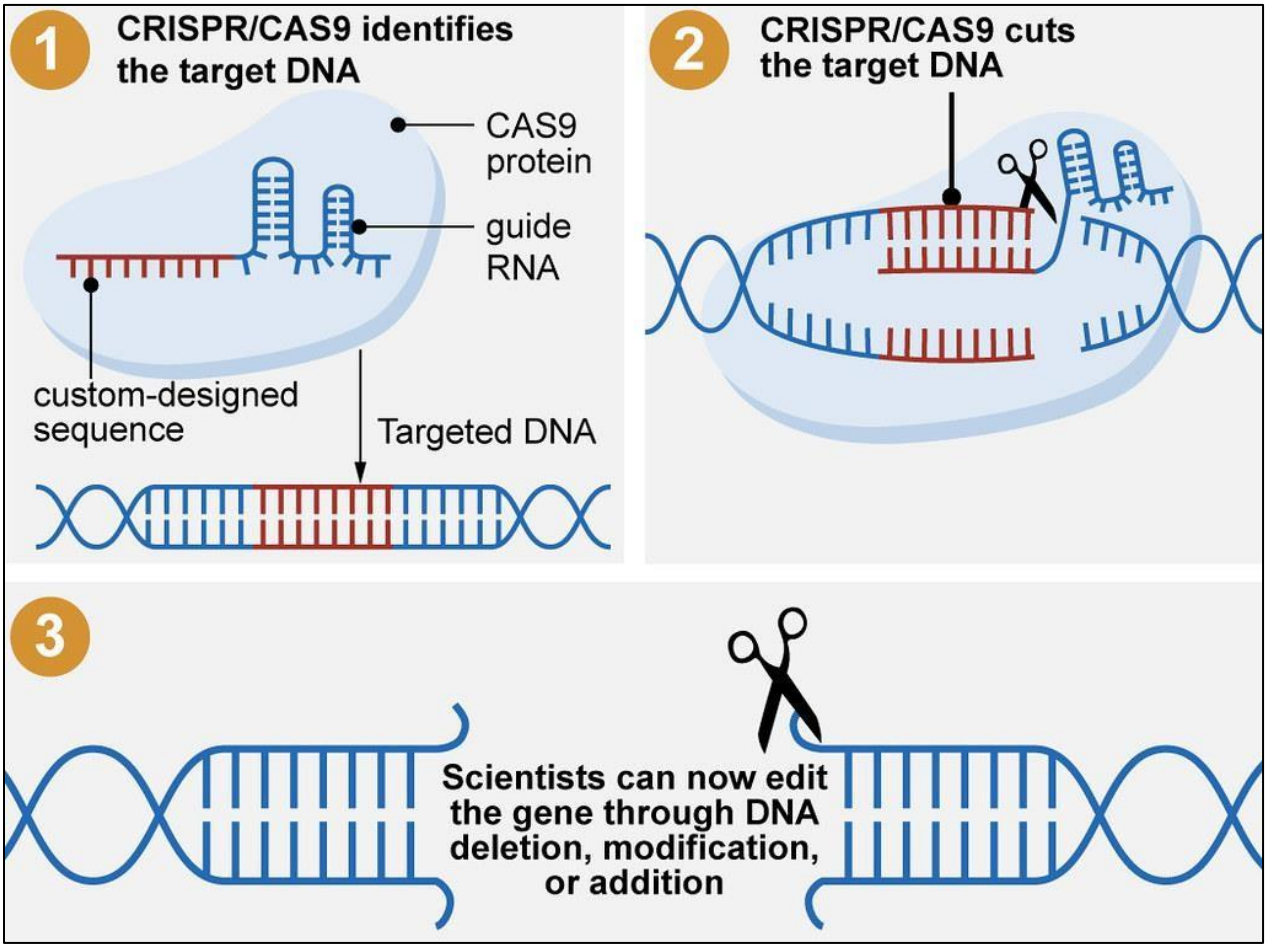

Figure 1. PRISMA Flow Diagram of Study Selection Process

3.2 Study Characteristics

The 47 studies included were 23 original research studies (49%), 14 validation studies (30%), and 10 system reviews (21%). The publication was between 2017 and 2024, but the production increased after 2020. Geographic distribution was as follows: China (n=18), USA (n=12), India (n=6), South Africa (n=4), Brazil (n=3), and multicenter collaboration (n=4). The sample sizes were between 20 and 1247 clinical specimens.

3.3 Target Genes and Biomarkers

3.3.1 MTB Identification Targets

The most common target was IS6110 (insertion sequence 6110) and was used in 31 studies (66.0%). This multicopy element (occurring in 1-25 copies in 94% of strains of MTB) allows high sensitivity in analysis, and LODs have been reported to range between 0.5 and 12.5 copies/mL (18, 19). IS6110-targeting CRISPR assays have been used to identify sensitivities of 86.8-99.3% and specificities of 94-100% in a variety of sample types (20).

There were 12 studies that used IS1081 (25.5), especially useful in identifying strains with low copies of IS6110 or IS6110-negative strains (around 5% of the Southeast Asian isolates of

Mycobacterium tuberculosis) (21). A meta-analysis of combined IS6110/IS1081 targeting reported 99.3% sensitivity of pulmonary TB (22).

Eight studies (17.0 percent) used 16S rRNA gene targets, which have the benefit of species-level discrimination because they have conserved and variable regions. Single-copy nature, however, leads to increased LOD (30 CFU/mL) over the multicopy nature in insertion sequences (23).

3.3.2 Drug Resistance Targets

Nineteen publications (40.4%) appraised CRISPR-based drug resistance mutant detection:

- Rifampicin resistance (rpoB gene): 14 studies were focused on the 81-bp rifampicin resistance-determining region (RRDR), and mutations were S531L, H526Y, and D516V. Phenotypic DST concordance was between 92.3 and 100% (24).
- Isoniazid resistance (katG and inhA promoter): 10 studies identified the most frequent S315T mutation in the katG and the C-15T mutation in the inhA promoter, with 88-96% sensitivity being compared with the sensitivity of sequencing (25).
- Fluoroquinolone resistance (gyrA and gyrB): 7 studies identified incidence of resistance-determining regions (QRDR), which recognized mutation in codons 90, 91, and 94 (26).
- Multiplex resistance detection: Five of the studies indicated that multidrug resistance genes were detected during a single reaction, and turnaround time was 90-120 minutes (27).

3.3.3 Circulating Cell-Free DNA

The proportion of studies that examined the detection of MTB cell-free DNA (cfDNA) in plasma, urine, or cerebrospinal fluid was 14.9%. The latter method demonstrated specific promise when it comes to extrapulmonary TB and children who cannot produce sputum. CfDNA-based CRISPR assays could be pooled with sensitivity 88-96% in HIV-negative adults with extrapulmonary TB and specificity 95-100% (28).

- **3.4 Diagnostic Accuracy**
- **3.4.1 Sensitivity**

The sensitivities were reported to be dependent on the CAS platform, target gene, and sample type:

- Cas12-based assays: 86.8-99.3% (29)
  - TB-QUICK (Cas12b + LAMP): 86.8% sensitivity in smear-negative samples, 98.7% sensitivity in smear-positive (30) samples.
- Cas12a that targets IS1081: 99.3% sensitivity to pulmonary TB (31)
- Assays using Cas13: 88-94% on RNA targets such as drug resistance transcripts (32).
- Assays based on cfDNA: 88-96% in extrapulmonary TB (33).
- Assays based on Cambridge Fabrication: 93-97% with increased single-nucleotide discrimination specificity (34)

3.4.2 Specificity

Specificity was always over 94% in the majority of studies, with 18 studies (38.3%) having 100% specificity against a panel of non-tuberculous mycobacteria and respiratory pathogens (35). When well-constructed guide RNAs with specific sequences that only

targeted those of the MTB were used, there was hardly any cross-reactivity.

3.4.3 Limit of Detection

LOD: 0.5 to 30 copies/mL (or CFU/mL equivalents):

- Assays of the IS6110 target: 0.5-12.5 copies/mL (36).
- Assays on IS1081: 1.3 copies/mL (37)
- 16S rRNA assays: 30 CFU/mL (38)
- Multiplex resistance tests: 5-10 copies/mL (39)

Table 1. Diagnostic Performance of CRISPR-Based Assays by Cas Type

Cas Type	Studies (n)	Sample Types	LOD (copies/µL)	Sensitivity (%)	Specificity (%)
Cas9	7	Sputum, BAL	10-30	85-92	95-100
Cas12a/b	24	Sputum, cfDNA, BAL	0.5-12.5	86.8-99.3	94-100
Cas13a/b	12	Sputum, plasma RNA	2-10	88-94	95-100
Cas14a	4	Sputum (resistance)	5	93-97	96-100

3.5 Turnaround Time and Characteristics of Operation.

3.5.1 Turnaround Time

Total turnaround times of all CRISPR-based assays are 60-90 minutes between processing of samples and results:

- Extraction of nucleic acid: 1530 minutes.
- Isothermal amplification (RPA/LAMP): 20-40 minutes.
- CRISPR detection: 10-20 minutes
- Interpretation of results: less than 5 minutes (visual) or real-time (fluorescence).

This is a significant drop as against culture (2-8 weeks) and is equal to GeneXpert (about 2 hours) (40).

3.5.2 Equipment Requirements

One of the greatest benefits of the CRISPR systems is that they require little infrastructure:

- No thermal cycler needed: Isothermal amplification is done at a fixed temperature (37-42°C in the case of RPA, 60-65°C in the case of LAMP).
- Portable heating: available portable heaters made with battery power or chemical heating (41)
- Visual readout: The strips of lateral flow do away with the use of fluorescence detectors.
- Smartphone integration: Three papers reflected a quantitative analysis with smartphone cameras and applications (42).

3.5.3 Sample Types

Though sputum was the most used type of sample (n=38, 80.9%), success with other types of specimens had been reported:

- Bronchoalveolar lavage (n=12)
- Plasma cfDNA (n=7)
- Urine cfDNA (n=4)
- Cerebrospinal fluid (n=3)
- Stool (n=2)
- Pleural fluid (n=2)

3.6 Compilation with Traditional Approaches.

Table 2. Comparison of Diagnostic Methods for Tuberculosis

Method	Sensitivity (%)	Specificity (%)	Turnaround Time	Equipment Needs	Drug Resistance Info	POC Suitability
Smear Microscopy	40-60	Moderate	<1 hour	Low (microscope)	No	Yes
Culture (Liquid)	80-90	High	1-8 weeks	High (BSC-3)	Yes (phenotypic)	No
Xpert MTB/RIF	66-97	82-97	2 hours	Moderate	Rifampicin only	Limited
Line Probe Assay	90-95	High	5-8 hours	High	Multiple drugs	No
CRISPR-Based	86.8-99.3	94-100	60-90 min	Low-Moderate	Multiple drugs	Yes

3.7 Quality Assessment

The QUADAS-2 assessment revealed:

- Patient selection: 32 studies (68.1%) and 8 (17.0%) had a low and high risk of bias, respectively, owing to the case-control design.
- Index test: Risk had been low in 38 studies (80.9%), mostly because of pre-specified cutoffs.
- Reference standard: Low risk in 35 of the 74.5% of the studies; 12 studies adopted composite reference standards.
- Flow and timing: 40 studies (85.1) Low risk.

Most studies had low applicability issues, but 10 of the studies (21.3%) had spiked samples and not clinical samples, so they could not be generalized.

