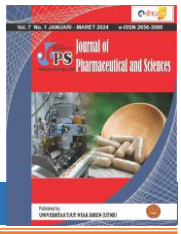
Journal of Pharmaceutical and Sciences
Electronic ISSN: 2656-3088 DOI: https://doi.org/10.36490/journal-jps.com
Homepage: https://journal-jps.com
REVIEW ARTICLE JPS. 2026, 9(1), 423-445



Biotechnological Advances in Tuberculosis Diagnosis: Global Developments and Their Applicability in Indonesia

Kemajuan Bioteknologi dalam Diagnosis Tuberkulosis: Perkembangan Global dan Penerapannya di Indonesia

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Abstract

Tuberculosis (TB) remains a major global health burden, and Indonesia is among the countries with the highest incidence and mortality rates. Early and accurate diagnosis is essential for effective TB control; however, conventional diagnostic methods such as sputum smear microscopy, culture, chest radiography, and the tuberculin skin test continue to face limitations in sensitivity, specificity, turnaround time, and operational feasibility. This narrative review synthesizes global biotechnological developments in TB diagnostics and evaluates their potential applicability within the Indonesian healthcare system. A structured literature search was conducted using PubMed, ScienceDirect, and Google Scholar, applying Population, Intervention, Comparison, and Outcome (PICO) based inclusion and exclusion criteria. Key advances in molecular diagnostics include conventional polymerase chain reaction (PCR), real-time PCR, automated nucleic acid amplification test (NAAT) platforms such as GeneXpert, Xpert Ultra, and Truenat, as well as loop-mediated isothermal amplification (LAMP). Emerging innovations including CRISPR-based assays, biosensor platforms, microfluidic lab-on-chip devices, and nanotechnology-enhanced systems demonstrate improved sensitivity, portability, and testing speed, with potential for point-of-care implementation, although many require further field-based validation. No single diagnostic tool is universally optimal, as suitability depends on infrastructure availability, workforce capacity, and population needs. In Indonesia, persistent challenges include limited laboratory networks, high diagnostic costs, supply-chain constraints, and variability in human resource competence. Strengthening diagnostic systems, expanding decentralized testing, integrating digital health technologies, and supporting local production of diagnostic materials are critical to enable sustainable adoption and accelerate progress toward national TB elimination targets.

***Keywords*: Tuberculosis Diagnostics, Molecular Detection Technologies, CRISPR-based Assays, Biosensor Platforms, Point-of-Care Innovations.**

Abstrak

Tuberkulosis (TB) masih menjadi salah satu beban kesehatan global utama, dan Indonesia termasuk di antara negara dengan tingkat insidensi dan mortalitas tertinggi. Diagnosis yang dini dan akurat merupakan komponen kunci dalam pengendalian TB; namun, metode diagnostik konvensional seperti mikroskopi hapusan dahak, kultur, radiografi dada, dan uji tuberkulin masih memiliki keterbatasan dalam sensitivitas, spesifisitas, waktu penyelesaian pemeriksaan, serta kelayakan operasional. Tinjauan naratif ini bertujuan untuk merangkum perkembangan bioteknologi terkini dalam diagnosis TB secara global serta mengevaluasi potensi penerapannya dalam sistem pelayanan kesehatan di Indonesia. Pencarian literatur terstruktur dilakukan menggunakan basis data *PubMed*, *ScienceDirect*, dan *Google Scholar* dengan menerapkan kriteria inklusi dan eksklusi berbasis *Population*, *Intervention*, *Comparison*, *and Outcome* (PICO). Perkembangan penting dalam diagnostik molekuler meliputi *polymerase chain reaction* (PCR) konvensional, *real-time* PCR, platform *nucleic acid amplification test* (NAAT) otomatis seperti *GeneXpert*, *Xpert Ultra*, dan *Truenat*, serta teknik

amplifikasi isotermal *loop-mediated isothermal amplification* (LAMP). Selain itu, inovasi yang sedang berkembang, termasuk diagnostik berbasis CRISPR, biosensor, perangkat mikrofluida *lab-on-chip*, dan sistem berbasis nanoteknologi, menunjukkan peningkatan sensitivitas, portabilitas, dan kecepatan pemeriksaan, dengan potensi penerapan sebagai diagnosis *point-of-care*, meskipun sebagian besar masih memerlukan validasi lapangan lebih lanjut. Tidak terdapat satu metode diagnostik yang bersifat universal, karena kesesuaiannya dipengaruhi oleh ketersediaan infrastruktur, kapasitas tenaga kesehatan, dan kebutuhan populasi. Di Indonesia, tantangan yang masih dihadapi mencakup keterbatasan jaringan laboratorium, tingginya biaya diagnostik, kendala rantai pasok, serta variasi kompetensi sumber daya manusia. Oleh karena itu, penguatan sistem diagnostik dan perluasan layanan pengujian terdesentralisasi menjadi langkah strategis untuk mendukung pencapaian target eliminasi TB nasional.

Kata Kunci: Diagnostik Tuberkulosis, Teknologi Deteksi Molekuler, Uji Berbasis CRISPR, Platform Biosensor, Inovasi Point-of-Care.

https://doi.org/10.36490/journal-jps.com.v9i1.1309

Article History:
Received: 07/11/2025,
Revised: 06/01/2026,
Accepted: 06/01/2026,
Available Online: 17/02/2026.

QR access this Article

Introduction

Tuberculosis (TB) remains a major global health threat despite decades of public health interventions and continuous advancements in diagnostic and therapeutic strategies. According to the World Health Organization (WHO) Global Tuberculosis Report, an estimated 10.8 million people developed TB in 2023, with 1.3 million deaths, reaffirming TB as the leading cause of death from a single infectious pathogen. The global burden is heavily concentrated in high-incidence countries, with Indonesia ranking second worldwide, reporting approximately 1.1 million new TB cases annually. These numbers highlight persistent gaps in early detection, treatment coverage, and transmission control [1].

One of the most significant barriers to TB elimination is the challenge of accurate and timely diagnosis [2]. Conventional diagnostic methods such as sputum smear microscopy, culture, chest radiography, and the tuberculin skin test continue to be widely used but suffer from major limitations [3]. Smear microscopy lacks sensitivity, particularly in children, HIV-positive individuals, and cases with low bacillary loads [4]. Culture, while considered the gold standard, requires weeks to yield results [5]. Radiography lacks specificity, and the tuberculin skin test is highly vulnerable to false-positive and false-negative outcomes [6]. These diagnostic gaps contribute to delayed treatment initiation, undetected infections, ongoing community transmission, and the rise of multidrug-resistant tuberculosis (MDR-TB) [7].

The limitations of established diagnostic methods underscore the urgent need for innovative technologies capable of providing rapid, accurate, and accessible TB detection [8]. Recent biotechnological advances have introduced powerful molecular and point-of-care platforms, including polymerase chain reaction (PCR), real-time PCR, automated nucleic acid amplification test (NAAT) systems such as GeneXpert MTB/RIF, Xpert Ultra, and Truenat, as well as isothermal amplification methods like loop-mediated isothermal amplification (LAMP) [9]. Emerging innovations including clustered regularly interspaced short palindromic repeats (CRISPR)-based detection systems, biosensor technologies, microfluidic lab-on-chip devices, and nanotechnology-enhanced assays offer unprecedented improvements in sensitivity, specificity, portability, and turnaround time [10]. These technologies hold particular promise for high-burden, resource-limited settings like Indonesia, where decentralized diagnostic access is essential.

Given the rapid pace of technological development, a comprehensive understanding of how these diagnostic platforms compare and how feasible they are for implementation in Indonesia is critically important [2]. This narrative review synthesizes global advancements in TB diagnostic biotechnology, evaluates performance across conventional, molecular, and emerging platforms, and assesses their potential integration within the Indonesian healthcare system. By identifying opportunities, limitations, and contextual considerations, this review aims to support strategic decision-making toward accelerating TB detection and advancing national elimination goals [11].

