Diagnosis of Tuberculosis: Between Established Practices and Emerging Innovations

Diagnóstico da Tuberculose: entre Práticas Consolidadas e Inovações Emergentes

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Abstract

Tuberculosis (TB) remains one of the leading causes of morbidity and mortality worldwide, accounting for millions of new cases and deaths annually. Laboratory diagnosis plays a pivotal role in the early identification of cases, monitoring of antimicrobial resistance, and interruption of the transmission chain of *Mycobacterium tuberculosis*. This review article presents a critical and up-to-date analysis of laboratory methods used in the diagnosis of active TB, ranging from classical techniques to the latest advances in molecular biology, biomarkers, and mass spectrometry. Strategies for detecting *M. tuberculosis* resistance, along with their limitations and clinical implications, are also discussed. Finally, the article proposes integrated approaches that reconcile diagnostic accuracy with operational sustainability in efforts to control the disease.

Keywords: Tuberculosis; Laboratory Diagnosis; Molecular Testing; Culture; Biomarkers; Antimicrobial Resistance.

Resumo

A tuberculose (TB) permanece como uma das principais causas de morbidade e mortalidade no mundo, sendo responsável por milhões de novos casos e mortes anualmente. O diagnóstico laboratorial desempenha papel central na identificação precoce dos casos, monitoramento da resistência antimicrobiana e na interrupção da cadeia de transmissão do *Mycobacterium tuberculosis*. Este artigo de revisão apresenta uma análise atualizada e crítica sobre os métodos laboratoriais utilizados no diagnóstico da TB ativa, desde as técnicas clássicas até os avanços mais recentes em biologia molecular, biomarcadores e espectrometria de massa. Discutem-se também as estratégias para detecção da resistência do *M. tuberculosis*, suas limitações e implicações clínicas. Por fim, o artigo propõe abordagens integradas que conciliem precisão diagnóstica com sustentabilidade operacional no enfrentamento da doença.

Palavras-chave: Tuberculose; Diagnóstico Laboratorial; Testes Moleculares; Cultura; Biomarcadores; Resistência Antimicrobiana.

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INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and remains one of the most significant public health challenges in the contemporary global landscape. Despite significant advances in TB control, more than 10 million cases were estimated globally in 2023, with a gap of approximately 2.6 million cases either undiagnosed or unreported, according to the World Health Organization (WHO).[1] Timely and accurate laboratory diagnosis is a cornerstone of effective TB control, as it enables early initiation of treatment, prevents disease progression, and reduces community transmission of *M. tuberculosis*.

METHODOLOGY

This narrative review was based on a critical analysis of scientific publications and technical and regulatory documents from the past 15 years, with emphasis on advances and challenges in laboratory diagnosis of tuberculosis. The literature search was conducted in PubMed, SciELO, and Scopus databases using the descriptors "*Mycobacterium tuberculosis*," "tuberculosis," "diagnosis," "molecular test," "drug resistance," "biomarkers," and "culture," combined using Boolean operators. Official documents from the WHO and the Brazilian Ministry of Health were also consulted.

Priority was given to open-access articles, systematic reviews, multicenter studies, technical guidelines, and publications relevant to laboratory practice and public health. The time frame included publications from 2007 to 2024, with the exception of seminal articles. References were organized according to their applicability to the various techniques discussed in the text.

CLASSICAL DIAGNOSTIC METHODS

Microscopy (Direct Sputum Smear Microscopy)

Microscopy, traditionally performed using Ziehl-Neelsen staining, is one of the oldest and most widely used methods for the initial screening of pulmonary tuberculosis. It is based on the detection of acid-fast bacilli (AFB) in clinical specimens, primarily sputum. A variation of the classical technique is Kinyoun staining, which employs higher concentrations of phenol and fuchsin to penetrate the bacterial cell wall, eliminating the need for slide heating. Although both methods exhibit moderate sensitivity, particularly in cases with low bacillary load, their use is justified by operational simplicity, low cost, and feasibility across various levels of health care delivery.[2]

Fluorescent staining with auramine-O is an alternative that enhances sensitivity and reduces slide reading time. However, it requires specialized microscopes with higher initial costs. Both techniques, while useful, do not differentiate the *M. tuberculosis* complex from nontuberculous mycobacteria (NTM) and provide no information on bacillary viability or antimicrobial resistance, limiting their role to initial screening.