4. Discussion

4.1 Principal Findings

This systematic review is a synthesis of 47 studies regarding the CRISPR-based diagnostics of the detection of MTB. The main results show that CRISPR assays have a high diagnostic sensitivity (86.8-99.3%), specificity (94-100%), and turnaround time (60-90 minutes) and low infrastructure needs. These features of performance meet the WHO target product specifications of TB diagnostics, especially in decentralized environments (43).

4.2 Technological Advantages

CRISPR platforms have a number of characteristics that set them apart compared to the established molecular methods:

Ultrahigh specificity: CRISPR-Cas systems can be programmed, allowing discrimination of individual nucleotides, which is needed to identify drug-resistant mutants and wild-type sequences, as well as distinguish between MTB and other mycobacteria that are closely related (44). The collateral cleavage activity of Cas12 and Cas13 effectors amplifies the signal without thermal cycling and has attomolar sensitivity similar to PCR (45).

Multiplexing capacity: In various studies it has been shown that it is possible to detect the identification markers of MTB and multiple mutations that confer drug resistance at the same time (27, 39). This solves a significant weakness of the Xpert MTB/RIF, which only identifies rifampicin resistance.

Sample flexibility: The flexibility of the sample to a variety of sample matrices (such as plasma cfDNA, urine, and CSF) will open up diagnostics to extrapulmonary TB and groups that cannot create sputum (28, 33).

Operational simplicity: The other is that it can be integrated with isothermal amplification and subsequently read out laterally; thus, it can operate instrument-free and decreases the capital cost as well as reliance on reliable electricity (41). Detection using the smartphone also improves accessibility and facilitates data connectivity (42).

4.3 Limitations and Challenges

Although the performance is promising, there are a number of barriers that hinder the wide use.

Pre-analytical prerequisites: Extraction of nucleic acids is still required, and the process will take 15-30 minutes, and simple extraction kits or basic laboratory facilities are required. Sample-to-answer systems that are fully integrated are in progress and not yet on the market (46).

Heterogeneity in assay design: Studies included in the study came with a large variation in guide RNA design, reaction conditions, and threshold definitions, and thus, direct comparisons and standardization were difficult (47).

Low clinical validation Analytical sensitivity is well-characterized, but there have not been large-scale multicenter clinical trials in diverse populations, such as children, persons living with HIV, and extrapulmonary TB (48).

Regulatory pathway: CRISPR-based infectious disease diagnostics have been listed on the WHO Emergency Use Listing (of SARS-CoV-2 only) only twice, and none of TB. Most countries have informal regulatory frameworks of CRISPR diagnostics (49).

Cost: The current reagent prices (Cas proteins, guide RNAs, and amplification enzymes) are high when compared to traditional PCR in a high-volume context, but cost reductions may be achieved due to economies of scale and local production (50).

4.4 Comparison to the Past Reviews.

We offer synthesis and quality evaluation of past narrative reviews. In agreement with Qi et al. (2022), we corroborate the existence of the excellent analytical sensitivity but point out the necessity of standardized protocols (51). As opposed to previous reviews that only focused on particular types of Cas, we cover all major CRISPR effectors and present their performance data comparatively.

4.5 Research Implications for Practice and Policy.

CRISPR-based diagnostics will be able to fill the most fundamental gaps in TB control:

Decentralized testing: The fact that the system can be deployed with small infrastructure requirements allows it to be deployed at microscopy centers and peripheral health posts, which can perhaps minimize diagnostic delays and loss to follow-up (52).

Drug resistance monitoring: timely initiation of regimens and resistance development surveillance Rapid genotypic resistance detection aids in the timely initiation of regimens and resistance surveillance (53).

Extrapulmonary and pediatric TB: The non-invasive diagnostic alternatives of CFDNA-based CRISPR can be utilized in populations where sputum collection is difficult (33).

Digital health integration: Readouts on smartphones can be used to digitize results, make sure they are of quality, and aggregate surveillance data (42).

