Review

Current Landscape of Molecular Diagnostic Tests for Tuberculosis and Drug Resistance: From Routine Practice to Emerging Technologies

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Review

Current Landscape of Molecular Diagnostic Tests for Tuberculosis and Drug Resistance: From Routine Practice to Emerging Technologies

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Abstract

The rapid and accurate diagnosis of *Mycobacterium tuberculosis* (MTB) infection, along with timely detection of drug resistance, is crucial for controlling tuberculosis (TB) globally. Over the past two decades, molecular diagnostic assays have transformed TB detection, ranging from centralized high-throughput platforms to portable point-of-care systems. Our review provides a comprehensive overview of key WHO-endorsed and emerging molecular assays for TB diagnosis and drug resistance detection. Cartridge-based systems such as Xpert MTB/RIF, