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Harnessing the synergy of CRISPR-Cas12 with isothermal amplification for precise and rapid detection of tuberculosis: a systematic review

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ABSTRACT:

— ***Objective:*** Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), remains a leading cause of infectious mortality globally. Conventional diagnostic tools face limitations in sensitivity, specificity, cost, and differentiation between latent and active TB. Emerging molecular systems like Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas12, especially when combined with isothermal amplification, offer a rapid, sensitive, and cost-effective alternative suitable for point-of-care settings. The aim of this study was to evaluate the diagnostic performance of CRISPR-Cas12 systems integrated with isothermal amplification techniques for MTB detection.

— ***Materials and Methods:*** A systematic review was conducted in PubMed, Scopus, ScienceDirect, Wiley, and SpringerLink to identify studies published between 2020 and 2025. Studies combining CRISPR-Cas12 with isothermal amplification were included. The Quality assessment of diagnostic accuracy studies-2 (QUADAS-2) tool was used to assess study quality.

— ***Results:*** Eight studies were included, employing various Cas12 systems and isothermal amplification strategies. Reported sensitivity ranged from 67.2% to 100%, and specificity from 95.2% to 100%. Several systems outperformed GeneXpert in some individual studies, especially in smear-negative samples.

— ***Conclusions:*** CRISPR-Cas12 combined with isothermal amplification demonstrates high diagnostic potential for TB detection, offering excellent sensitivity and specificity, rapid turnaround time, and suitability for point-of-care testing.

— ***Keywords:*** CRISPR-Cas12, Diagnostics, Isothermal amplification, Mycobacterium tuberculosis, Tuberculosis.

— ***Abbreviation:*** AFB: Acid-Fast Bacilli; AUC: Area under the curve; BALF: Bronchoalveolar lavage fluid; CFU: Colony Forming Units; CPA: Cross-priming amplification; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; ERA: Enzymatic recombinase amplification; HDA: Helicase-dependent amplification; IFN-γ: Interferon-γ; IGRA: Interferon-γ release assays; LAMP: Loop-mediated isothermal amplification; LoD: Limit of detection; MCDA: Multiple cross displacement amplification; MTB: *Mycobacterium tuberculosis*; NASBA: Nucleic acid sequence-based amplification; NAT: Nucleic acid amplification test; PCR: Polymerase chain reaction; QUADAS-2: Quality assessment of diagnostic accuracy studies-2; ROC: Receiver operating characteristic; RPA: Recombinase polymerase amplification; SDA: Strand displacement amplification; ssDNA: Single-stranded DNA; TB: Tuberculosis; TST: Tuberculin skin test.

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INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB). According to the Global Tuberculosis Report (2023), there were 10.8 million new TB cases, reflecting an estimated 4.6% increase in incidence compared to the levels reported in 2020[1]. With about 1.25 million deaths, TB remains the most significant contributor to infectious disease-related mortality. Globally, Indonesia ranks second in terms of the highest TB burden and has the second-largest gap between estimated case numbers and reported cases[2].

TB transmission can occur through the air when a person with active TB releases droplets containing bacteria. After exposure to MTB, individuals may develop latent TB infection, an asymptomatic condition or progress directly to active disease depending on their immune status[3]. In active TB, symptoms may include persistent coughing and sometimes with blood, fever, significant weight loss, and night sweats[4]. Given that TB symptoms may remain subclinical in early stages, prompt detection is vital to initiate rapid intervention and interrupt the chain of transmission. Early detection enables rapid intervention, reduces the risk of complications, and breaks the chain of transmission[5].

The tuberculin skin test (TST) and Interferon-γ release assays (IGRA) are widely used reference systems to assess immune responses to TB infection[6]. The TST involves the intradermal administration of purified protein derivative, whereas IGRA relies on either whole blood or peripheral blood mononuclear cells to measure IFN-γ production. These systems reflect the continuous advancement of early TB diagnostic strategies, which include symptom-based screening, imaging modalities, biomarker discovery, and the evaluation of diagnostic system[7]. However, the intradermal nature of the TST, while causing some procedural discomfort, often leads to false-positive/negative results due to cross-reactivity with the BCG vaccine and non-tuberculous mycobacteria. In addition, the TST may produce local skin reactions such as erythema, and interpretation can be affected by prior Bacillus Calmette-Guérin vaccination[8]. Both TST and IGRA share similar limitations, including inaccuracy in distinguishing latent TB from active TB, distinguishing new infections from old infections, and predicting progression from latent TB to active TB[9].

