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## Exploring the diagnostic synergy of isothermal amplification-integrated CRISPR technology for tuberculosis: a systematic review

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To address the problems linked with *Mycobacterium tuberculosis* (MTB) detection, we need an accurate, sensitive, and rapid detection method for efficient epidemiological management of tuberculosis (TB). Nucleic acid-based diagnosis of TB is more sensitive and specific but primarily requires trained workers and costly infrastructure. Isothermal amplification methods have paved the way for efficient and rapid diagnosis of TB due to their negligible infrastructure requirements; however, they sometimes suffer from drawbacks such as false-positive results and challenges in primer design. With progress in clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein (Cas)-integrated nucleic acid detection methods, the above limitations are being overcome in pathogen detection. The combination of CRISPR with any suitable isothermal amplification techniques such as recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP) offers several advantages due to its higher sensitivity, specificity, versatility and reproducibility as a point-of-care detection technique. Thus, in this systematic review, we aimed to provide a comprehensive overview of the various isothermal amplification methods coupled with CRISPR-based TB diagnostic studies that are reported in the literature. About 12 articles were included in this review using predefined selection criteria. Data were extracted for detailed review from PubMed, Google Scholar and ScienceDirect, and diagnostic efficiency was evaluated. The data uncovered that most of the studies were conducted in China, with *IS6110* and *IS6108* as the major target genes employed. The most used detection methods were based on fluorescence and lateral flow. Analytical sensitivity, defined by the limit of detection, ranged between 10 and 20 copies per  $\mu\text{L}$ . Diagnostic sensitivity and specificity were consistently high, ranging from 95 to 100%. Taken together, the synergy between isothermal amplification methods and CRISPR-Cas technique could serve as a potential alternative to qPCR, GeneXpert, and conventional acid-fast staining, particularly in low-resource regions for easy and rapid TB diagnosis.

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## Introduction

Tuberculosis (TB), caused by the pathogen *Mycobacterium tuberculosis* (MTB), persists as a major global health threat and ranks among the foremost infectious diseases responsible for high morbidity and mortality worldwide. According to the World Health Organization (WHO), TB continues to be a major global health challenge, with an estimated 10 million people developing TB and 1.5 million deaths in 2024.<sup>1</sup> TB primarily affects the lungs but can also involve other organs, leading to extrapulmonary TB. Despite significant advances in medicine, TB continues to pose a serious public health challenge, especially in resource-limited settings where timely and accurate diagnosis remains a critical barrier to effective treatment.<sup>2</sup>

Isothermal amplification techniques, including loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), rolling circle amplification (RCA), and recombinase-aided amplification (RAA), offer significant advantages over traditional PCR methods. These methods amplify DNA/RNA at a constant temperature, eliminating the need for a thermal cycler, which makes them more accessible and portable for use in low-resource settings.<sup>3–5</sup> LAMP has been widely used for the detection of various pathogens, including MTB, due to its simplicity, high sensitivity, and specificity.<sup>6,7</sup> RPA and RAA also offer similar advantages, including rapid amplification times and the ability to operate at ambient temperatures, enhancing their suitability for field diagnostics.<sup>8–10</sup> Clustered regularly interspaced short palindromic repeats (CRISPR)-associated systems, CRISPR-Cas, have revolutionized genetic research, but their application in diagnostics has gained significant attention only recently. CRISPR-Cas systems have been adapted for use in molecular diagnostics, particularly for pathogen detection, due to their high specificity, sensitivity, and ability to detect minute quantities of nucleic acid.<sup>11,12</sup> CRISPR-based diagnostics, such as SHERLOCK and DETECTR, offer the potential for rapid, portable, and cost-effective testing for infectious diseases, including TB.<sup>13–17</sup> The integration of CRISPR-Cas systems with various isothermal amplification techniques, such as LAMP, RPA, RCA, and RAA, holds promise for creating highly sensitive and specific diagnostic platforms that can be used in resource-limited settings for the early detection of TB.<sup>18–21</sup>

## Literature survey and inclusion of studies

A systematic literature survey was carried out to identify and select relevant studies focusing on the application of isothermal amplification techniques in MTB diagnostics. The search was conducted across three scientific databases: PubMed, Google Scholar, and ScienceDirect. The time frame for the search spanned from the year 2020 to 2025. The primary keywords used during the database queries were “*Mycobacterium tuberculosis*”,