Unlike previous reviews that primarily emphasize the technical performance of individual diagnostic methods, this review presents a structured comparative analysis across diagnostic platforms and provides context-specific implementation considerations aligned with the hierarchical structure and resource variability of the Indonesian healthcare system.

Methods

Narrative Review Methodology

This narrative review aims to synthesize scientific evidence on global biotechnological advances in tuberculosis (TB) diagnostics and evaluate their potential applicability within the Indonesian healthcare context. The narrative review method was selected due to its flexibility in integrating diverse scientific sources, enabling both a comprehensive global assessment and a country-specific contextual analysis.

Literature Search Strategy

A structured literature search was conducted using three major scientific databases: PubMed, ScienceDirect, and Google Scholar. The search strategy employed combinations of the following keywords linked with Boolean operators: "tuberculosis diagnosis", "TB biotechnology", "molecular diagnostics", "CRISPR-based TB detection", "tuberculosis biosensor", "microfluidic TB testing", and "point of care TB diagnostics". The search was limited to articles published within the last 10 years to ensure the inclusion of recent technologies and research developments. Additionally, backward citation screening was performed to identify other relevant publications that might not have been captured in the initial search.

Inclusion and Exclusion Criteria

To ensure methodological rigor and maintain a focused scope, the inclusion and exclusion criteria for this narrative review were organized using the Population, Intervention, Comparison, and Outcome (PICO) framework. This structured approach helps define the target population, characterize the diagnostic interventions under evaluation, determine relevant comparators, and clarify the outcomes used to assess diagnostic performance. Applying the PICO model enhances transparency in study selection and strengthens the overall reliability of the review. A detailed summary of the PICO-based inclusion and exclusion criteria is presented in Table 1.

Table 1. PICO Framework for Inclusion and Exclusion Criteria.

Component	Inclusion Criteria	Exclusion Criteria
P (*Population/Problem*)	Studies involving individuals with suspected or confirmed *Mycobacterium tuberculosis* infection, and research evaluating tuberculosis (TB) diagnostic tools.	Studies unrelated to tuberculosis (TB) diagnosis or not involving infectious disease diagnostics.
I (*Intervention*)	Diagnostic methods designed to detect *Mycobacterium tuberculosis*, including conventional, molecular, and emerging biotechnological platforms.	Diagnostic tools not intended for tuberculosis (TB) detection or incapable of identifying *Mycobacterium tuberculosis*.
C (*Comparison*)	Studies comparing performance across diagnostic methods.	Studies lacking comparative assessment or failing to provide sufficient methodological detail.
O (*Outcome*)	Measures of diagnostic accuracy including sensitivity, specificity, turnaround time,	Outcomes unrelated to diagnostic performance such as treatment outcomes,

	feasibility, and applicability in low-resource settings.	epidemiological modelling, or vaccine effectiveness.
S (*Study Design*)	Peer-reviewed articles published in English within the last ten years, including original research and review articles.	Editorials, opinion pieces, non-scientific publications, and studies lacking adequate methodological description.

Study Selection Process

All records retrieved from PubMed, ScienceDirect, and Google Scholar were exported to Mendeley for reference management and deduplication. A total of 612 records were identified through database searching, and 38 additional records were identified through backward citation screening. After removing 176 duplicates, 474 records remained for title and abstract screening. During this stage, 356 records were excluded for not meeting the PICO-based eligibility criteria. The full texts of 118 articles were assessed for eligibility, and 48 articles were excluded with reasons, including not focused on TB diagnostics (n = 18), insufficient methodological detail (n = 12), outside the publication window (n = 7), non-English (n = 5), and outcomes not aligned with diagnostic performance (n = 6). Finally, 70 articles were included in the narrative synthesis. Title/abstract screening and full-text eligibility assessment were conducted by two reviewers independently; any disagreements were resolved through discussion until consensus was reached. In total, 98 references were cited; 70 articles formed the evidence base for thematic synthesis, while the remaining 28 references were used as background context (e.g., guidelines or reports).

Review and Analysis Process

A thematic narrative synthesis was performed to organize and interpret the included literature. For each included article, key information was extracted using a standardized matrix, including technology/platform type, sample type and target/biomarker, reported performance indicators (e.g., sensitivity/specificity or comparable measures), turnaround time, and operational requirements (equipment, infrastructure, and training). Key information from each included article was reviewed qualitatively and classified based on shared technological principles, intended use settings, and implementation considerations. Theme development followed a hybrid approach: a deductive structure aligned with the review scope (conventional diagnostics, molecular diagnostics, and emerging biotechnological innovations), followed by inductive refinement based on recurring concepts identified during full-text review (e.g., CRISPR-Cas systems, biosensor modalities, microfluidic integration, and nanomaterial-enhanced assays). Initial codes were used to group studies into themes and subthemes, and the thematic structure was iteratively refined through author discussion until consensus was reached. The final synthesis was organized into the following thematic groups:

1. Conventional Diagnostic Methods: sputum smear microscopy, *Mycobacterium tuberculosis* culture, chest radiography, and the tuberculin skin test (TST).
2. Molecular Diagnostic Technologies: conventional PCR, real-time PCR (qPCR), NAAT platforms (GeneXpert, Xpert Ultra, Truenat), and loop-mediated isothermal amplification (LAMP).
3. Emerging Biotechnological Innovations: CRISPR-Cas12/13-based TB diagnosis, biosensor-based TB detection, microfluidic/lab-on-chip technology, and nanotechnology-enhanced TB assays.

Justification for Methodology

The narrative review method was chosen for its ability to synthesize information from diverse scientific sources, providing a comprehensive overview of advancements in TB diagnostics and identifying knowledge gaps and areas requiring further research. This approach supports the identification of opportunities and challenges in implementing new diagnostic technologies in developing countries like Indonesia while considering local contextual factors.

Discussion

Limitations of Conventional Diagnostic Methods

Conventional diagnostic methods for tuberculosis (TB), including sputum smear microscopy, *Mycobacterium tuberculosis* culture, chest radiography, and the tuberculin skin test (TST), have long been the foundation of TB diagnosis, particularly in settings with limited laboratory infrastructure [3]. Although these

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

methods are inexpensive, widely available, and operationally straightforward, their diagnostic performance remains insufficient for the timely and accurate identification of active TB cases, which is critical for effective treatment and control of the disease [12].

Sputum Smear Microscopy

Sputum smear microscopy is a longstanding diagnostic technique used to detect acid-fast bacilli (AFB) in sputum specimens obtained from the lower respiratory tract. Visualization relies on Ziehl-Neelsen or auramine staining, both of which highlight the lipid-rich cell walls characteristic of *Mycobacterium tuberculosis* [13]. Auramine-based fluorescent staining generally offers higher sensitivity than the Ziehl-Neelsen method; however, the diagnostic yield of both techniques is strongly dependent on the bacillary load within the specimen [14]. As a result, patients with paucibacillary disease, children, and individuals living with HIV frequently produce false-negative results because the bacillary concentration does not reach the microscopic detection threshold [13]. In HIV-associated tuberculosis, atypical clinical manifestations and impaired immune responses further reduce smear positivity rates, making it less reliable in these patient groups [15]. Although auramine staining may enhance detection, its usefulness is limited by fluorescence artifacts, the need for specialized fluorescence microscopy, and lower specificity when sample quality is inadequate [16]. These inherent limitations prevent smear microscopy from reliably detecting early active TB, leading to delays in treatment initiation and contributing to ongoing transmission within the community [3], [13]. Despite its limited sensitivity, sputum smear microscopy continues to play a role in many high-burden settings due to its low cost, minimal infrastructure requirements, and ease of implementation. However, reliance on smear microscopy alone is increasingly recognized as insufficient for achieving early case detection targets, particularly in countries such as Indonesia where a substantial proportion of TB cases remain undiagnosed or are detected at advanced stages of disease [17].