With the progress of molecular technologies and the increasing demand for higher diagnostic precision, a novel detection system known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has been introduced. CRISPR represents an adaptive immune mechanism that naturally occurs in bacteria and archaea[10]. When bacteria invade, CRISPR uses gRNA/Cas proteins to recognize and specifically cleave foreign genes as a form of defense[11]. CRISPR-Cas12 can control and cut target DNA in trans-cleavage activity, enabling real-time detection of specific targets. CRISPR-Cas12 using the ITP-CRISPR system can detect the SARS-CoV-2 virus with high accuracy, achieving a sensitivity of 93.8% and specificity of 100%[12].

In pathogen detection, isothermal amplification systems, including Recombinase Polymerase Amplification (RPA), Loop-mediated Isothermal Amplification (LAMP), Nucleic Acid Sequence-Based Amplification (NASBA), Helicase-Dependent Amplification (HDA), and Strand Displacement Amplification (SDA), have been developed as more sensitive, rapid, and precise alternatives to the Polymerase Chain Reaction (PCR). While PCR remains the conventional gold standard for nucleic acid amplification in diagnostic applications, its application is limited by the need for sophisticated and costly instrumentation[13]. Therefore, isothermal amplification has become an alternative due to its simplicity and flexibility both in the field and in the laboratory[14]. Additionally, isothermal amplification is superior in terms of sensitivity and specificity[15,16].

This study attempted to review and discuss the use of CRISPR-Cas12 as a diagnostic approach for MTB detection. It also highlights the essential role of isothermal amplification techniques, which serve as a critical component in enhancing the sensitivity and specificity of CRISPR-Cas12-based detection by facilitating the amplification of target genes.

MATERIALS AND METHODS

Registration

The present systematic review adhered to the PRISMA 2020 guidelines[17] and was prospectively registered in PROSPERO (www.crd.york.ac.uk/PROSPERO/view/CRD420251120778).

Focused Question

The focused question in this study is "How does the integration of CRISPR-Cas12 with isothermal amplification techniques improve the accuracy and speed of MTB detection?" The PICOS model was used to formulate this question, where P (Population) refers to patients or samples infected with MTB, I (Intervention) refers to the use of the CRISPR-Cas12 system combined with isothermal amplification, C (Comparison) refers to conventional detection systems such as bacterial culture, conventional PCR, or GeneXpert, O (Outcomes) includes diagnostic accuracy (sensitivity, specificity, LoD, detection time), and S (Study type) refers to experimental, pre-clinical, and diagnostic validation studies conducted both *in vitro* and *ex vivo*, published in peer-reviewed scientific journals.

Eligibility Criteria

This systematic review incorporated primary research articles that specifically applied CRISPR-Cas12 systems combined with isothermal amplification techniques for detecting MTB. To ensure methodological quality and relevance, only studies meeting the following criteria were considered: peer-reviewed articles published between January 2020 and April 2025, availability in English, and reporting at least one quantitative diagnostic accuracy metric, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), or area under the receiver operating characteristic curve (AUC). Crucially, to ensure clinical applicability, this review included only studies involving clinical samples [e.g., sputum, bronchoalveolar lavage fluid (BALF), plasma]; studies relying exclusively on spiked specimens or artificial mock samples without clinical validation were excluded. Studies were excluded if they were reviews, conference abstracts, editorials, letters, or commentaries. Additionally, studies were not retrieved if they did not utilize CRISPR-Cas12 detection systems, lacked integration with isothermal amplification, involved only theoretical or computational modeling, or failed to provide sufficient diagnostic performance data for extraction and synthesis.