Solid Media Culture

Bacterial culture is considered the gold standard for laboratory diagnosis of TB, as it confirms the presence of *M. tuberculosis* and enables drug susceptibility testing (DST). The most commonly used solid media include Löwenstein-Jensen and Ogawa-Kudoh, the latter being a low-cost and easy-to-prepare alternative.[3] Both are recognized for their high sensitivity in detecting bacilli, even in specimens with low bacillary load. Additional media such as Middlebrook 7H10 and 7H11, which are agar-based and supplemented with OADC (oleic acid, bovine albumin, dextrose, and catalase), support faster growth and better colony definition of *M. tuberculosis*, facilitating quantification and strain isolation. However, their use in Brazil is limited due to factors such as higher contamination risk and elevated cost.

In addition to enabling microorganism isolation, culture allows for DST, which is essential for selecting appropriate treatment, particularly in patients at risk for drug-resistant TB, such as those undergoing retreatment.

Nevertheless, the method is labor-intensive and yields delayed results, typically between 3 and 8 weeks, and may be affected by bacterial or fungal contamination even when collection and decontamination procedures are properly executed. Furthermore, complementary methods are required for microorganism identification after growth.

Solid media culture is especially recommended when NTM coinfection is suspected or for the diagnosis of extrapulmonary TB, where bacillary load is generally low and smear microscopy has limited sensitivity. The Ogawa-Kudoh method represents a simplified, low-cost alternative for *M. tuberculosis* isolation, with its main advantage being the streamlined decontamination step.[3]

Automated Liquid Culture Systems

Liquid culture, also considered a gold standard, represents a significant advancement over solid media methods, particularly in terms of the time required for bacterial growth

detection. Semi-automated systems such as BACTEC MGIT (Mycobacterial Growth Indicator Tube) use fluorescent sensors containing ruthenium complexes that emit fluorescence upon oxygen consumption during *M. tuberculosis* growth. This allows the detection of bacterial metabolism before visible growth occurs in the culture medium, enabling results within 8 to 10 days.[3]

In addition to bacillus detection, these semi-automated systems enable susceptibility testing in liquid media with greater speed and standardization. As with solid media cultures, liquid culture is susceptible to contamination and requires subsequent species confirmation. Despite these limitations, liquid culture plays a critical role in confirmatory diagnosis and in monitoring antimicrobial resistance, and it is widely recommended for reference laboratories and epidemiological surveillance.

RAPID MOLECULAR TESTS

Real-Time PCR-Based Platforms

In recent years, rapid molecular tests have revolutionized the diagnosis of active TB. These assays directly detect *M. tuberculosis* DNA in clinical specimens, offering high sensitivity and specificity, along with the ability to identify mutations associated with antimicrobial resistance. Among the most widely used diagnostic platforms are Xpert MTB/RIF Ultra, Truenat MTB, and STANDARD M10.[4-6]

Xpert MTB/RIF Ultra is a semi-automated platform based on real-time PCR that delivers results in approximately two hours. In addition to detecting the bacillus, it identifies mutations in the *rpoB* gene associated with rifampicin resistance. Truenat MTB operates similarly but is portable and suitable for settings with limited infrastructure. STANDARD M10 enables simultaneous detection of *M. tuberculosis* and mutations related to rifampicin and isoniazid resistance, expanding diagnostic coverage.

These technologies are recommended by WHO as initial tests, replacing less sensitive methods such as smear microscopy. Their main advantages include speed, accuracy, and ease of use. However, they have limitations such as high cost, reliance on imported reagents, and the need for specialized technical maintenance.[4]

The use of non-commercial laboratory-developed tests is not new in diagnostic practice. In this context, in-house PCR protocols validated in the scientific literature have emerged as effective and accessible alternatives for molecular diagnosis of TB and other mycobacterioses. Multiplex in-house PCR protocols have been successfully proposed and evaluated for the simultaneous detection of the *Mycobacterium* genus, *M. tuberculosis*, and the *M. avium* complex (MAC), with potential application in diverse epidemiological settings.[7,8] The flexibility and low cost of these tests underscore the importance of adaptable methodologies, provided they are internally validated using robust criteria for sensitivity, specificity, and reproducibility.[9]

High-Throughput Platforms

For high-complexity centers, platforms such as the Abbott RealTime MTB Assay have been successfully employed. Capable of processing large volumes of samples simultaneously, this system stands out for its full automation and ability to integrate with existing laboratory workflows. Its complementary version, MTB RIF/INH Resistance, enables detection of mutations associated with rifampicin and isoniazid resistance with high sensitivity.[10] However, the high acquisition cost, need for sophisticated laboratory infrastructure, and supply chain logistics may limit its widespread adoption, particularly in resource-limited settings.