“isothermal amplification techniques”, “CRISPR”, “LAMP”, “RPA”, “RAA” and “RCA”. In PubMed, a total of 107 articles were retrieved, with publication dates ranging from 2003 to 2025. When the keyword “CRISPR” was included in the PubMed search, only 3 articles were found, all of which were published between 2020 and 2025. On Google Scholar, the same keyword filters yielded 4170 results. All eligible studies were included regardless of access status. Further narrowing the search with the term “CRISPR” brought the number down to 509. ScienceDirect returned 98 relevant results under the initial search conditions. Combining the results from all three databases, a total of 610 articles were identified for initial consideration. Given the recent emergence and integration of these isothermal amplification techniques into MTB diagnostics, the literature search was limited to studies published between January 2020 and March 2025. The screening process began with the exclusion of 64 articles written in languages other than English, leaving 546 English-language articles. From this pool, 207 articles were excluded owing to duplication or because they focused on organisms other than MTB. This step reduced the number of eligible articles to 339. Further evaluation led to the removal of 210 articles because they did not include both isothermal amplification techniques and CRISPR-based diagnostic applications. The inclusion of CRISPR as one of the diagnostic components was a mandatory criterion for all selected studies. This refinement resulted in 129 articles specifically addressing MTB diagnostics using isothermal amplification technologies. A closer inspection of these 129 studies resulted in the exclusion of 84 articles due to their irrelevance of content. The remaining 65 articles were then examined for methodological consistency and relevance. These articles included studies on isothermal amplification methods such as loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), rolling circle amplification (RCA), and recombinase-aided amplification (RAA). Of these, 53 articles were excluded, which were categorized as review papers, editorials, book chapters, commentaries, or conference abstracts, as they did not constitute original research. Ultimately, 12 articles fulfilled all the inclusion criteria and were finally considered (Fig. 1); their study characteristics are listed in Table 1 and discussed in subsequent sections.

## Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification method that operates at a constant temperature, typically between 60 °C and 65 °C, and is facilitated by the use of a set of four to six primers that target different regions of the DNA. This technique enables the amplification of DNA in a rapid, simple, and cost-effective manner without the need for



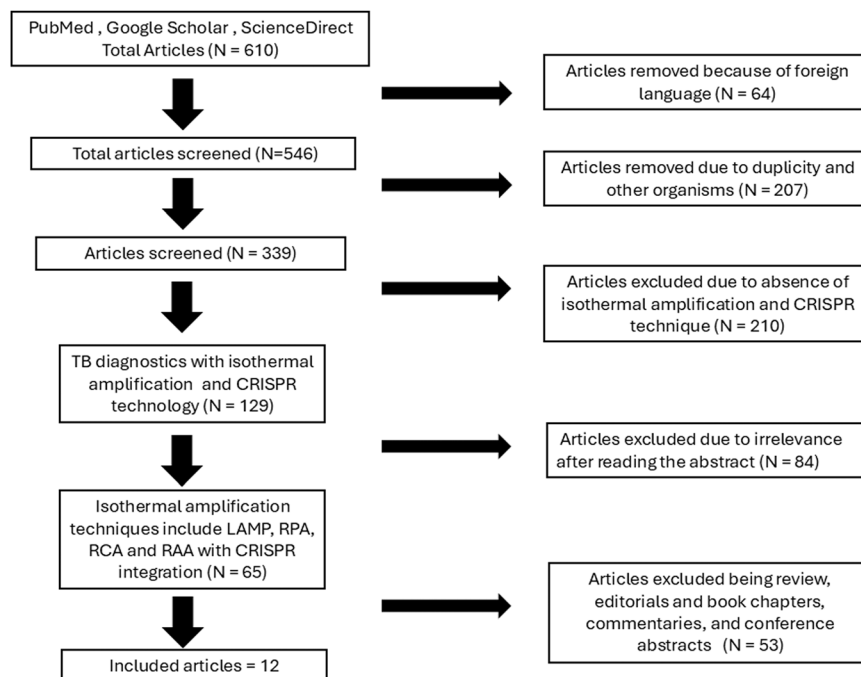


Fig. 1 PRISMA flowchart illustrating the literature search and screening strategy used in the study.

sophisticated thermal cyclers.<sup>3</sup> LAMP has shown considerable promise in the detection of MTB owing to its sensitivity and specificity. Studies have demonstrated that LAMP can detect low concentrations of MTB DNA in sputum samples, even in the presence of other respiratory pathogens.<sup>22</sup> Recent advancements in CRISPR-based detection have overcome these issues by enhancing the specificity of LAMP, ensuring a more accurate and rapid diagnostic approach.<sup>7,23,24</sup>

## Recombinase polymerase amplification (RPA)

Recombinase polymerase amplification (RPA) is another isothermal amplification technique that is faster than traditional PCR, as it operates at a constant temperature of 37–42 °C. RPA employs recombinase enzymes to facilitate strand displacement and a polymerase for amplification, making it an efficient method for DNA detection.<sup>9</sup> Notable success has been achieved in RPA-based MTB detection, and due to its rapid amplification duration, results are often achieved within 20 minutes, making it more advantages in terms of minimal infrastructure requirements and operational simplicity.<sup>25</sup> The combination of RPA with CRISPR technology further enhances the specificity and sensitivity of the assay, making it ideal for field-based diagnostics.<sup>26,27</sup> The high specificity of CRISPR ensures that the amplified product is accurately detected, even at low concentrations of MTB DNA, providing a highly sensitive tool for TB detection.<sup>8,27,28</sup>

## Rolling circle amplification (RCA)

Rolling circle amplification (RCA) is an isothermal amplification technique that uses a single-stranded DNA or RNA template to generate long, concatemeric DNA products. From a small number of samples, RCA has the ability to produce large quantities of DNA, making it an attractive method in diagnostics.<sup>29</sup> RCA has been utilized to detect MTB with high efficiency in clinical samples.<sup>11</sup> When integrated with CRISPR technology, RCA can be used to further enhance detection sensitivity. CRISPR-Cas systems, such as Cas12 and Cas13, can be utilized for the specific detection of amplified products, enabling real-time monitoring of the DNA amplification process and reducing false positives.<sup>17,29</sup> This combination offers a promising tool for the rapid and accurate detection of TB in resource-limited settings.