Search Strategy

A thorough electronic search of the literature was performed by two authors (BCB and IAS) in March-April 2025 across PubMed, Scopus, ScienceDirect, Wiley Online Library, and SpringerLink databases. This search aimed to identify original diagnostic accuracy studies evaluating the integration or application of CRISPR-Cas12 systems in conjunction with isothermal amplification techniques for MTB detection. The search strategy using MeSH terms and a combination of free-text keywords was applied. The search strategies in the selected databases are available in Supplementary Table 1.

Study Selection and Data Extraction

All studies fulfilling the eligibility criteria underwent full-text assessment, and pertinent information was systematically extracted using a standardized form tailored to evaluate the diagnostic synergy between CRISPR-Cas12 and isothermal amplification techniques for TB detection. While PPV, NPV, and AUC were included as eligible outcome measures, the data extraction and synthesis primarily focused on sensitivity and specificity, as these were the only metrics consistently reported across all included studies.

For studies that reported multiple CRISPR-based nucleic acid amplification tests (NATs) targeting MTB, data from each distinct system were extracted independently to ensure a comprehensive and methodologically consistent analysis. Both reviewers independently conducted the search, screening, and data extraction. Disagreements were resolved through discussion, and consensus was achieved in all cases.

Assessment of Methodological Quality

Study quality was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) instrument, which evaluates potential bias and applicability issues across four domains: patient selection, index test, reference standard, and flow and timing. For each domain, signaling questions were used to judge the risk of bias as low, high, or unclear. If any single signaling question was judged as high risk, the entire domain was considered high risk. If two or more questions were judged as unclear, the domain's risk was considered unclear. Conversely, if all questions or at least two were judged as low risk, the domain was considered low risk. Applicability was also assessed in the domains of patient selection, index test, and reference standard. Quality assessments were performed independently by two reviewers (BCB and IAS). Discrepancies were addressed and settled through discussion until consensus was achieved.

RESULTS

Search Result

A total of 1,235 relevant records were initially identified, of which 259 duplicates were removed, leaving 976 studies for screening. Title and abstract evaluation led to the exclusion of 957 articles, after which 19 studies underwent full-text review. Finally, eight studies[16,18-24] met all requirements and were included in this study. Figure 1 presents a detailed study selection process, including the specific reasons for study exclusion.

Characteristics of the Included Studies

The eight included studies were published between 2020 and 2025, with seven studies[16,18-20,22-24] conducted in China and one study[21] from the United States. The sample sizes ranged from 28 to 293 clinical samples; these figures represent the total number of clinical specimens evaluated and do not necessarily correspond to the number of unique individual patients. The types of CRISPR-Cas systems utilized were predominantly Cas12a (six studies)[16,18-20,23,24] and Cas12b (two studies)[21,22]. All studies employed fluorescence-based readouts as the system of result interpretation. No study reported the use of lateral flow strips or other detection formats. These studies focused on integrating CRISPR-Cas12 with various isothermal amplification techniques, including LAMP, MCDA, RPA, CPA, and one-pot sys-