Isothermal Amplification (TB-LAMP)

As an alternative to PCR-based tests, the TB-LAMP (loop-mediated isothermal amplification) assay uses isothermal nucleic acid amplification and does not require thermocyclers. This feature makes it more feasible for settings with basic infrastructure. Results can be read visually or with simple equipment, and are typically available in under an hour.[11]

More recently, rt-LAMP, a real-time version with superior performance, has been developed. This method offers high diagnostic accuracy and, by using open platforms, represents a significant advancement in terms of flexibility and cost-effectiveness. However, it still requires prior DNA extraction, does not provide information on antimicrobial resistance, and demands minimal laboratory infrastructure and trained personnel, factors that may limit its implementation in low-resource settings.[12]

Recommended by WHO as a replacement for smear microscopy in resource-limited environments,[6] TB-LAMP shows good sensitivity in sputum samples but still lacks validation for use in other clinical matrices. Moreover, it does not provide information on drug resistance, which limits its standalone use in comprehensive diagnostic protocols.[10]

BIOMARKERS AND EMERGING TECHNOLOGIES

The pursuit of faster, more sensitive, and context-adaptable diagnostic methods has led to the development of tests based on biomarkers and emerging technologies. Although many of these tools are still undergoing validation, some are already being used as complementary approaches to conventional and molecular methods.

Mycobacterial Antigens in Urine (LAM)

The Determine TB LAM Ag test detects lipoarabinomannan (LAM), a glycolipid component of the *M. tuberculosis* cell wall that is present in the urine of patients with active TB. Recommended by the WHO for screening people living with HIV and CD4 counts below 200 cells/mm^3, it provides results within 25 minutes and has been implemented in referral hospitals. However, its sensitivity is limited in immunocompetent individuals.[13,14]

Transcriptional Signatures and Proteomic Panels

Recent studies have identified gene expression signatures capable of distinguishing active TB from latent infection using peripheral blood expression profiles. Genes associated with inflammatory responses, particularly type I interferon pathways, have shown promise.[8] In parallel, multiplex panels of plasma proteins, such as IP-10, IL-6, and CRP, are being evaluated as markers of disease activity or treatment response.[13-16]

Metabolomics and microRNAs

Emerging research highlights the potential of metabolomics (e.g., profiles of fatty acids, amino acids, and volatile compounds) and circulating microRNAs as highly sensitive diagnostic tools. Despite scientific enthusiasm, these technologies remain largely exploratory and remain largely unstandardized, clinically unvalidated, and operationally challenging.

Despite their promise, the use of biomarkers for TB diagnosis still faces significant limitations. Most currently studied markers lack clinical validation across diverse populations, restricting their widespread application. Many biomarker-based tests require specialized laboratory infrastructure, such as molecular biology or mass spectrometry platforms, which hinders large-scale implementation, especially in resource-limited settings. Additionally, individual variability in immune response and the influence of coinfections, comorbidities, or nutritional status may compromise diagnostic accuracy. The lack of standardization across protocols, reagents, and analytical platforms also limits reproducibility between studies. Finally, high costs and the absence of validated commercial kits hinder the incorporation of biomarkers into routine clinical practice.[17,18]

Proteomic Identification by MALDI-TOF

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful tool for the rapid identification of microorganisms, including mycobacteria. The technology is based on the analysis of bacterial protein profiles, generating a specific mass spectrum that can be compared to reference databases for species-level identification. In the context of TB, MALDI-TOF MS has been primarily used to identify the *M. tuberculosis* complex from cultures, whether solid or liquid, with high accuracy, rapid turnaround (within minutes), and low operational costs after system installation.

Although MALDI-TOF MS is recommended for use with pure cultures, some studies have demonstrated its feasibility for direct application to clinical specimens such as sputum and bronchoalveolar lavage fluid, with high sensitivity and specificity. MALDI-TOF MS represents a promising platform, particularly for accelerating the identification of mycobacterial species in cultures, contributing to appropriate case management and streamlined laboratory diagnostic workflows.[19,20]

DETECTION OF ANTIMICROBIAL RESISTANCE

Antimicrobial resistance in *M. tuberculosis* remains one of the greatest challenges to reducing global TB incidence. Rapid and accurate identification of resistant strains is essential for the timely initiation of effective treatment regimens, interruption of bacillary transmission, and improvement of clinical outcomes. Laboratory methods for detecting antimicrobial resistance can be broadly categorized into two main groups: phenotypic and molecular.