***Mycobacterium tuberculosis* Culture**

Culture-based diagnosis offers markedly higher sensitivity than sputum smear microscopy because it directly detects viable *Mycobacterium tuberculosis* growing on solid or liquid media [18]. Solid culture systems such as Löwenstein-Jensen allow the observation of characteristic colony morphology, while liquid culture platforms, including automated systems, enhance detection by continuously monitoring metabolic activity [13]. Despite these advantages, culture methods are constrained by the inherently slow growth rate of *Mycobacterium tuberculosis*, with visible colonies typically requiring two to eight weeks of incubation [19]. This extended turnaround time limits the usefulness of culture in urgent clinical decision-making and delays the confirmation of infectious cases. Consequently, reliance on culture alone can hinder the timely initiation of therapy and diminish the effectiveness of public health interventions aimed at rapid case detection and interruption of transmission [20]. Although culture remains the diagnostic gold standard and is essential for drug-susceptibility testing, its prolonged processing time, high biosafety requirements, and need for specialized laboratory infrastructure limit its suitability as a frontline diagnostic tool. In high-burden countries such as Indonesia, culture-based diagnosis is therefore more appropriately positioned as a confirmatory and reference method rather than a primary screening tool for early TB detection [21].

Chest Radiography

Chest radiography is widely used as a supportive diagnostic tool for pulmonary tuberculosis because it is rapid, accessible, and capable of providing immediate visualization of lung abnormalities. However, its diagnostic value is limited by poor specificity, as radiographic findings such as infiltrates, nodules, fibrotic streaks, cavitary lesions, and pleural effusions often resemble abnormalities caused by bacterial or fungal infections, malignancies, or chronic pulmonary diseases, making it unreliable for distinguishing active tuberculosis from other conditions [22]. Interpretation of chest radiographs is highly dependent on the experience of the radiologist, resulting in significant variability in accuracy. This can lead to both overdiagnosis and missed diagnoses [23]. Additionally, chest radiography cannot differentiate active disease from residual post-treatment changes, as sequelae of previous tuberculosis may mimic radiologic patterns of active infection [24]. Due to these limitations, chest radiography functions primarily as a screening or supportive modality. It must be complemented by microbiological or molecular diagnostic tests to confirm active tuberculosis. In high-burden settings such as Indonesia, chest radiography remains valuable for triaging

suspected cases and assessing disease severity, but it should not be relied upon as a standalone diagnostic tool due to its limited specificity and operator-dependent interpretation [22].

Tuberculin Skin Test (TST)

The tuberculin skin test (TST) evaluates delayed-type hypersensitivity to purified protein derivative (PPD). However, its diagnostic performance is highly inconsistent [6]. In regions with widespread Bacillus Calmette–Guérin (BCG) vaccination, such as Indonesia, prior immunization often results in false-positive results due to antigenic cross-reactivity between BCG strains and PPD [25]. Environmental exposure to non-tuberculous mycobacteria further compromises the test's specificity, rendering it unreliable for distinguishing between true *Mycobacterium tuberculosis* infection and other mycobacterial infections. In contrast, individuals with impaired cell-mediated immunity including people living with HIV, malnourished patients, children, and the elderly frequently show false-negative results despite active or latent infection [26]. Technical factors, such as improper intradermal administration and variability in measuring induration among different examiners, also introduce inconsistencies. Collectively, these limitations significantly reduce the diagnostic value of TST, restricting its role to a supportive tool rather than a confirmatory method for TB diagnosis, particularly in endemic regions where high diagnostic accuracy is essential. Consequently, in high-burden countries such as Indonesia, TST is more appropriately used for latent TB screening in specific populations and should not be relied upon as a standalone test for diagnosing active tuberculosis [27].

Taken together, the limitations of smear microscopy, culture, chest radiography, and the tuberculin skin test highlight that conventional methods fail to provide the speed and sensitivity needed for effective TB control. These constraints emphasize the need for more advanced diagnostic technologies that enable earlier case detection and reduce ongoing transmission [3].

Advances in Molecular Diagnostic Technologies

Advances in molecular diagnostic technologies have significantly enhanced the speed and accuracy of tuberculosis (TB) detection. Unlike conventional methods that depend on microscopic visualization or the inherently slow growth of mycobacteria, nucleic acid amplification techniques enable the direct identification of Mycobacterium tuberculosis DNA from clinical specimens. This approach substantially reduces diagnostic turnaround time and improves sensitivity, particularly in cases with low bacillary loads [21]. Technologies such as conventional polymerase chain reaction (PCR), real-time PCR, automated nucleic acid amplification test (NAAT) platforms, isothermal amplification methods, and molecular drug-resistance assays have become essential components of modern TB diagnostics and hold strong potential for broader adoption in healthcare systems within high-burden countries, including Indonesia. However, despite their superior analytical performance, the implementation of molecular diagnostic technologies remains uneven, as their adoption is often constrained by higher costs, dependence on laboratory infrastructure, and the need for trained personnel, particularly in decentralized and resource-limited healthcare settings [28].

Conventional PCR

Conventional PCR is a foundational nucleic acid amplification method that detects *Mycobacterium tuberculosis* by selectively amplifying species-specific DNA sequences through repeated cycles of denaturation, annealing, and extension [29]. Compared with conventional diagnostic techniques such as sputum smear microscopy, PCR offers substantially higher sensitivity and specificity, as it can identify very small amounts of mycobacterial DNA. This makes PCR particularly valuable for diagnosing paucibacillary TB presentations, including those occurring in children, individuals living with HIV, and patients with extrapulmonary disease [30]. However, PCR accuracy is highly dependent on sample quality, efficiency of DNA extraction, and strict contamination control. Inadequate laboratory handling may lead to failed amplification or false-positive results. In addition, the requirement for specialized equipment, controlled laboratory environments, and trained personnel limits the routine use of conventional PCR in decentralized and primary healthcare settings. Despite these operational challenges, conventional PCR remains a cornerstone of molecular TB diagnostics, owing to its rapid processing time and reliable ability to directly detect *Mycobacterium tuberculosis* genetic material in a wide range of clinical specimens [31].

Real-Time PCR (quantitative PCR, qPCR)

Real-time PCR (qPCR) is an advanced version of conventional PCR that enables the simultaneous detection and quantification of *Mycobacterium tuberculosis* DNA using fluorescent probes that monitor

Electronic ISSN : 2656-3088
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amplification in real time during each thermal cycle [32]. This technique provides higher sensitivity than conventional PCR due to its ability to differentiate specific amplification signals from nonspecific reactions, thereby enhancing diagnostic accuracy, especially in specimens with low bacillary loads [33]. Its faster turnaround time, quantitative measurement capability, and reduced contamination risk further increase its clinical value. However, qPCR requires more sophisticated instrumentation, rigorous quality control procedures, and personnel with specialized training. In addition, the dependence on stable laboratory infrastructure and higher operational costs may limit the routine implementation of qPCR in decentralized healthcare settings and primary-level facilities. Despite these operational requirements, qPCR remains one of the most reliable and precise molecular methods for the rapid detection of *Mycobacterium tuberculosis* across diverse clinical environments [21].

NAAT Platforms (GeneXpert, Ultra, Truenat)

Automated nucleic acid amplification test (NAAT) platforms such as GeneXpert Mycobacterium tuberculosis/rifampicin (MTB/RIF), Xpert Ultra, and Truenat have fundamentally transformed TB diagnostics by delivering rapid, sensitive, and standardized results. These cartridge-based systems integrate DNA extraction, amplification, and detection into a fully closed and automated workflow, thereby minimizing contamination risk and reducing the dependency on highly skilled laboratory personnel [34]. GeneXpert MTB/RIF enables the simultaneous detection of *Mycobacterium tuberculosis* and rifampicin resistance within approximately two hours, while Xpert Ultra offers substantially improved sensitivity, particularly in paucibacillary cases, due to its multi-copy genetic targets [35]. Truenat, a portable NAAT platform capable of operating on battery power, further expands diagnostic access by enabling reliable testing in remote or resource-limited settings with minimal infrastructure requirements [36]. However, despite their operational advantages, the widespread implementation of NAAT platforms remains constrained by high cartridge costs, instrument maintenance requirements, and dependence on stable supply chains, which may limit sustainability and scalability in decentralized healthcare settings. Nevertheless, NAAT platforms have become essential tools for accelerating case detection, enabling earlier treatment initiation, and strengthening TB control strategies, especially in high-burden countries like Indonesia [37].