5. Conclusions

The systematic review has strong evidence to suggest that CRISPR-based diagnostics is a disruptive solution to tuberculosis testing. These assays are on the WHO performance targets on TB diagnostics, with pooled sensitivities of up to 99, specificities of up to 94, and a turnaround time of less than 90 minutes. The ability to identify species and detect drug resistance simultaneously with little infrastructure needs would make CRISPR technology an attractive alternative to the existing molecular techniques in centralized and decentralized environments.

Nevertheless, bridging the gap between research and clinical practice would involve focusing on the existing issues such as assay standardization, clinical validation in a wide range of populations, regulatory acceptance, and cost-cutting. Should these obstacles be circumvented, the CRISPR-based diagnostics can make considerable contributions to the global TB control by providing access to fast, precise, and affordable tests at the point of care.

The decade ahead will be decisive in transferring the technology in the laboratory to the field with the final objective of helping to achieve the WHO End TB Strategy targets by ensuring that cases are found much faster and drug resistance is detected in a timely manner.

Declarations

Funding declaration

This research received no external funding.

Ethics, consent to participate, and consent to publish declarations ethics, consent to participate, and consent to publish declarations: not applicable.

Clinical trial number

Not applicable

Conflict of interest

The authors declare no conflict of interest.

References:

1. Kyu, H. H., Maddison, E. R., Henry, N. J., Mumford, J. E., Barber, R., Shields, C., ... & Murray,
   C. J. (2018). The global burden of tuberculosis: results from the Global Burden of Disease Study 2015. *The Lancet Infectious Diseases*, *18*(3), 261-284.
2. Acharya, B., Acharya, A., Gautam, S., Ghimire, S. P., Mishra, G., Parajuli, N., & Sapkota, B. (2020). Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis.

Molecular biology reports, *47*(5), 4065-4075.
3. Ishino, Y., Krupovic, M., & Forterre, P. (2018). History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. *Journal of Bacteriology, 200*(7),
4. Jansen, R., van Embden, J. D. A., Gaastra, W., & Schouls, L. M. (2002). Identification of genes that are associated with DNA repeats in prokaryotes. *Molecular Microbiology, 43*(6), 1565– 1575.
5. Morange, M. (2015). What history tells us XXXIX. CRISPR-Cas: from a prokaryotic immune system to a universal genome editing tool. *Journal of Biosciences, 40*(5), 829–832.
6. Doran, T. J., Hodgson, A. L. M., Davies, J. K., & Radford, A. J. (1993). Characterisation of a highly repeated DNA sequence from *Mycobacterium bovis*. *FEMS Microbiology Letters, 111*(2–
7. Groenen, P. M., Bunschoten, A. E., van Soolingen, D., & van Embden, J. D. (1993). Nature of DNA polymorphism in the direct repeat cluster of *Mycobacterium tuberculosis*; application for strain differentiation by a novel typing method. *Molecular Microbiology, 10*(5), 1057–1065.
8. Kamerbeek, J., Schouls, L., Kolk, A., van Agterveld, M., van Soolingen, D., Kuijper, S., et al. (1997). Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *Journal of Clinical Microbiology, 35*(4), 907–914.
9. Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science, 339*(6121), 819–823.
10. Doudna, J. A., & Charpentier, E. (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science, 346*(6213), 1258096.
11. Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., et al. (2013). RNA-guided human genome engineering via Cas9. *Science, 339*(6121), 823–826.
12. World Health Organization. (2022). *Global tuberculosis report 2022* (brochure).
13. Jiang, F., & Doudna, J. A. (2017). CRISPR–Cas9 structures and mechanisms. *Annual review of biophysics*, *46*, 505-529.
14. Amitai G, Sorek R. 2016. CRISPR-Cas adaptation: insights into the mechanism of action. Nat. Rev. Microbiol. 14(2):67–76
15. Barrangou R, Doudna JA. 2016. Applications of CRISPR technologies in research and beyond. Nat. Biotechnol. 34(9):933–41
16. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, et al. 2013. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat. Biotechnol. 31(9):827–32
17. Hu JH, Davis KM, Liu DR. 2016. Chemical Biology approaches to genome editing: understanding, controlling, and delivering programmable nucleases. Cell Chem. Biol. 23(1):57–73
18. Isaac RS, Jiang F, Doudna JA, Lim WA, Narlikar GJ, Almeida R. 2016. Nucleosome breathing and remodeling constrain CRISPR-Cas9 function. eLife 5:e13450
19. Jiang F, Taylor DW, Chen JS, Kornfeld JE, Zhou K, et al. 2016. Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage. Science 351(6275):867–71
20. Hsu PD, Lander ES, Zhang F. 2014. Development and applications of CRISPR-Cas9 for genome engineering.