## Recombinase-aided amplification (RAA)

Recombinase-aided amplification (RAA) is another isothermal amplification method similar to RPA, but it uses recombinase enzymes to bind to single-stranded DNA, forming a heteroduplex with a primer, which allows for amplification at a constant temperature.<sup>10</sup> RAA has been shown to provide fast, accurate, and sensitive results for MTB detection, often in less than 30 minutes. The major advantage of RAA over traditional PCR is its ability to perform amplification at lower temperatures, making it more suitable for point-of-care diagnostics in low-resource settings. Coupling RAA with



**Table 1** Characteristics of the included studies (*n* = 12)

S. no.	Author	Country	Sample size	Type of specimen	Target gene	Method of detection	Sensitivity	Specificity	Reference method	Isothermal techniques used
1	Compiro <i>et al.</i> , 2024 (ref. 34)	Thailand	82	Sputum	<i>rpoB</i>	Gel electrophoresis and fluorescence	1 copy per reaction	100%	AFB culture	RPA, CRISPR
2	Xiao <i>et al.</i> , 2023 (ref. 27)	China	147	Sputum	<i>IS6110</i> , <i>IS1081</i>	Gel electrophoresis, fluorescence, lateral flow, RT-PCR	4 copies per $\mu\text{L}$	100%	GeneXpert	RPA, CRISPR
3	Liu <i>et al.</i> , 2022 (ref. 8)	China	49	Sputum	<i>rpsL</i>	Fluorescence	100 fg	100%	Sequencing	RPA, CRISPR
4	Ren <i>et al.</i> , 2023 (ref. 10)	China	401	BALF, sputum, pus	<i>IS1081</i>	RT-PCR	1 copy per $\mu\text{L}$	100%	ZN staining, AFB culture, GeneXpert	RPA, CRISPR
5	Li <i>et al.</i> , 2024 (ref. 30)	China	151	Sputum	<i>IS6110</i> , <i>IS1081</i>	Gel electrophoresis, fluorescence	1 copy per $\mu\text{L}$ , 10 copies per $\mu\text{L}$	100%	GeneXpert	RAA, CRISPR
6	Sam <i>et al.</i> , 2021 (ref. 23)	China	148	BALF, sputum	<i>IS6110</i>	RT-PCR	1.3 copy per $\mu\text{L}$	95.20%	GeneXpert, AFB culture	LAMP, CRISPR
7	Wang <i>et al.</i> , 2021 (ref. 24)	China	44	Sputum	<i>IS6110</i>	Colorimetric, gel electrophoresis, lateral flow, RT-PCR	10 copies per reaction	100%	ZN staining, AFB culture, GeneXpert	LAMP, CRISPR
8	Sun <i>et al.</i> , 2024 (ref. 29)	China	38	BALF, sputum	<i>rpov</i>	Gel electrophoresis, fluorescence	$10^4$ amplicons	100%	Sequencing	RCA, CRISPR
9	Xu <i>et al.</i> , 2020 (ref. 25)	China	193	Sputum	<i>IS6110</i> , <i>IS1081</i>	Gel electrophoresis, fluorescence, RT-PCR	99.29%	100%	AFB culture	RPA, CRISPR
10	Zhang <i>et al.</i> , 2023 (ref. 35)	China	504	BALF, sputum, tissue, hydrothorax	<i>IS6110</i> , <i>IS1081</i>	Gel electrophoresis, fluorescence, RT-PCR	3.13 CFU $\text{mL}^{-1}$	100%	BACTEC960 culture	RAA, CRISPR
11	Thakku <i>et al.</i> , 2023 (ref. 28)	USA	52	Blood	<i>IS6110</i> , <i>IS1081</i>	Lateral flow	25 copies per $\mu\text{L}$	100%	AFB culture, GeneXpert, qPCR	RPA, CRISPR
12	Kaushik <i>et al.</i> , 2025 (ref. 7)	India	232	Sputum, urine, serum	<i>IS6110</i>	Lateral flow	1 ag	100%	ZN staining, AFB culture, GeneXpert	LAMP, CRISPR

CRISPR systems can significantly enhance TB diagnostics by improving both sensitivity and specificity with high precision, ensuring accurate TB detection even in samples with low DNA concentrations.<sup>16,30</sup> There are few key differences (Table 2) between LAMP, RPA/RAA, RCA and RCA in terms of primer design, amplification efficiency, specificity, cost and field suitability. LAMP, which requires 4–6 primers targeting 6–8 regions, offers high specificity and inhibitor resistance, but primer design is complex and the method is sensitive to temperature fluctuations—false positives due to primer dimers are well-documented. RPA/RAA, using only two primers and operating at 37–42 °C, delivers rapid results ( $\leq 20$  min), tolerates greater temperature variation, and is easier to design; however, it relies on proprietary enzymes

that increase cost and may cause non-specific amplification without probe integration. RCA uses simple padlock probes and a single primer, but its reaction is slower ( $\sim 1$  h) and requires circular template preparation; it lends itself to multiplexing *via* concatemer production PMC provides a concise visual overview of these comparisons.

## Mechanism of CRISPR-Cas in diagnostics

CRISPR-based diagnostics exhibit great sensitivity, specificity, speed, and cost-effectiveness. This makes them ideal for point-of-care testing, especially in resource-limited settings. The CRISPR-Cas system, originally discovered as a bacterial

**Table 2** Comparison of different isothermal amplification techniques

Method	Temp	Primers	Speed	Specificity	Cost/supply	Field suitability
LAMP	60–65 °C	4–6	$\sim 60$ min	High, but primer-dimer risk	Moderate	Heater required
RPA	37–42 °C	2 (+ probe)	5–20 min	Good, non-specific possible	High (proprietary)	Low-equipment viable
RAA	37–42 °C	2 (+ probe)	$\sim 20$ min	Similar to RPA	Similar	Similar
RCA	37–65 °C	1 (+ padlock)	$\sim 60$ min	Very high	Dependent on ligase	Moderate



immune mechanism, has been adapted for molecular diagnostics due to its high specificity and sensitivity. The CRISPR-Cas system functions by utilizing a guide RNA (gRNA) to direct the Cas protein (typically Cas9, Cas12, or Cas13) to a specific DNA or RNA target. Upon recognition of the target sequence, Cas proteins induce a detectable change, such as DNA cleavage in the case of Cas12 or cleavage of ssRNA in the case of Cas13, making it an ideal tool for diagnostic purposes.<sup>7,24,31</sup> For example, the CRISPR-Cas12 and Cas13 systems are often coupled with isothermal amplification techniques to increase the sensitivity of pathogen detection.<sup>28</sup> The key advantage of using CRISPR in diagnostics is its ability to recognize specific sequences with high accuracy, even in the presence of closely related sequences, which makes it far more specific than traditional PCR-based methods.<sup>7,17,32</sup> Diagnosing MTB begins with isolating the nucleic acid from the patient sample. The extracted nucleic acid is processed and amplified using isothermal techniques like recombinase polymerase amplification (RPA) or loop-mediated isothermal

amplification (LAMP). These methods are preferred because they require less equipment than traditional PCR. Using RPA or LAMP accelerates the diagnostic process, enabling rapid and sensitive detection of MTB. This makes the CRISPR-Cas system a promising diagnostic tool for facilities with limited infrastructure (Table 2).

As an isothermal technology, LAMP works at a constant temperature, which simplifies the amplification step. In contrast, PCR requires complex equipment, such as a thermal cycler. The rapid and efficient nature of LAMP enables amplification in just 30 to 60 minutes. Its integration with Cas12b, a thermostable CRISPR-associated nuclease, boosts the system's effectiveness by enabling one-pot detection. This method streamlines workflows, shortens diagnostic time, reduces dependence on complex equipment, and lowers the risk of cross-contamination. Upon target recognition (*cis*-cleavage), Cas12 (DNA target) and Cas13 (RNA target) undergo conformational changes that induce robust *trans*-cleavage, enabling non-specific cutting of nearby single-stranded nucleic acid reporters (*e.g.*, ssDNA for Cas12, ssRNA for Cas13).