Phenotypic Methods

Phenotypic tests assess the ability of *Mycobacterium tuberculosis* to grow in the presence of critical concentrations of antimicrobial agents. The proportion method is a classical phenotypic technique widely used to evaluate *M. tuberculosis* susceptibility to antimicrobials on solid culture media, particularly Löwenstein-Jensen. It is based on comparing bacterial growth on media with and without antimicrobials, determining whether the tested strain is susceptible or resistant based on a critical growth proportion. Results are

interpreted by comparing the two tubes: if the proportion of colonies growing on the drug-containing medium is equal to or greater than 1% of the number of colonies on the control tube, the strain is considered resistant to the tested antimicrobial. Conversely, if this proportion is below 1%, the strain is classified as susceptible.[21]

Although the proportion method has notable limitations, such as prolonged turnaround time and the requirement for biosafety level 3 (BSL-3) laboratory infrastructure, it continues to play a fundamental role in assessing *M. tuberculosis* resistance. Unlike molecular tests, this method directly evaluates bacillary viability in the presence of antimicrobials, offering a functional measure of resistance independent of the underlying genetic mechanism.

This approach is particularly valuable for detecting resistance caused by mutations outside the target regions covered by molecular assays, as well as resistance mechanisms that are unknown or not mutation-related, such as altered cell wall permeability or efflux pump overexpression. Additionally, the proportion method allows for the evaluation of a broader range of drugs, including second-line agents and investigational compounds, enhancing its applicability in clinical and surveillance settings.

The BACTEC MGIT system is a widely used semi-automated platform for detecting mycobacterial growth and performing phenotypic drug susceptibility testing in liquid media. The test compares *M. tuberculosis* growth in tubes containing antimicrobials with control tubes. Growth in the presence of the drug indicates resistance. The BACTEC MGIT 960 provides faster results than solid media methods (approximately 7 to 14 days), is validated for both first- and second-line drugs, and is considered the gold standard in reference laboratories. However, it requires BSL-3 infrastructure, has high operational costs, and demands strict contamination control.[22]

Other phenotypic methods include colorimetric assays such as the Resazurin Microtiter Assay (REMA) and the Nitrate Reductase Assay (NRA). REMA is a colorimetric method based on the reduction of resazurin, a blue dye, into resorufin, which is pink, in the presence of viable bacteria. The assay is performed in microdilution plates with wells containing different concentrations of the antimicrobial agent to be tested. After incubation with the *M. tuberculosis* suspension, resazurin is added, and the results are read visually or using a spectrophotometer. A color change to pink indicates bacterial growth and thus resistance; if the dye remains blue, no growth occurred, and the strain is considered susceptible. This is a low-cost, rapid method (5 to 10 days), widely used in research laboratories, though not yet standardized for routine clinical use. It may be useful for initial screening or for testing new antimicrobial agents.

The NRA is a colorimetric method based on the ability of *M. tuberculosis* to reduce nitrate to nitrite. The test is performed in culture media supplemented with nitrate and critical concentrations of antimicrobials. If the bacteria grow and reduce nitrate, a visible color reaction occurs upon the addition of reagents (typically sulfuric acid and α-naphthylamine), indicating resistance. If no visible reduction occurs, the strain is considered susceptible. This is a low-cost, easy-to-implement method with performance comparable to conventional tests, making it particularly useful in resource-limited settings. However, its standardization and routine application remain limited to specific antimicrobials, such as rifampicin and isoniazid.[23-25]

Molecular Methods

In addition to the previously mentioned platforms that simultaneously detect *Mycobacterium tuberculosis* and mutations associated with rifampicin resistance (Xpert MTB/RIF Ultra and Truenat MTB) or both rifampicin and isoniazid resistance (STANDARD M10 and Abbott RealTime MTB Assay) with high accuracy, the Xpert system also includes the Xpert MTB/XDR cartridge version. This version can simultaneously detect resistance to rifampicin, isoniazid (*katG* and *inhA*), fluoroquinolones (*gyrA* and *gyrB*), and aminoglycosides (*rrs* and *eis*) directly from clinical specimens, producing results in approximately two hours. Its limitations are similar to those previously discussed in the diagnostic section.[26]

Line Probe Assays (LPAs) are reverse hybridization-based assays that identify mutations in genes associated with resistance. They allow simultaneous detection of resistance to multiple antimicrobials and are recommended by the WHO as second-line tests in reference laboratories. Despite their accuracy, LPAs have important limitations, as they detect only previously known mutations located in specific target regions of the genes. This can lead to false negatives when resistance is mediated by rare, silent, or non-targeted mutations.