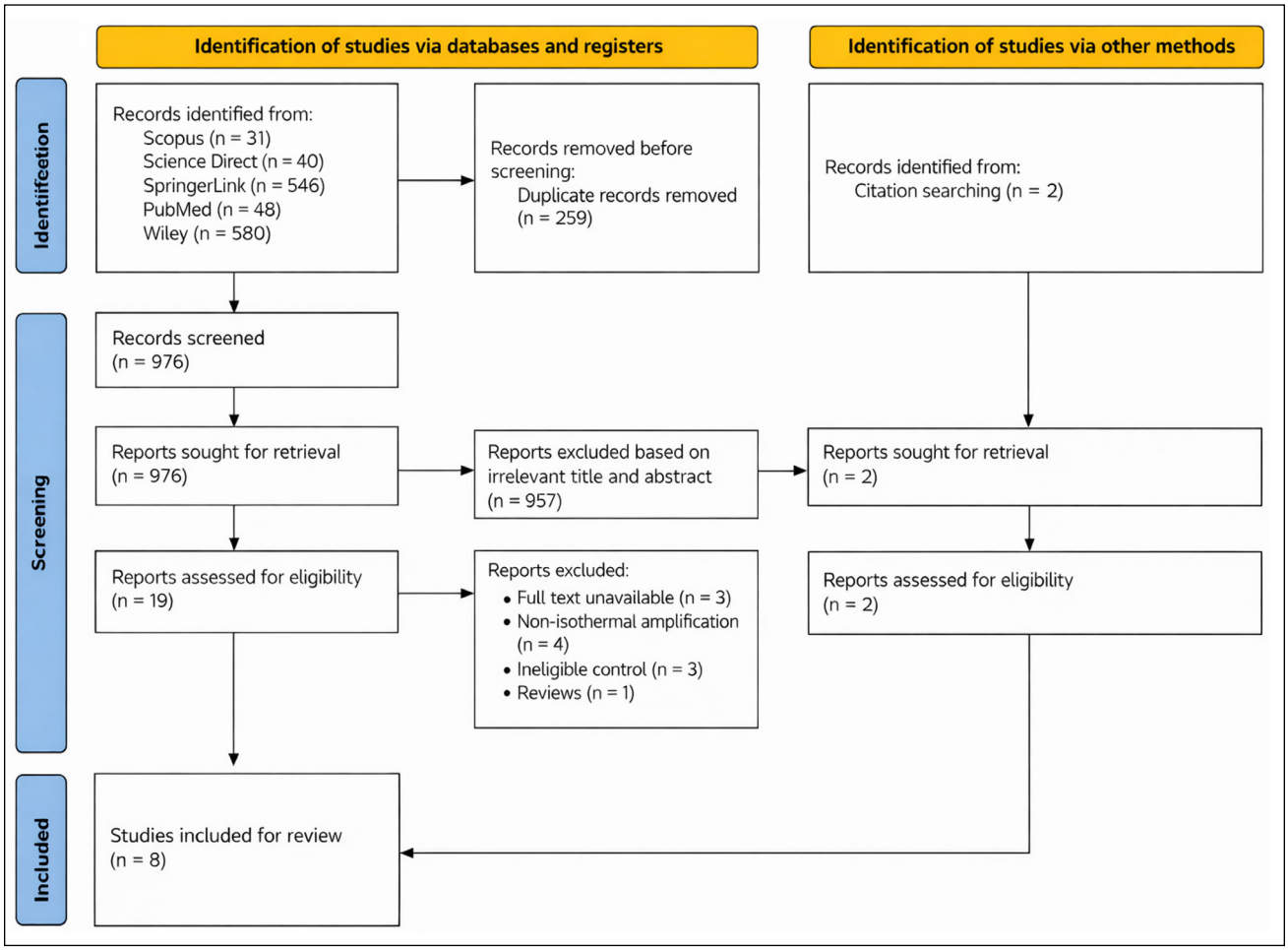

Figure 1. PRISMA diagram.

tems for MTB detection. The characteristics of the studies are summarized in Table 1.

Quality Assessments

Figure 2A shows the individual quality assessment using QUADAS-2, while Figure 2B summarizes the proportion of risk of bias in each domain. Overall, most studies showed a low risk of bias. In domain D1 (patient selection), one study[19] (12.5%) raised some concerns due to the lack of a detailed description of the patient selection system, while seven studies[16,18,20-24] (87.5%) had low risk. In domain D2 (index test), two studies[20,24] (25%) were classified as some concerns, possibly due to unclear implementation of the index test in a blinded manner against the reference standard. The remaining six studies[16,18,19,21-23] (75%) were assessed as having low risk in this domain. For domain D3 (reference standard), one study[19] (12.5%) also raised some concerns, primarily because the diagnostic validity of the reference standard used was not explicitly described, while seven studies[16,18,20-24] (87.5%) had low risk. Meanwhile, in domain D4 (flow and timing), only one study (12.5%) showed some concerns, and the remaining seven studies[16,18-23] (87.5%) had low risk, indicating good consistency in the flow and timing of test implementation. Overall, no study was assessed as high risk. In terms of overall bias assessment, five studies (62.5%) were assessed as having a low overall risk of bias, and three studies (37.5%) were categorized as having some concerns. These results indicate that the majority of studies have high methodological quality and are suitable as a basis for assessing the diagnostic accuracy of CRISPR-Cas12 for detecting MTB.