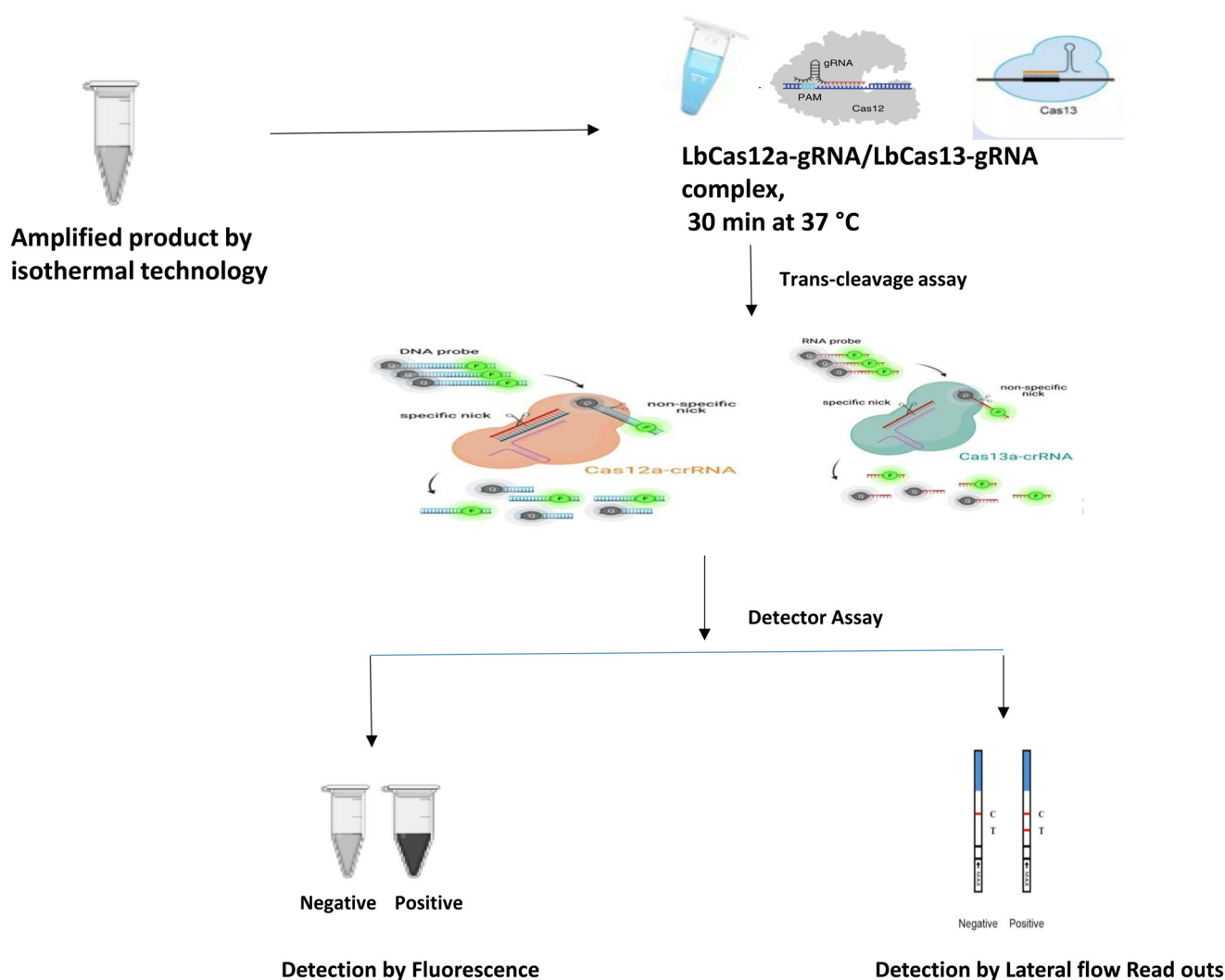


Fig. 2 Schematic showing isothermal amplification and CRISPR-Cas12- and Cas13-mediated detector assay.





for Cas13). This collateral activity provides signal amplification, wherein a single target molecule can activate multiple reporter-cleavage cycles, increasing assay sensitivity. Detection outputs include:

- Fluorophore-quencher reporters, yielding fluorescence upon reporter cleavage.
- Lateral flow strips, where labelled reporters produce visual bands, as shown in Fig. 2. Moreover, CRISPR-based assays can be performed rapidly and without sophisticated equipment, making them well-suited for point-of-care diagnostics.<sup>16</sup>

Another CRISPR-based diagnostic method for TB detection is DETECTR, which utilizes Cas12 for DNA detection.<sup>26</sup> DETECTR detects MTB DNA in a two-step manner, in which isothermal methods like LAMP first amplify the DNA/RNA, followed by precise identification *via* CRISPR-Cas12-based detection.<sup>33</sup> This combination not only enhances sensitivity but also ensures faster results compared to traditional methods such as culture and PCR. The ability of CRISPR-Cas12 system to generate a signal in the presence of amplified DNA further enhances the speed and accessibility of diagnostic testing, which is crucial for the timely management of TB cases.<sup>28</sup>

The sensitivity of CRISPR-based assays is also noteworthy. Because the Cas proteins can be programmed to target highly specific sequences, the system can detect even small quantities of genetic material, often down to a single copy of the target sequence.<sup>7</sup> This level of sensitivity is particularly beneficial when dealing with pathogens present in low concentrations in clinical samples, such as MTB. Additionally, CRISPR-based diagnostics can be adapted for the detection of multiple pathogens in a single assay, offering a multiplexing capability that further enhances diagnostic accuracy and utility.