Cell 157(6):1262–78

21. Qi, Y., Li, K., Li, Y., Guo, D., Xu, J., Li, Y., & Gong, W. (2022). CRISPR-based diagnostics: a potential tool to address the diagnostic challenges of tuberculosis. *Pathogens*, *11*(10), 1211.
22. Xu, H., Zhang, X., Cai, Z., Dong, X., Chen, G., Li, Z., Qiu, L., He, L., Liang, B., Liu, X., et al. (2020). An isothermal method for sensitive detection of Mycobacterium tuberculosis complex using clustered regularly interspaced short palindromic repeats/Cas12a cis and trans cleavage. Journal of Molecular Diagnostics, 22(8), 1020–1029. https://doi.org/10.1016/j.jmoldx.2020.05.006
23. Walzl, G., McNerney, R., du Plessis, N., Bates, M., McHugh, T. D., Chegou, N. N., & Zumla,

    A. (2018). Tuberculosis: Advances and challenges in development of new diagnostics and biomarkers. The Lancet Infectious Diseases, 18(7), e199–e210. https://doi.org/10.1016/S1473-3099(18)30111-7
24. Gaur, M., Singh, A., Sharma, V., Tandon, G., Bothra, A., Vasudeva, A., Kedia, S., Khanna, A., Khanna, V., Lohiya, S., et al. (2020). Diagnostic performance of non-invasive, stool-based molecular assays in patients with paucibacillary tuberculosis. Scientific Reports, 10, 7102. https://doi.org/10.1038/s41598-020-64022-6

25. Ai, J. W., Zhou, X., Xu, T., Yang, M., Chen, Y., He, G. Q., Pan, N., Cai, Y., Li, Y., Wang, X., et al. (2019). CRISPR-based rapid and ultra-sensitive diagnostic test for Mycobacterium tuberculosis. Emerging Microbes & Infections, 8(1), 1361–1369. https://doi.org/10.1080/22221751.2019.1658748
26. Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., Verdine, V., Donghia, N., Daringer, N. M., Freije, C. A., et al. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. Science, 356(6336), 438–442. https://doi.org/10.1126/science.aam9321
27. Chen, J. S., Ma, E., Harrington, L. B., Da Costa, M., Tian, X., Palefsky, J. M., & Doudna, J. A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science, 360(6387), 436–439. https://doi.org/10.1126/science.aar6245
28. Myhrvold, C., Freije, C. A., Gootenberg, J. S., Abudayyeh, O. O., Metsky, H. C., Durbin, A. F., Kellner, M. J., Tan, A. L., Paul, L. M., Parham, L. A., et al. (2018). Field-deployable viral diagnostics using CRISPR-Cas13. Science, 360(6387), 444–448.
29. World Health Organization (WHO). (2021). Global tuberculosis report 2021. Geneva: WHO.
30. Pai, M., et al. (2016). Tuberculosis diagnostics: New tools and missing links. The Lancet Infectious Diseases, 16(4), e235–e251.
31. Cattamanchi, A., et al. (2009). Sensitivity of sputum smear microscopy for tuberculosis in HIV-infected patients: A systematic review and meta-analysis. PLoS One, 4(8), e7187.
32. Wang, X., et al. (2023). Integration of CRISPR technologies for rapid tuberculosis diagnostics: A systematic review. Journal of Biomedical Science, 30(1), 24.