Mechanism of CRISPR-Cas12 Combined with Isothermal Amplification

Multiple amplification systems have been utilized across recent studies to optimize sensitivity and field applicability. Xu et al[24] utilized RPA targeting IS1081, combined with Cas12a-based detection *via* collateral cleavage of single-stranded DNA (ssDNA) reporters. Xiao et al[16] employed a similar RPA-Cas12a strategy but targeted both IS1081 and IS6110, and further enhanced specificity through graphene oxide-assisted suppression of nonspecific signals. Gan et al[18] integrated enzymatic recombinase amplification (ERA) with both lateral flow and real-time fluorescence systems to detect IS1081. Similarly, Jia et al[19] applied multiple cross displacement amplification (MCDA)

Table 1 . The characteristics of the included studies.

Study (reference)	Sensitivity	Specificity	Limit of detection (LoD)	Sample type (clinical samples)	Reference test	Time detection
Xu et al[24]	99.29% (139/140)	100% (53/53)	4.48 fmol/L	193 sputum samples (140 positive and 53 negatives for MTB)	Mycobacterial culture	4 hours, including 1 hour of MTB DNA extraction, 1 hour for MTB DNA amplification by RPA, and 2 hours for detection of Cas12a/gRNA system
Gan et al[18]	a. Fluorescence 100% (32/32) b. Lateral flow 93.8% (30/32)	a. Fluorescence 100% b. Lateral flow 100%	a. 9 copies/µL b. 90 copies/µL	60 total samples, 32 for fluorescence and 28 for lateral flow	Commercial qPCR detection	50 minutes for fluorescence 60 minutes for lateral flow
Wang et al[23]	79.5% (35/44)	100%	50 fg of genomic DNA (~10 copies) per reaction	44 double-blinded clinical sputum samples	AFB smear microscopy, conventional culture, and Xpert MTB/RIF	1 hour, including rapid template extraction (15 min), LAMP amplification (approximately 40 min), and CRISPR-Cas12a-based detection (within 5 min)
Lin et al[20]	94% (17/18)	100% (8/8)	-	28 BALF samples, 10 samples from healthy donors and 18 MTB-positive samples from TB patients.	Culture, Xpert MTB/RIF, TB-DNA, and AFB smear microscopy	2 hours
Jia et al[19]	100% (77/77)	100% (19/19)	40 fg/reaction (~8 copies)	Sputum microscopy	Sputum smear extraction, 40 min and GeneXpert MTB/RIF	60 minutes (15 min amplification, 5 min detection)
Sam et al[22]	86.8% (59/68)	95.2% (20/21)	1.3 copies/µL	Pulmonary samples (sputum, BALF) and plasma	AFB smear, MTB culture, and GeneXpert MTB/RIF	2 hours
Peng et al[21]	67.2%	96.7%	0.8 copies/µL (H37Rv DNA); 50 CFU/mL	Sputum	Composite reference standard (culture, AFB smear, Xpert, and clinical follow-up)	80 minutes (from extraction to result)
Xiao et al[16]	74.8% (80/107)	100% (40/40)	4 copies/µL	Sputum	Xpert MTB/RIF and Mycobacterial culture	Within 1 hour (<60 min)

BALF: Bronchoalveolar lavage fluid; CFU: Colony Forming Units; MTB: *Mycobacterium tuberculosis*; AFB: Acid-Fast Bacilli; PCR: Polymerase chain reaction; RPA: Recombinase polymerase amplification; Loop-mediated Isothermal Amplification (LAMP); Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas12.

targeting the *sdaA* gene, enabling rapid visual readouts under blue light.