LAMP (Loop-Mediated Isothermal Amplification)

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification technique that enables the rapid detection of *Mycobacterium tuberculosis* by amplifying target DNA at a constant temperature using four to six specially designed primers, resulting in high amplification efficiency without the need for thermal cycling [38]. Compared with conventional PCR, LAMP offers a significantly faster turnaround time, a more simplified workflow, and greater tolerance to sample-derived inhibitors, making it particularly suitable for deployment in resource-limited settings. LAMP assays can produce detectable amplification signals within 30–60 minutes, which can be visualized through turbidity, fluorescence, or colorimetric changes, facilitating integration into point-of-care diagnostic platforms [39]. However, maintaining analytical specificity requires meticulous primer design, and the risk of carryover contamination remains a concern when laboratory workflow is not strictly controlled. In addition, variability in assay standardization and limited large-scale field validation may affect the consistency of LAMP performance across different healthcare settings. Despite these challenges, the speed, operational simplicity, and minimal equipment requirements of LAMP highlight its strong potential as a scalable and accessible diagnostic tool for expanding TB testing capacity, especially in high-burden countries seeking low-complexity molecular solutions [40].

Taken together, advancements in molecular diagnostic technologies including PCR-based methods, automated NAAT platforms, and isothermal amplification techniques have collectively improved the speed, sensitivity, and reliability of TB detection. Nevertheless, differences in cost, infrastructure requirements, and technical complexity continue to influence their feasibility and scale of implementation across healthcare systems. These molecular tools significantly strengthen modern TB diagnostic strategies and support broader adoption in high-burden settings [41].

Emerging Biotechnological Innovations

Emerging biotechnological innovations are reshaping the landscape of tuberculosis diagnostics by introducing highly sensitive and potentially portable detection platforms that aim to overcome the limitations

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

of conventional microscopy and centralized molecular testing. These technologies leverage advances in genome-editing systems, biosensor engineering, nanotechnology, and microfluidic integration to shorten diagnostic turnaround time and reduce dependence on complex laboratory infrastructure. While these approaches align with global priorities for early and decentralized TB detection, particularly in high-burden and resource-limited settings, most remain in early validation stages. Consequently, their technical robustness, clinical reliability, and feasibility for real-world implementation require careful and critical evaluation [42].

CRISPR-Based TB Diagnostics

Clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostic assays represent one of the most promising emerging innovations in TB detection because they offer exceptional sensitivity, rapid turnaround time, and molecular precision that outperform many existing diagnostic approaches [43]. These assays generally incorporate an isothermal amplification step such as recombinase polymerase amplification or loop-mediated isothermal amplification (LAMP) followed by activation of CRISPR-associated (Cas)12 or Cas13 enzymes, which produce a fluorescent or colorimetric signal upon recognizing *Mycobacterium tuberculosis*–specific DNA or RNA sequences [44]. This mechanism supports ultrasensitive detection, including the identification of single molecules and specific drug-resistance mutations, while requiring equipment that is simpler and more portable than conventional PCR platforms. CRISPR-based tests can deliver results in under one hour, operate at low constant temperatures, and be adapted to paper-based, lateral-flow, or microfluidic formats, making them theoretically suitable for decentralized point-of-care (POC) use [45].

Despite their considerable diagnostic potential, CRISPR-based TB assays face several unresolved technical and implementation challenges that limit their immediate translation into routine clinical use [46]. Most CRISPR-based platforms still rely on a pre-amplification step, such as recombinase polymerase amplification or LAMP, which increases assay complexity and raises the risk of cross-contamination, particularly in low-resource settings where strict workflow separation is difficult to maintain. In addition, although off-target effects are more commonly discussed in genome-editing applications, nonspecific collateral cleavage activity in CRISPR-based diagnostics may contribute to false-positive signals if assay design, guide RNA specificity, and reaction conditions are not rigorously optimized [47].

Further limitations relate to the lack of protocol standardization and insufficient real-world validation. Many CRISPR-based TB diagnostics remain at the proof-of-concept or early clinical evaluation stage, with few large-scale or multicenter studies conducted under true field conditions [46]. Reagent stability represents a major barrier, as CRISPR enzymes and associated reporters often require cold-chain storage, which can be difficult to maintain in tropical climates such as Indonesia. Moreover, although CRISPR-based diagnostics are frequently described as low-cost technologies, the initial costs associated with assay development, regulatory approval, and manufacturing scale-up remain substantial. Importantly, most reported CRISPR-based TB assays have been evaluated in laboratory or semi-controlled environments, and evidence supporting their performance in genuine point-of-care settings such as primary health centers without trained laboratory personnel remains limited [48]. This highlights a critical gap between laboratory feasibility and real-world implementation.

Biosensor-Based TB Detection

Biosensor-based diagnostic systems represent a rapidly evolving class of tuberculosis (TB) detection tools that combine biological recognition elements with physicochemical transducers to generate measurable signals upon target binding. Based on the type of transducer employed, TB biosensors can be broadly classified into electrochemical, optical, and piezoelectric platforms, each offering distinct analytical advantages and operational limitations [49].

Electrochemical biosensors detect changes in current, voltage, or impedance following biomolecular interactions and are among the most widely explored platforms for TB diagnosis due to their high sensitivity, low power requirements, and compatibility with miniaturized electronics. These systems have been developed to detect TB-specific biomarkers including ESAT-6 and CFP-10 proteins in sputum, as well as the lipoarabinomannan (LAM) antigen in urine, which is particularly relevant for HIV-associated TB. However, electrochemical signals may be susceptible to interference from complex biological matrices, necessitating careful surface functionalization and sample preparation to maintain specificity and signal stability [50].

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Optical biosensors, including fluorescence-based, surface plasmon resonance (SPR), and surface-enhanced Raman scattering (SERS) platforms, offer high analytical sensitivity and real-time detection capabilities. These systems have demonstrated strong performance in detecting ESAT-6 and CFP-10 antigens, as well as the LAM antigen through immunoassay-based optical readouts. Nevertheless, their reliance on optical components, stable light sources, and precise alignment increases system complexity, which may limit their suitability for low-resource point-of-care environments [51].

Piezoelectric biosensors measure mass changes on a sensor surface through frequency shifts, enabling label-free detection with high specificity. Quartz crystal microbalance and surface acoustic wave–based biosensors have been explored for detecting ESAT-6, CFP-10, and LAM antigens using antibody- or aptamer-functionalized surfaces. However, these systems are highly sensitive to environmental fluctuations such as temperature and vibration, constraining their applicability outside controlled laboratory conditions [52].

Rather than generically "enhancing sensitivity," nanotechnology modulates biosensor performance through shape- and structure-dependent physicochemical effects [53]. For instance, gold nanorods provide anisotropic geometries with tunable longitudinal plasmon resonances, which can improve signal-to-background ratios and facilitate directional electron transfer in electrochemical or SPR-based assays [54]. In contrast, gold nanostars, characterized by sharp branched tips, generate intense localized electromagnetic "hot spots," significantly amplifying optical signals in SERS- or fluorescence-based detection formats. These shape-dependent differences directly influence assay performance parameters such as detection limits, signal reproducibility, and dynamic range, but may also introduce challenges related to synthesis reproducibility and long-term stability [53].

Despite promising analytical performance, most biosensor-based TB diagnostics remain at the proof-of-concept or early validation stage. Many platforms described as point-of-care systems have primarily been evaluated under laboratory or semi-controlled conditions and still require trained operators, multistep sample preparation, or auxiliary equipment. As a result, these devices function more accurately as lab-on-a-chip systems rather than true point-of-care tools suitable for primary healthcare facilities such as community health centers without laboratory expertise [54]. Additional challenges include limited standardization across biosensor designs, signal instability in sputum-based samples, uncertainty in long-term durability, and scalability of manufacturing processes [55], [56]. Consequently, further system-level optimization and large-scale clinical validation are required before biosensor technologies can be reliably implemented in routine TB control programs [57].