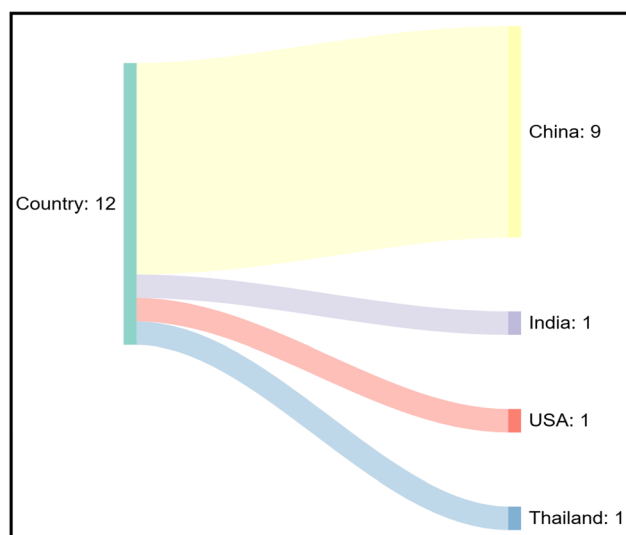


Fig. 3 Country-wise distribution of included studies ( $n = 12$ ) reported in the present investigation.

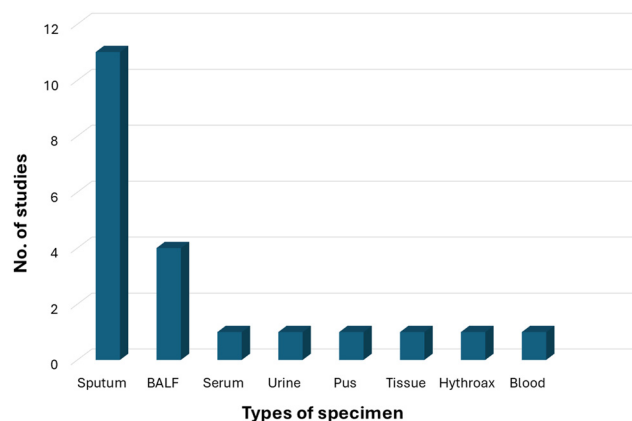


Fig. 4 Distribution of types of specimens used for the detection of MTB in the included studies ( $n = 12$ ).

## Characteristics of the included studies

Fig. 3, a Sankey diagram, shows the number of studies from different countries focusing on the use of any isothermal amplification techniques combined with CRISPR to detect MTB. Of the 12 studies included, China leads by a wide margin with 9 studies. India, the USA and Thailand each contributed one study. The figure highlights China's strong advocacy for the use of isothermal amplification-linked CRISPR diagnostics for TB. Fig. 4, a bar chart, shows different types of samples used in the 12 studies included in our study on CRISPR-based diagnostics for MTB. Sputum was the most commonly used specimen, appearing in 11 of the studies. Bronchoalveolar lavage fluid (BALF) came next, used in 4 studies. Other types of specimens, *viz.* serum, urine, tissue, blood, hydrothorax, and pus, were each used individually in just 1 study. Although sputum remains the preferred choice for MTB detection, this figure also reflects a small but notable interest in exploring other sample types. Fig. 5, a bar chart, illustrates the distribution of target genes used across all the studies in our analysis. Each bar represents the number of articles that utilized a specific gene as their molecular target for detection or analysis. Among all, *IS6110* was the most frequently used target gene, appearing

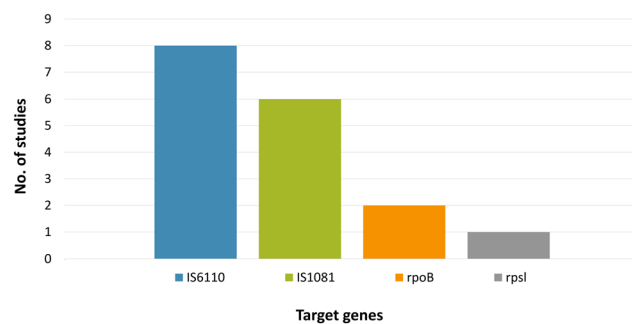


Fig. 5 Distribution of target genes reported in the included studies ( $n = 12$ ).



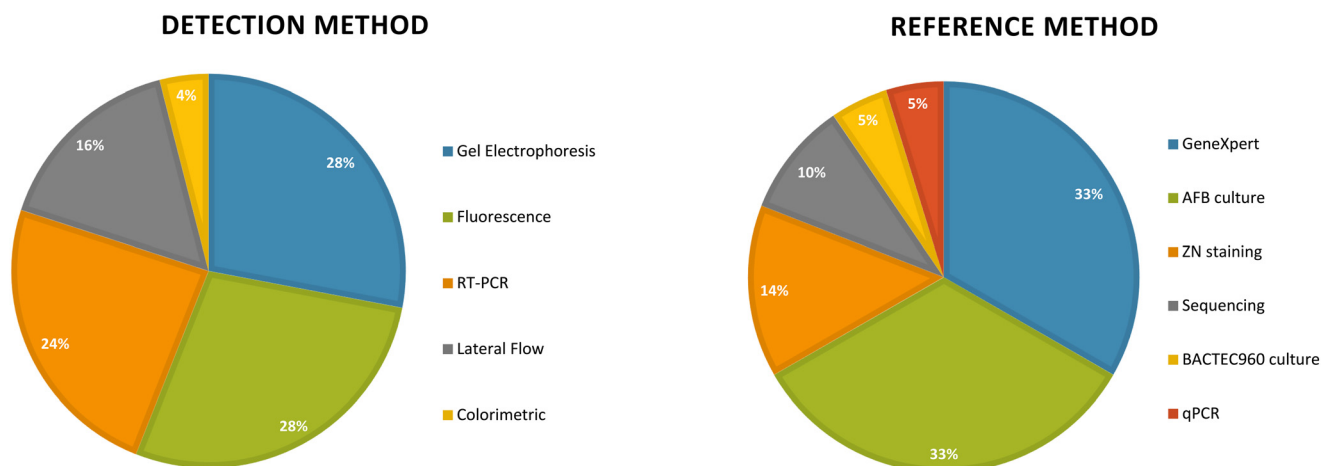


Fig. 6 Distribution of types of detection (left) and reference (right) methods for MTB in the included studies ( $n = 12$ ).

in 8 studies, highlighting its popularity and potential reliability in diagnostic assays. *IS1081* emerged as the second most frequently targeted gene, reported in 6 studies. The use of *rpob* and *rpsl* was relatively limited, featuring in only 2 and 1 articles, respectively. This figure emphasizes the variability in gene targets chosen by different researchers and underscores the prominence of *IS6110* in the current body of literature. Fig. 6, two pie charts, shows the distribution of different detection methods (left) and reference standard methods (right) reported in 12 studies that focused on CRISPR-based diagnostics for MTB. We separated them to make it easier to compare how often each method was used. For detection, gel electrophoresis and fluorescence-based assays were the most common, each used in 28% of the studies (7 of 12). RT-PCR came next at 24% (6 studies), followed by lateral flow methods at 16% (4 studies). A colorimetric method was used in just one study, making up 4%. On the reference side, GeneXpert and AFB culture were the most frequently used comparators, each appearing in 33% of the included studies. The ZN staining and Sanger sequencing methods covered 14% and 10% of the studies, respectively. Other methods like BACTEC960 and qPCR made up the remaining references, accounting for 5% of the total. By showing these two charts side by side, the figure gives a clear picture of the methods being used to detect MTB and how their performance was validated. Fig. 7, a bar graph, shows the utilization of various isothermal techniques alongside CRISPR in studies focused on MTB diagnostics. The combination of RPA and CRISPR is featured in six studies, while LAMP and CRISPR appear in three studies. RAA and CRISPR are used in two studies, and RCA with CRISPR is used in one study. This figure highlights the growing trend of combining CRISPR with isothermal methods for rapid and sensitive MTB detection. The analytical sensitivity (limit of detection) of the assays ranged from approximately 10 to 20 copies per  $\mu\text{L}$ . Their diagnostic sensitivity and specificity—when compared against reference standards—were typically between 95% and 100%. Only one study showed a specificity of 95%. Most of the studies used the DNA isolation method for the detection of the MTB pathogen, but one study innovatively used

RNA as a template for the detection of MTB. In addition, the researchers used different types of samples such as sputum, urine, serum, BALF, tissue, pus, blood, and hydrothorax.

Recent advances showcase a range of CRISPR-based tests for the rapid, sensitive, and specific detection of *M. tuberculosis*. For example, WATSON uses genome-wide tiled amplification combined with Cas13 detection to identify fragmented cfDNA in patient plasma, providing better sensitivity than single-locus tests and working well with lateral flow formats. On the amplification-free side, a Cas12a-GFET platform combines CRISPR detection with graphene field-effect transistors to directly measure serum DNA without pre-amplification. This setup achieves a detection limit of about  $2.4 \times 10^{-18}$  M and provides results in under 5 minutes.

Methods that involve amplification, like MTB-MCDA-CRISPR, use isothermal multiple cross displacement amplification (MCDA) before Cas12a detection. This approach achieves 40 fg sensitivity in less than an hour and performs similarly to Xpert assays. Another isothermal method combines ERA (exonuclease-based amplification) with Cas12a, completing tests in about 40 minutes and offering near-perfect predictive values, making it suitable for

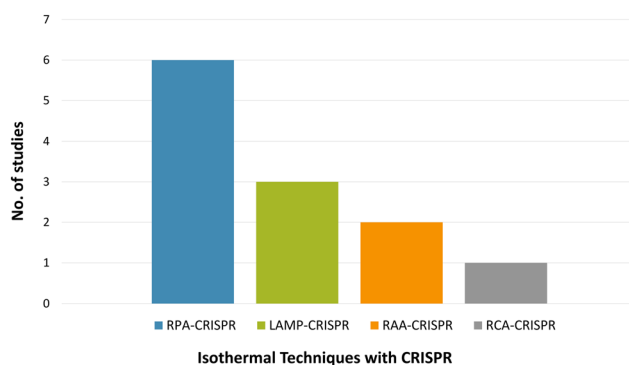


Fig. 7 Distribution of isothermal techniques combined with CRISPR in MTB diagnostics across the selected studies ( $n = 12$ ).