LAMP was employed by Wang et al[23] and Sam et al[22], who developed the loop-mediated isothermal amplification coupled with CRISPR-Cas12a-mediated diagnostic (LACD) and a platform nominated as TB-QUICK, which combines loop-mediated isothermal amplification and CRISPR-Cas12b detection using Cas12a and Cas12b, respectively; both demonstrated high clinical sensitivity within their respective study populations; specifically, the LACD system and TB-QUICK reported sensitivities of 79.5% and 86.8%, which exceeded the performance of GeneXpert observed in those specific cohorts.

To further streamline workflows, Peng et al[21] proposed a contamination-resistant, one-pot closed-tube

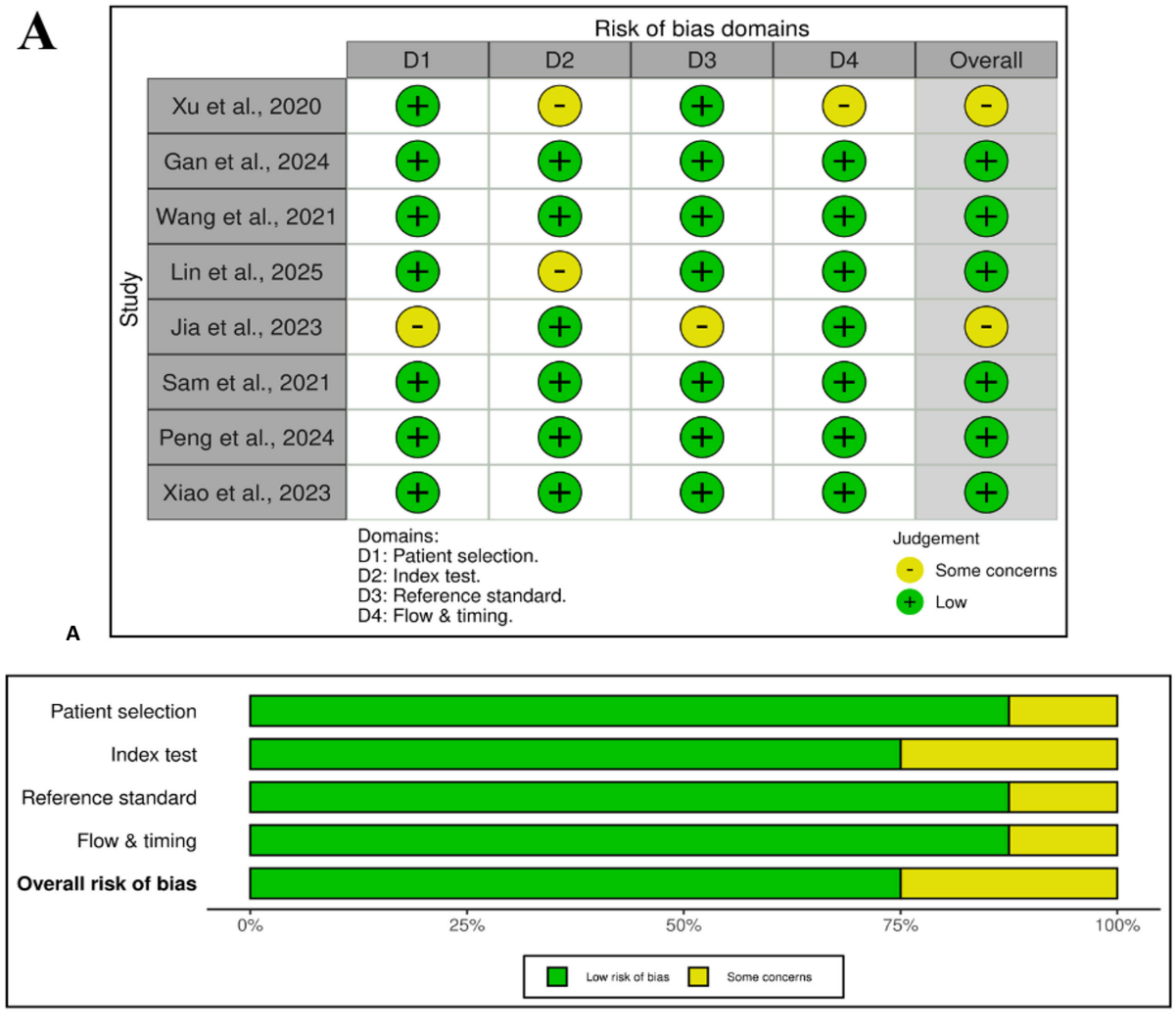

Figure 2. Risk of bias assessment using QUADAS-2. **A**, Risk of bias and applicability concerns graph. **B**, Risk of bias and applicability concern summary.