Microfluidic / Lab-on-Chip TB Testing

Microfluidic and lab-on-chip technologies are reshaping TB diagnostics by integrating multiple laboratory procedures into compact, semi-automated platforms capable of processing microliter-scale samples with high analytical precision. These systems typically incorporate microchannels, valves, reaction chambers, and embedded sensors to enable sample lysis, nucleic acid extraction, isothermal amplification, and on-chip detection within workflows that can be designed as closed and contamination-controlled, thereby reducing reliance on extensive laboratory infrastructure [58]. By leveraging controlled microscale fluid dynamics through pressure-driven pumping, centrifugal actuation, or capillary-driven flow microfluidic TB assays can shorten turnaround time (often <1 hour, depending on assay design) while minimizing reagent consumption and reducing, though not eliminating, operator involvement. Innovations such as droplet microfluidics, paper-based analytical devices, and disposable cartridge architectures have further supported the development of portable formats intended for decentralized and resource-constrained settings. When paired with optical, electrochemical, or fluorescence-based readouts, these devices can detect *Mycobacterium tuberculosis* DNA, antigen targets, and drug-resistance markers through integrated workflows [59].

Despite their technical sophistication, most microfluidic and lab-on-chip TB diagnostic platforms have been evaluated predominantly in controlled laboratory environments or limited pilot studies, with relatively few large-scale and multicenter evaluations conducted under true field conditions [60]. Many systems described as point-of-care (POC) tools still require multiple manual steps (e.g., specimen loading, reagent addition, cartridge sealing), precise fluid handling, external power sources, or dedicated readers, limiting feasibility in primary healthcare facilities such as community health centers without laboratory specialists. In practice, a substantial portion of these platforms function more accurately as "lab-on-a-chip" systems rather than genuine POC tests, as real-world performance is influenced by operational constraints often minimized

in laboratory studies, including sputum viscosity and debris, risks of channel clogging or leakage, ambient temperature and humidity, and biosafety and waste-management requirements [61].

Additional barriers include challenges in scalable manufacturing and quality control (e.g., maintaining microfabrication tolerances, reliable bonding and sealing, and device-to-device reproducibility), cartridge standardization, and cost-effective mass production without compromising analytical performance [62]. Furthermore, comprehensive clinical validation in real-world healthcare environments remains limited, creating a persistent gap between laboratory performance and routine clinical deployment. Consequently, while microfluidic and lab-on-chip technologies hold strong promise for future TB diagnostics, their translation into truly point-of-care solutions suitable for widespread implementation will require further engineering optimization, operational simplification, and rigorous field-based validation in representative care settings [60], [62].

Nanotechnology-Enhanced TB Assays

Nanotechnology-based diagnostic approaches have been explored to improve tuberculosis (TB) detection by incorporating engineered nanomaterials as functional components of the assay rather than as generic "sensitivity boosters." Nanomaterials such as gold nanoparticles, quantum dots, graphene derivatives, and magnetic nanobeads can enhance assay performance through defined mechanisms, including increased probe loading capacity, improved electron-transfer kinetics, target preconcentration, and shape-dependent optical signal enhancement. These materials are commonly integrated into optical, electrochemical, and colorimetric sensing platforms to detect *Mycobacterium tuberculosis* DNA/RNA targets, antigenic biomarkers, or cell-wall components, and they have been incorporated into lateral-flow formats, portable biosensors, and microfluidic cartridges to support compact diagnostic designs intended for decentralized testing [63]. In addition, functionalized nanoparticles can be configured to detect specific genetic mutations linked to drug resistance, enabling more rapid screening for multidrug-resistant tuberculosis (MDR-TB) and supporting timely treatment decisions [57].

A key advantage of nanotechnology lies in structure–function tuning that directly affects assay readout. For example, gold nanorods exhibit anisotropic geometries with tunable longitudinal plasmon resonances, which can improve signal-to-background ratios in surface plasmon resonance (SPR) and enhance electron-transfer–related signal generation in electrochemical configurations by providing high-aspect-ratio conductive interfaces [64]. In contrast, gold nanostars, characterized by sharp branched tips, generate intense localized electromagnetic "hot spots" that can yield substantially stronger signal enhancement in surface-enhanced Raman scattering (SERS) or fluorescence-coupled detection compared with smoother nanostructures, thereby lowering practical detection thresholds in optical assays. These shape-dependent effects can expand dynamic range and improve analytical detectability, but they may also influence assay reproducibility because nanostar synthesis and tip morphology are often more variable than rod-based formulations [65].

Despite promising analytical performance, most nanotechnology-enhanced TB diagnostics remain evaluated primarily under controlled laboratory conditions, and evidence supporting performance in real-world point-of-care (POC) environments is still limited [66]. Practical deployment is constrained by manufacturing and standardization challenges, including scalability of nanomaterial production, batch-to-batch variability (e.g., size/shape distributions and surface functionalization density), and long-term material stability. Environmental sensitivity such as performance drift with temperature and humidity may be particularly relevant in tropical regions and can affect both nanoparticle integrity and assay signal consistency [67].

Importantly, the translation of nanomaterial-enabled assays into robust, user-friendly, and cost-effective devices suitable for primary healthcare settings remains challenging [62], [68]. Many nanotechnology-enabled readouts (e.g., fluorescence, SERS, or high-resolution optical detection) still require dedicated readers, controlled optics, or stable power, which can shift the system closer to a *lab-on-a-chip* paradigm rather than a true POC test deployable in community health centers without laboratory specialists. In addition, large-scale clinical validation across diverse field settings remains scarce, sustaining a gap between laboratory-level performance and routine implementation in TB programs [68]. Consequently, while nanotechnology-enhanced diagnostic systems show strong potential to strengthen TB detection through mechanistically improved assay designs, further device-level optimization, operational simplification, and rigorous field-based validation are required before widespread deployment [64].

Taken together, emerging biotechnological innovations provide a robust foundation for improving TB diagnostics through faster, more sensitive, and more accessible testing modalities. CRISPR-based assays, biosensor technologies, microfluidic systems, and nanotechnology-enhanced platforms collectively address limitations of conventional diagnostic tools and may support expanded early TB detection in resource-constrained settings. Nevertheless, persistent challenges related to technical complexity, standardization, field validation, and true point-of-care feasibility highlight the gap between laboratory innovation and real-world implementation. Although further optimization and comprehensive clinical validation remain necessary, continued advances across these technologies underscore their potential to strengthen global efforts toward more timely TB diagnosis [69].

Comparative Evaluation of Diagnostic Platforms

The accurate diagnosis of tuberculosis (TB) is critical for effective disease management and control, especially in regions with high TB burden. A variety of diagnostic tools are available, each with its own advantages and limitations, varying in terms of diagnostic performance, turnaround time, operational complexity, and approximate cost per test factors that critically influence feasibility and adoption in resource-constrained settings such as Indonesia [69]. Table 2 presents a comparative evaluation of these TB diagnostic methods, summarizing their diagnostic performance, turnaround time, operational complexity, approximate cost, strengths, limitations, and relevance to the Indonesian healthcare system.

As shown in Table 2, there is no single diagnostic method that is ideal in all settings. Each diagnostic tool has distinct advantages depending on the clinical context, available resources, and the specific needs of the population. While methods like GeneXpert *Mycobacterium tuberculosis*/rifampicin (MTB/RIF) and real-time *polymerase chain reaction* (PCR) provide rapid results and high sensitivity, their high costs and infrastructure requirements can limit their implementation in resource-limited settings. On the other hand, traditional methods such as sputum smear microscopy and tuberculin skin test are still widely used, although they have limitations, particularly in detecting early-stage TB or cases with low bacillary loads [41]. The appropriate choice of diagnostic method depends on the available resources, making it crucial to understand the strengths and limitations of each tool for informed decision-making in TB screening, diagnosis, and treatment.