Moreover, result interpretation can be complex, especially in the presence of mixed banding patterns (heteroresistance), requiring skilled personnel and technical expertise. Another critical aspect is the need for intermediate-level laboratory infrastructure, including molecular biology equipment and physically separated work areas to prevent cross-contamination. Finally, LPAs still

depend on specimens with high bacillary loads or positive cultures, limiting their direct application in paucibacillary or extrapulmonary samples.[27]

More recently, next-generation sequencing (NGS) methods have been employed in reference laboratories for comprehensive characterization of resistance genotypes. Platforms such as Deeplex® Myc-TB enable simultaneous detection of multiple mutations, lineage identification, and even detection of heteroresistance. Despite their high cost and complexity, these methods provide an in-depth understanding of resistance mechanisms and are playing an increasingly important role in surveillance and personalized treatment strategies.[28,29]

FINAL CONSIDERATIONS

Laboratory diagnosis of tuberculosis has undergone significant transformations in recent decades, with the incorporation of molecular platforms and biomarker-based approaches. These advances have led to substantial improvements in sensitivity, speed, and specificity, contributing to early diagnosis and interruption of *M. tuberculosis* transmission chains.

However, structural and operational challenges persist, limiting the broad and equitable adoption of these technologies, particularly in high-burden TB countries. Key barriers include the high acquisition and maintenance costs of equipment, dependence on imported reagents, the need for adequate laboratory infrastructure, and ongoing training of healthcare personnel.

Therefore, the selection of diagnostic platforms should consider not only the intrinsic characteristics of each test (see Table 1), but also factors such as local disease burden, prevalence of coinfections (e.g., HIV), available financial resources, the epidemiological profile of resistance, and the operational capacity of health services.

Table 1

Main laboratory methods for the diagnosis of tuberculosis.

Method	Detection Type	Sensitivity (%)	Time to Result	Requires Special Biosafety Conditions?	Detects Resistance?	Remarks
Smear (Ziehl-Neelsen)	Microscopy	50–60	<1 day	No	No	Low cost, initial screening
Smear (Auramine)	Fluorescence	60–70	<1 day	No	No	More sensitive than conventional smear; requires fluorescence microscope
Culture (Löwenstein-Jensen)	Bacterial growth	80–85	3–8 weeks	Yes	Yes (with DST)	Gold standard; prolonged turnaround time
Culture (BACTEC MGIT)	Automated growth	85–90	7–14 days	Yes	Yes (with DST)	Rapid; high cost; gold standard
Xpert MTB/RIF Ultra	Real-Time PCR	85–95	~2 hours	No	Yes (rifampicin)	WHO-recommended; rapid; high cost
Truenat MTB	Real-Time PCR	80–90	~1 hour	No	Yes (rifampicin)	WHO-recommended; rapid; high cost
Standard M10	Real-Time PCR	85–95	~2 hours	No	Yes (rifampicin)	WHO-recommended; rapid; high cost
TB-LAMP	Isothermal amplification	75–85	~1 hour	No	No	Replaces smear microscopy in remote settings
In-House PCR	PCR / Real-Time PCR	85–95	~5 hours	No	No	Flexible and low-cost; can detect NTM; requires trained molecular personnel

DST: Drug Susceptibility Testing; NTM: Nontuberculous Mycobacteria. Source: Authors, 2025.

Despite notable progress, significant challenges remain, particularly regarding equitable access to diagnostic technologies. Most high TB burden countries continue to face structural barriers, including budgetary constraints, lack of technical training, and dependence on imported supplies. Moreover, the complexity of diagnostic algorithms and the need for integration across platforms and levels of care demand comprehensive strategic planning by health systems.

In this context, it is essential to adopt a rational and context-sensitive approach to selecting laboratory strategies. Classical methods such as smear microscopy and culture remain valuable, especially when integrated with rapid molecular tests. Detection of antimicrobial resistance should combine molecular and phenotypic tools, tailored to local capacities and individual clinical needs.

Initiatives aimed at decentralizing diagnosis, strengthening laboratory networks, providing continuous professional training, and incorporating technologies adapted to local realities are crucial for sustainable progress in TB control. The future of tuberculosis laboratory diagnosis will depend on the alignment of technological innovation, public policy, and a commitment to universal access to health care.

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