system combining cross-priming amplification (CPA) with Cas12b. Distinctively, Lin et al[20] introduced an amplification-free intracellular detection strategy by electroporating Cas12a-crRNA complexes into macrophages, achieving MTB identification within 30 minutes.

Diagnostic Accuracy of CRISPR-Cas12 with Isothermal Amplification

The diagnostic performance of CRISPR-Cas12 coupled with isothermal amplification demonstrates high precision; however, its superiority over conventional systems is not uniform. Some studies[18,19,22,23] reported higher sensitivity than GeneXpert, while others[16,21] showed lower or comparable performance, sample type, and bacterial load. For example, while sensitivity in some systems reached 79.5%-86.8%, surpassing Xpert MTB/RIF in those cohorts, studies by Xiao et al[16] and Peng et al[21] documented sensitivities that were either comparable or lower than the Xpert system, reflecting the challenges of molecular detection across different clinical contexts. Across the evaluated studies, specificity was notably robust; Xiao et al[16], Xu et al[24], Gan et al[18], Jia et al[19], Lin et al[20], and Wang et al[23] all reported an absolute specificity of 100%, while Sam et al[22] and Peng et al[21] maintained high specificity levels at 95.2% and 96.7%, respectively. Nevertheless, the diagnostic performance of CRISPR-Cas12 combined with isothermal amplification exhibited variable sensitivity, with reported values reaching 100% in studies by Gan et al[18] and Jia et al[19] and decreasing to 67.2% in the study conducted by Peng et al[21], thereby highlighting the influence of differences in system design and target sequence selection on detection efficacy. Despite this variability, this system demonstrated strong overall sensitivity, with Gan et al[18] and Jia et al[19] achieving 100% detection rates, followed closely by Xu et al[24] (99.29%) and Lin et al[20] (94%). Furthermore, in comparative analyses where the system sensitivity was variable, it consistently outperformed standard diagnostic modalities. For instance, Wang et al[23] reported a sensitivity of 79.5%, which was significantly higher than Xpert

MTB/RIF (61.4%), culture (45.5%), and AFB smear (36.4%). Similarly, Sam et al[22] observed a sensitivity of 86.8%, surpassing both culture (66.7%) and Xpert (70.4%). This superiority was further corroborated by Xiao et al[16] (74.8%) and Peng et al[21] (67.2%), who both documented higher sensitivity rates for CRISPR-Cas12 compared to the Xpert assay (63.6% and 64.2%, respectively) and culture systems, highlighting the molecular systems' efficacy in detecting cases that traditional protocols might miss.

DISCUSSION

Evidence from the eight reviewed studies demonstrates that CRISPR-Cas12-based diagnostics, integrated with isothermal amplification (e.g., LAMP, RPA, MCDA, CPA), detect MTB with a sensitivity range of 67.2% to 100%. Systems such as TB-QUICK, ERA-Cas12a, and MCDA-Cas12a consistently outperformed conventional systems like GeneXpert. For instance, Wang et al[23] reported 96.30% sensitivity and 100% specificity, surpassing GeneXpert within the same cohort. These findings are supported by robust methodological quality, as 62.5% of studies were categorized as low risk in QUADAS-2 assessments.