Roadmap for Implementation in Indonesia: Bridging the Gap Between Technology and Reality

Although comparative evaluation indicates that diagnostic accuracy alone does not determine the "best" test (Table 2), implementation in Indonesia requires a roadmap that explicitly links technology choice with health-system readiness, financing, specimen referral logistics, and digital reporting [71], [72]. This is particularly salient in a high-burden setting where improved test performance may not translate into population-level impact if cases remain under-notified or patients are lost along the diagnostic-to-treatment cascade [73]. Indonesia's national TB inventory study (2023–2024) estimated 15.6% under-reporting in routine surveillance, indicating that a meaningful share of TB detected in service delivery is not captured through routine notification. Under-reporting is further shaped by the mixed-provider landscape; among recorded private-sector facilities, only 37.7% were connected to the national TB information/reporting system, emphasizing that diagnostic scale-up must incorporate public–private mix (PPM) integration and interoperable data flows [74]. National planning guidance recommends a minimum of one rapid molecular test (TCM) instrument per district/city and sets a strategic target to shift diagnostic testing toward molecular platforms, reaching 75% of diagnostic examinations using TCM by 2024 [75].

Tiered Diagnostic Packaging Aligned with Indonesia's Service Hierarchy

A pragmatic approach for Indonesia is a tiered diagnostic package that matches tools to facility capability while standardizing referral triggers, turnaround-time (TAT) targets, and data integration requirements [76].

Tier 1: Primary Care (FKTP/Puskesmas and Peripheral Facilities).

Given heterogeneous infrastructure and human resources, Tier 1 should prioritize rapid triage, standardized specimen collection, and reliable referral for confirmatory testing, rather than universal deployment of complex platforms [77]. Operational priorities include: (i) structured symptom and risk screening; (ii) access to chest radiography through fixed or mobile referral pathways where available [78]; and (iii) standardized sputum collection and packaging SOPs to maximize downstream NAAT yield [79]. The

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objective is to reduce patient attrition by minimizing repeat visits and ensuring prompt result return via streamlined specimen referral and communication pathways [80].

Tier 2: District-Level Hospitals and Intermediate Laboratories.

Tier 2 is the optimal locus for expanded deployment of automated NAAT/TCM platforms as frontline bacteriological confirmation, particularly for priority groups (e.g., HIV co-infection, children, severe disease, or suspected drug resistance) [81]. Implementation should be paired with instrument uptime planning (service agreements, preventive maintenance), routine QA/QC and contamination control, and standardized interpretation and clinical action algorithms to shorten time-to-treatment initiation [72], [76].

Tier 3: Provincial/National Referral Laboratories and Advanced Centers.

Tier 3 should function as a quality and innovation anchor: providing confirmatory testing for complex cases, more comprehensive drug-resistance testing where available, training and external quality assessment (EQA) support for lower tiers, and structured pilot evaluation of emerging innovations (e.g., CRISPR-based assays, biosensors, microfluidic platforms, and nanotechnology-enhanced methods) in representative Indonesian field settings prior to wider diffusion [82], [83].

This tiered model operationalizes "appropriate technology by setting" into a deployable service strategy and emphasizes effective coverage diagnosis that reliably results in treated and notified cases rather than nominal placement of devices [81].

System Enablers That Determine Feasibility at Scale

To bridge technological promise and routine delivery, the roadmap should incorporate four enabling pillars:

1. Infrastructure and quality systems. Molecular and advanced diagnostics require stable electricity, biosafety procedures, appropriate storage for consumables, and consistent quality management [84]. A national–regional QA architecture (standardized SOPs, EQA, and performance monitoring) is essential to preserve accuracy during decentralization [82].
2. Workforce capacity and competency-based training. A tiered training strategy should be institutionalized: specimen handling and biosafety at Tier 1; instrument operation, contamination control, and result interpretation at Tier 2; and troubleshooting, quality oversight, and method validation at Tier 3 [85].
3. Supply-chain resilience and procurement continuity. Cartridge- and reagent-dependent platforms are vulnerable to stockouts and procurement delays. Forecasting linked to burden and utilization, buffer stock policies for high-burden districts, and strengthened distribution to remote/archipelagic geographies should be treated as core implementation requirements [86].
4. Specimen referral networks and TAT governance. Where confirmatory testing remains centralized, specimen transport and result communication become binding constraints. The roadmap should define transport schedules, packaging standards, sample tracking, and TAT benchmarks (collection-to-result; result-to-treatment initiation) to reduce diagnostic delay and pre-treatment loss to follow-up [87].

Financing Alignment and Sustainability within JKN

Sustained scale-up requires alignment with routine financing mechanisms rather than project-dependent support. Indonesia's JKN framework provides a policy anchor through standardized reimbursement structures across primary and referral care. Within this context, diagnostic algorithms should be operationally compatible with reimbursement pathways, including clarity on coding/bundling for molecular testing and referral-based diagnostics. Strategic purchasing and bundled diagnostic packages can reduce cost variability, improve service continuity during procurement disruptions, and encourage appropriate utilization across tiers [88], [89].

Digital Integration and Interoperability to Improve Notification and Continuity of Care

Given persistent under-reporting and limited private-provider connectivity, diagnostic expansion must be coupled with interoperable data exchange to reduce manual reporting burden and improve notification completeness [74]. Building on lessons from earlier national digital initiatives, Indonesia already uses SITB-connected software (e.g., GxAlert integration) to transmit GeneXpert results into the national TB information system, which can be extended through SATUSEHAT TB interoperability to strengthen real-time reporting,

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case tracking, and linkage-to-treatment. SATUSEHAT provides an implementable pathway: the TB interoperability playbook specifies integration stages and standardized data submission across the care continuum (e.g., patient identity, encounters, diagnostic workflows, episodes of care, and outcomes) via defined FHIR resources [90]. Embedding interoperability requirements into diagnostic roll-out enables automated transmission of laboratory/imaging results, strengthens longitudinal case tracking, and lowers barriers for private providers to participate in national reporting and follow-up. National policy direction also supports leveraging technology and data integration to accelerate TB elimination, providing a favorable context to institutionalize these requirements within implementation planning [76].

Phased Rollout to Balance Feasibility and Innovation

A staged approach improves feasibility and reduces the risk of premature diffusion of tools not yet operationally robust:

1. Phase 1 (0–12 months): Optimization and connectivity. Strengthen specimen referral, QA/QC, workforce training, and SATUSEHAT-aligned interoperability, prioritizing private-provider onboarding to address under-notification [76].
2. Phase 2 (12–24 months): Targeted decentralization. Expand molecular capacity strategically to high-burden districts using readiness-based site selection and iterative monitoring (uptime, invalid/error rates, stockout frequency, and TAT) [81].
3. Phase 3 (24–36 months): Controlled adoption of emerging innovations. Introduce CRISPR/biosensor/microfluidic/nanotechnology tools via structured pilots linked to field validation, cost-effectiveness assessment, and interoperability readiness prior to scale-up [83].

Overall, an Indonesia-specific roadmap should prioritize effective coverage over nominal deployment, embedding financing alignment and interoperability into diagnostic expansion so that innovation translates into measurable gains in notification completeness and timely treatment initiation within a mixed-provider health system [81].

Operational Challenges in Diagnostic Implementation

Despite substantial advances in TB diagnostics, translating these technologies into routine clinical practice presents operational challenges that directly determine scalability and real-world impact [35]. In Indonesia, implementation constraints are amplified by heterogeneous service readiness across geographies and a mixed-provider care landscape, where gaps in notification and connectivity can blunt the public health value of improved diagnostic performance. The national TB inventory study (2023–2024) estimated 15.6% under-reporting in routine surveillance and documented limited connectivity among recorded private-sector facilities (37.7% connected to the national TB reporting platform), underscoring that implementation barriers are not only technical but also systemic [74].

Infrastructure and maintenance capacity remain major constraints, particularly in rural and underserved settings. Many molecular and next-generation tools require stable electricity, adequate biosafety infrastructure, appropriate storage conditions for consumables, and routine calibration or preventive maintenance. Where power stability and maintenance pathways are weak, instrument downtime and higher invalid/error rates can lead to nominal availability without reliable service continuity [2].