lateral flow readings. A simple Cas12a assay targeting *IS6110* can be completed in under 15 minutes, detecting roughly 3 CFU mL<sup>-1</sup> while clearly distinguishing *M. tuberculosis* from other bacteria. An *in situ* Cas12a-based assay applied to macrophages and BALF samples achieved 94% sensitivity, compared to 67–78% for culture or Xpert, and 100% specificity. Although most reviewed assays reported excellent sensitivity (95–100%) and low limits of detection (10–20 copies per  $\mu$ L), our analysis highlights several significant limitations rooted in study design and implementation. First, half of the studies employed case-control designs using convenience samples, potentially introducing spectrum bias—leading to inflated accuracy compared to real-world performance in heterogeneous populations. Second, there was considerable heterogeneity in reference standards, with some studies using GeneXpert and others relying on culture or qPCR for confirmation. This raises concerns about verification bias and limits the validity of cross-study comparisons. Third, inconsistent fluorescence or signal thresholds across studies may explain the variability in reported specificity (85–100%), emphasizing the need for standardized cut-off values. Fourth, only one study explicitly addressed negative or discordant results, suggesting the presence of publication bias, where studies reporting less favorable outcomes may remain unpublished.

Lastly, a multiplexed RCA-Cas12a system that targets the *rpoB* gene improves point-of-care detection for resistance and strain typing, though complete performance data were not provided. Overall, these platforms highlight important trends: genome-wide tiling, electrical sensing without amplification, fast isothermal amplification, and direct application to clinical samples. They deliver sub-femtomolar sensitivity, high specificity, and turnaround times of under an hour, demonstrating strong promise for near-patient TB diagnostics.

## Limitations and future directions

Despite the numerous advantages, several challenges remain associated with the integration of CRISPR and isothermal amplification techniques. One of the primary concerns is the sensitivity and detection limits of these integrated systems. Although CRISPR enhances the specificity of isothermal amplification, detecting very low concentrations of DNA can still be challenging. In some cases, false negatives may occur if the amplification process is not efficient enough to generate a detectable signal.<sup>16</sup> To mitigate this, optimizing the amplification conditions and improving CRISPR systems for better sensitivity is crucial.<sup>8</sup> Another significant challenge is the potential for false positives or negatives. False positives may result from non-specific amplification or contamination, whereas false negatives may occur if the CRISPR system does not efficiently detect low amounts of the target DNA. These issues can be overcome by carefully designing CRISPR guide RNAs to ensure high specificity, implementing stringent quality control measures, and using internal controls to

confirm the validity of the results.<sup>7,17,25,32</sup> Scalability and implementation in resource-limited settings are also present critical challenges. Although CRISPR-integrated isothermal amplification assays are cost-effective and portable, scaling them for widespread use in low-resource settings requires significant investment in infrastructure, training, and supply chains. Moreover, ensuring the availability of reagents and consumables, such as CRISPR enzymes and primers, could pose challenges in remote regions.<sup>20</sup> Addressing these issues will require collaboration among researchers, healthcare providers, and policymakers to ensure that these technologies are accessible and sustainable. Finally, regulatory and ethical considerations must be considered when developing CRISPR-based diagnostic tools. The use of gene-editing technologies in diagnostics raises concerns about potential misuse, data privacy, and regulatory approval processes. It is essential to establish clear regulatory frameworks to ensure that CRISPR-based diagnostics are safe, reliable, and compliant with ethical standards. Additionally, public acceptance and understanding of CRISPR technology will be vital for its successful integration into clinical practice. To address these limitations, future research should: (a) prioritize prospective, field-based studies in mixed patient cohorts to reduce spectrum bias; (b) adopt uniform reference standards or parallel validation methods to minimize verification bias; (c) establish harmonized detection threshold guidelines, ideally validated across multiple platforms; and (d) pre-register protocols and commit to publishing both positive and negative results to mitigate publication bias. Such targeted strategies will strengthen the scientific rigor, reproducibility, and real-world applicability of isothermal amplification-CRISPR diagnostics for MTB.

## Conclusion

The integration of isothermal amplification technologies, such as LAMP, RPA, RCA, and RAA, with CRISPR-Cas systems has the potential to revolutionize TB diagnostics. These integrated systems combine the strengths of both amplification techniques and CRISPR, resulting in highly sensitive, specific, and rapid diagnostic platforms. Isothermal amplification methods enable efficient DNA amplification without the need for complex thermal cycling, making them ideal for field-based applications. When paired with the ability of CRISPR to specifically target and cleave DNA or RNA, these systems can detect MTB with unprecedented accuracy, even in samples containing low amounts of DNA. This integration enhances the speed and cost-effectiveness of TB diagnosis, making it more accessible in resource-limited settings.

## Conflicts of interest

There are no conflicts to declare.





## Data availability

Since it is review therefore the data that support the findings of this study are publicly available.

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