Cas12a was the dominant effector (6/8 studies)[16,18-20,23,24], primarily paired with low-temperature amplification systems like RPA and ERA. This combination proved highly effective; Gan et al[18] and Jia et al[19] achieved 100% sensitivity using Cas12a with ERA and MCDA, respectively. In contrast, Cas12b is thermostable and suitable for high-temperature systems (e.g., LAMP, CPA). However, the Cas12b system showed greater performance fluctuation, ranging from 86.8% (TB-QUICK) to 67.2% (CPA-based). While Cas12b simplifies the workflow, careful optimization of the reaction buffer is essential to prevent enzyme inhibition.

Contrary to the assumption that multi-targeting guarantees higher sensitivity, optimization appears more critical. Xiao et al[16] employed a dual-target strategy (IS6110 and IS1081) but achieved only 74.8% sensitivity step significantly lower than the single-target system (>99%) reported by Xu et al[24] and Jia et al[19]. This suggests that increasing target complexity without harmonizing primer kinetics may reduce amplification efficiency. Regarding sample types, Lin et al[20] demonstrated that targeting intracellular MTB in BALF *via* macrophage electroporation yielded 94% sensitivity. This indicates that bypassing extraction losses by directly targeting intracellular pathogens may be superior for paucibacillary samples.

The observed sensitivity variations reflect a trade-off between Limit of Detection (LoD) and workflow complexity. "One-pot" systems (e.g., Peng et al[21], 67.2%) reduce contamination risks but often suffer from competitive inhibition between reagents. Conversely, two-step systems (Xu et al[24] and Jia et al[19]) consistently yielded sensitivities >99% by allowing optimal enzymatic conditions for each step, despite higher contamination risks. Notably, reported LoD values did not always correlate linearly with clinical sensitivity. This discrepancy confirms that pre-analytical factors, specifically the difficulty of releasing DNA from the thick MTB cell wall in sputum, remain a primary bottleneck, regardless of the intrinsic sensitivity of the CRISPR system.

Furthermore, the versatility of CRISPR-Cas12a is continuously expanding beyond conventional nucleic acid amplification, with recent studies[25,26] demonstrating its potential in detecting non-nucleic acid targets and utilizing novel platforms such as photoelectrochemical assays.

This review has several limitations warranting caution. First, substantial heterogeneity exists across studies regarding sample sizes (28-293), Cas protein subtypes, and amplification methods (e.g., RPA *vs.* LAMP), complicating direct comparisons. Second, pre-analytical variability in sample collection and processing introduces potential bias. Third, the small sample sizes in most studies increase the risk of overfitting and limit generalizability, further compounded by the geographic predominance of studies from China. Finally, with only eight studies meeting the inclusion criteria out of 976 screened, this field remains in an early developmental stage. Consequently, large-scale, multicenter, and standardized studies are required to validate these findings for clinical and point-of-care implementation.

CONCLUSIONS

This systematic review highlights the promising diagnostic performance of CRISPR-Cas12 integrated with isothermal amplification for MTB detection. The included studies consistently demonstrated high sensitivity and specificity, with several systems showing comparable or higher diagnostic accuracy in some studies[16,21-23]. The integration of various isothermal amplification techniques with Cas12 systems underscores the versatility and adaptability of this molecular approach. While the overall methodological quality of the studies was acceptable, the current evidence is geographically limited and subject to heterogeneity in design and implementation. In conclusion, CRISPR-Cas12 integrated with isothermal amplification demonstrates strong potential for MTB detection, with reported sensitivity and specificity ranging from 67.2% to 100% and from 95.2% to 100%, respectively. Although several systems achieved diagnostic accuracy comparable to or exceeding that of GeneXpert, the observed heterogeneity across studies emphasizes the need for standardized protocols and a harmonized system.

ETHICS APPROVAL AND INFORMED CONSENT:
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The authors have no conflicts of interest to declare.

Availability of Data and Materials:
All data generated or analyzed during this study are included in this published article. Other data may be requested through the corresponding author.

Authors' Contributions:
Conceptualization: BCB, IAS; data curation: BCB, IAS; formal analysis: BCB, IAS; investigation: BCB, IAS; methodology: BCB, IAS; project administration: BCB; software: BCB, IAS; writing – original draft: BCB, IAS; writing – review and editing: BCB, IAS. All authors read and approved the final version of the manuscript.

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