Financial barriers and procurement fragility also impede sustainability. High acquisition and maintenance costs for automated NAAT systems, qPCR platforms, and emerging microfluidic devices can strain program budgets. In addition, recurring costs for single-use cartridges, reagents, and consumables create long-term expenditure commitments. These costs are compounded by logistical barriers including stockouts, delayed procurement cycles, and distribution constraints to remote/archipelagic areas which can interrupt testing continuity and force reliance on slower alternatives [91].

Human resource limitations and quality management gaps can degrade test performance at scale. High-performing platforms often require trained staff capable of executing complex workflows, adhering to contamination control measures, and interpreting results appropriately. Under high workload and limited staffing, pre-analytical errors (e.g., inadequate specimen quality/volume, suboptimal storage, transport delays) and inconsistent SOP adherence can reduce sensitivity even when assays are analytically robust. Strengthening competency-based training, QA/QC oversight, and EQA systems is therefore essential to preserve accuracy in routine use [92].

Table 2. Comparison of Tuberculosis Diagnostic Methods.

Diagnostic Tool	Diagnostic Performance	Turnaround Time	Operational Complexity	Approximate Cost per Test	Key Strengths	Key Limitations	Recommended Use Case in Indonesian Healthcare Tier	References
Sputum Smear Microscopy	Sensitivity: 50-60%; Specificity: >95%. Performance is reduced in pediatric, HIV-associated, and paucibacillary TB and is highly dependent on bacillary load and sputum quality.	Minutes to hours; results can be available quickly.	Very low; minimal equipment, easy to use.	Very Low	Low cost; widely available; rapid results, especially in primary health settings.	Low sensitivity, particularly in early TB, low bacillary load, and HIV patients; requires high-quality sputum.	Tier I–II: initial triage tool where NAAT is not immediately available; refer for NAAT/culture in high-risk groups (HIV, children, paucibacillary) or when clinical suspicion remains high despite a negative smear.	[13], [14], [15], [16]
***Mycobacterium tuberculosis* Culture**	Sensitivity/Specificity: >95% (reference standard). Highly effective for detecting drug-resistant TB, but limited by slow growth in solid media (2-8 weeks); liquid culture systems provide faster results.	2 to 8 weeks for solid media; liquid media provides faster results (days to weeks).	High complexity; requires a BSL-2 lab environment, specialized equipment, and trained personnel.	High	Gold standard for diagnosis; essential for drug resistance testing and confirming TB diagnosis.	Slow results, requires high-quality lab infrastructure, and specialized training. Contamination risks.	Tier III (primary): reference confirmation and drug-resistant TB workup (culture ± phenotypic DST); not recommended for first-line screening at Tier I due to long turnaround time and infrastructure needs.	[18], [19], [20]
Chest Radiography	Sensitivity: moderate; Specificity: low. Detects pulmonary abnormalities suggestive of TB but cannot confirm active disease or reliably differentiate TB from other lung conditions.	Immediate results; visual diagnosis available within minutes after the image is taken.	Moderate; requires X-ray equipment, radiologist for interpretation, radiation safety protocols.	Moderate	Rapid results; non-invasive; useful for screening and triaging TB suspects.	Low specificity; cannot confirm active TB; may show abnormalities that overlap with other diseases.	Tier I–II: supportive screening and assessment of pulmonary involvement; use to guide referral for bacteriological confirmation (NAAT/culture), not as a standalone diagnostic test.	[22], [23], [24]
Tuberculin Skin Test (TST)	Sensitivity / Specificity: variable. False positives may occur due to BCG vaccination, and false negatives are common in immunocompromised individuals (e.g., HIV, malnutrition).	48-72 hours; results depend on delayed hypersensitivity reaction.	Very low; requires simple intradermal injection and reading by a healthcare worker.	Very Low	Low cost; widely available; suitable for latent TB screening, especially in BCG-vaccinated populations.	Low specificity (false positives in BCG-vaccinated individuals); false negatives in HIV, malnutrition, or children.	Tier I–II: latent TB infection (LTBI) screening in risk groups; not recommended for diagnosing active TB—positive results require clinical assessment and bacteriological testing if symptomatic.	[25], [26], [27]

Conventional PCR	Sensitivity: 80–95%; Specificity: >95%. Enables early detection of active TB but is highly dependent on sample quality and DNA extraction efficiency.	2-4 hours; results can be obtained within a few hours.	High; requires specialized equipment (thermal cycler), trained personnel, and DNA extraction procedures.	High	Rapid and accurate; can detect low bacillary load; ideal for early TB detection, especially in immunocompromised patients.	Requires high-quality samples and DNA extraction; risk of contamination; expensive and requires specialized laboratory setup.	Tier III: use in advanced diagnostic centers for targeted molecular confirmation and selected resistance targets; not recommended for routine primary-care workflows.	[29], [30], [31]
Real-Time PCR (qPCR)	Sensitivity: 85–98%; Specificity: >95%. Allows quantitative detection of MTB DNA and supports drug-resistance monitoring; suitable for low bacillary load samples.	1-2 hours; rapid results due to real-time monitoring during amplification.	High; requires specialized equipment, trained personnel, and strict contamination control.	High	Rapid results; quantitative; highly sensitive for detecting latent TB and drug resistance.	Expensive; requires high-quality samples and laboratory infrastructure; contamination risk.	Tier III: reference/teaching hospitals and provincial/national labs for high-accuracy molecular detection and expanded resistance monitoring where QA/QC capacity is established.	[32], [33]
GeneXpert MTB/RIF	Sensitivity: 85–92%; Specificity: >95%. Rapid detection of MTB and rifampicin resistance within approximately 2 hours; performance may vary with bacillary load.	±2 hours; provides results much faster than culture or conventional PCR.	Moderate; requires cartridges, equipment maintenance, and trained personnel.	High	Rapid results; detects MTB and rifampicin resistance simultaneously; easy-to-use, and WHO-approved.	High cartridge cost; requires stable electricity, maintenance, and trained operators.	Tier II (core placement): first-line rapid bacteriological confirmation and rifampicin resistance screening; Tier I (selective): only where TCM network, stable electricity, cartridge supply, and maintenance are assured.	[34]
Xpert Ultra	Sensitivity: 90–95% (improved for paucibacillary TB); Specificity: >95%. Enhanced performance compared with GeneXpert MTB/RIF, particularly in HIV-associated and pediatric TB.	<2 hours; provides results in less than 2 hours, similar to GeneXpert MTB/RIF.	Moderate to high; requires cartridges, specialized equipment, electricity, and trained personnel.	High	Higher sensitivity, especially for paucibacillary TB and HIV patients; detects drug resistance quickly; rapid results.	High cartridge cost; requires electricity and maintenance; limited availability in some areas.	Tier II–III: preferential use for paucibacillary disease (e.g., HIV, pediatric, selected extrapulmonary specimens) and settings needing higher sensitivity; deploy where training/QA and volume justify use.	[35]
Truenat MTB	Sensitivity: 80–95%; Specificity: >95% (sample-dependent). Portable and	<1 hour; results available in less than 1 hour,	Moderate; requires cartridge, battery, training, and	Moderate	Portable and affordable; easy to use in field settings and remote	Lower accuracy with poor sample quality; limited to	Tier I (Puskesmas/remote, selective): near-POC	[36]

	battery-operated; diagnostic accuracy may be reduced with poor-quality samples.	offering quick results for triage and diagnosis.	regular maintenance.		areas; rapid results for early TB detection.	MTB detection; not suitable for drug resistance testing.	molecular testing to reduce referral delays in rural/remote settings; Tier II: scale-up option to expand NAAT coverage across districts when QA and supply chains are in place.	
LAMP (Loop-Mediated Isothermal Amplification)	Sensitivity: 80–95%; Specificity: 90–98%. Isothermal amplification enables rapid detection, though performance depends on primer design and contamination control.	30-60 minutes; results can be obtained in 30 to 60 minutes, faster than conventional PCR and culture.	Low to moderate; requires primers, minimal equipment, and trained personnel for handling and interpreting results.	Low–Moderate	Fast and simple; low cost; no need for thermal cycler; portable and useful in resource-limited settings.	Primer design can be challenging; susceptible to contamination; does not provide drug resistance information.	Tier I (selective): rapid near-POC testing in remote sites with limited lab infrastructure; requires strict contamination control and clear referral pathways for discordant/complex cases; Tier II: complementary testing where operational readiness permits.	[38], [39], [40], [41]
CRISPR-Based Assays	Analytical sensitivity: single-copy detection reported; clinical sensitivity: variable and under validation. Capable of detecting MTB DNA and drug-resistance mutations, but large-scale field data remain limited.	<1 hour; provides rapid diagnosis.	Low to Moderate; lateral-flow and isothermal formats reduce instrumentation needs, though pre-amplification, reagent handling, and workflow control may still require trained operators.	Low–Moderate	Very rapid results; high analytical sensitivity; potential for low-cost deployment; capable of detecting drug-resistance mutations.	Requires specialized reagents; pre-amplification increases workflow complexity and contamination risk; limited standardization and field validation.	Tier III (validation/pilot): clinical validation, standardization, and implementation studies; Tier I–II (future/conditional): potential decentralized near-POC use once validated and integrated into national algorithms.	[44], [45], [47]
Biosensor-Based TB Detection	Analytical sensitivity: high; clinical performance: under validation. Detects TB biomarkers (antigens, lipids, or DNA) in small sample volumes; standardization and field validation remain challenges.	Minutes to hours; results available rapidly, depending on the biosensor technology and sample processing.	Low to moderate; requires biosensors, special reagents, and trained personnel for handling and interpreting results.	Moderate	Non-invasive; rapid results; affordable; small sample volume; can be used in point-of-care settings.	Development stage; field validation required; commercial availability still limited.	Tier I (future/conditional): potential rapid screening/triage tool at Puskesmas/remote outreach if robust, low-maintenance formats mature; results should be confirmed by NAAT until sufficient validation supports standalone use.	[49], [50], [68], [70]

Microfluidic / Lab-on-Chip	Analytical sensitivity: high; clinical performance: variable. Enables rapid detection of MTB DNA, antigens, and resistance markers; performance may be affected by sample quality and device complexity.	<1 hour; results available in less than 1 hour, often between 30 to 60 minutes.	Moderate to high; requires specialized microfluidic devices, trained personnel, and quality control for optimal performance.	Moderate–High	Rapid results; portable, low cost, suitable for point-of-care testing; effective for TB screening and drug resistance detection.	Susceptible to contamination; requires precise sample handling; limited capacity for complex testing (e.g., drug resistance).	Tier III (evaluation/pilot): performance validation and operational studies; Tier I–II (future/conditional): candidate near-POC platforms for remote screening/rapid workflows once cost, durability, and QA integration are demonstrated.	[58], [59], [60]
Nanotechnology-Based Assays	Analytical sensitivity: high (including single-molecule detection in some formats); clinical performance: under validation. Assay performance may be influenced by sample quality and environmental conditions.	Minutes to hours; results available in minutes to hours, depending on the type of assay.	Moderate to high; requires nanomaterials, specialized equipment, and trained personnel for operation and interpretation.	High	High sensitivity; portable, low-cost, and rapid results; capable of drug resistance detection and single molecule detection.	Sensitive to sample quality; requires specialized reagents and equipment; limited availability of nanomaterials.	Tier III (evaluation): validation for accuracy and resistance detection claims; Tier I–II (future/conditional): potential POC deployment only after field validation confirms reliability, affordability, and supply chain feasibility.	[57], [63], [66], [69]

Operational barriers also include patient-centered and pathway-related factors. Poor sputum quality, difficulty obtaining specimens from children or severely ill individuals, long travel distances to diagnostic centers, and variable health-seeking behavior reduce the effectiveness of screening and confirmatory testing. Where confirmatory testing is centralized, weak specimen transport systems and slow result communication prolong turnaround time and increase pre-treatment loss to follow-up [76].

Finally, data fragmentation and incomplete reporting, especially across private-sector care, can limit impact even when diagnostic capacity increases [85]. Under conditions of limited connectivity, results may not translate into complete notification, timely linkage-to-care, or longitudinal case tracking. Interoperability requirements operationalized through SATUSEHAT TB guidance provide a practical pathway to standardize and automate data exchange across the TB care continuum and reduce manual reporting burdens when adopted alongside diagnostic expansion [76].

Taken together, these constraints underscore that maximizing the value of advanced TB diagnostics in Indonesia requires concurrent investment in infrastructure and maintenance, workforce training and quality systems, supply-chain resilience, patient-centered referral pathways, and interoperable digital reporting so that diagnostic innovation translates into timely treatment initiation and complete notification across Indonesia's diverse healthcare landscape [2].

Future Perspectives in TB Diagnostic Development

Future directions in tuberculosis (TB) diagnostic development are expected to focus on overcoming long-standing limitations in sensitivity, accessibility, and decentralization by leveraging rapid advancements in molecular biology, nanotechnology, and digital health [93]. One of the most transformative pathways involves the refinement of ultra-sensitive point-of-care (POC) diagnostics, designed to detect Mycobacterium tuberculosis at extremely low bacterial loads. Innovations such as CRISPR-based detection systems, next-generation biosensors, and portable microfluidic platforms offer the potential to deliver laboratory-grade accuracy directly at the community level, providing faster diagnosis for children, individuals living with HIV, and paucibacillary cases that are often missed by conventional methods [94].

Equally promising is the development of multiplexed and syndromic diagnostic platforms, enabling simultaneous identification of TB alongside other respiratory pathogens. This approach can streamline clinical decision-making, reduce diagnostic delays, and enhance case detection efficiency in high-burden settings [95]. Advances in nanotechnology including functionalized nanoparticles, quantum dot-based probes, and nano-enhanced biosensors are anticipated to improve biomarker detection, facilitate detection of drug-resistance mutations, and support precision-guided treatment strategies [96].

In the future, integration of digital health technologies will play an increasingly critical role. Cloud-connected diagnostic devices, artificial intelligence-assisted interpretation, automated data transmission, and real-time epidemiological dashboards can strengthen surveillance, enhance case management, and improve linkage to care. Expanding local manufacturing capacity, reducing costs of reagents and cartridges, and improving environmental stability of diagnostic materials will be essential to ensure widespread implementation and sustainability [97].

Overall, future TB diagnostic development aims to bridge the gap between scientific innovation and real-world applicability. By combining technological breakthroughs with strengthened health-system readiness, next-generation diagnostic tools have the potential to significantly accelerate early detection, reduce transmission, and advance global TB elimination efforts particularly in resource-limited countries such as Indonesia [98].

Conclusions and Future Directions

Biotechnological advances have markedly improved the accuracy, speed, and accessibility of tuberculosis diagnosis compared with conventional methods, with NAAT platforms and isothermal amplification enabling earlier and more reliable detection, and emerging approaches (CRISPR-based diagnostics, biosensors, microfluidics, and nanotechnology) offering strong potential for decentralized testing in Indonesia. To translate these innovations into measurable gains in case detection and timely treatment, Indonesian policymakers should prioritize (1) strengthening regional technical capacity for NAAT maintenance and instrument uptime, (2) stabilizing reagent/cartridge supply chains and specimen referral logistics, and (3) enforcing quality assurance with interoperable digital reporting across public and private

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services. Future research should focus on (1) multi-site clinical validation of CRISPR-based and biosensor platforms across diverse Indonesian settings, (2) implementation and cost-effectiveness studies of tiered diagnostic algorithms aligned with health-system readiness, and (3) development and field evaluation of rapid assays that expand drug-resistance detection beyond rifampicin to support scalable, real-world deployment.

Conflict of Interest

The authors declare that there are no potential conflicts of interest that could influence the results, interpretation, or writing of this article.

Acknowledgment

The authors would like to express their gratitude to the Department of Pharmacy, Faculty of Health Sciences, Universitas Singaperbangsa Karawang, for the academic support, facilities, and guidance provided throughout the preparation of this article.

Supplementary Materials

No supplementary materials are provided for this article.

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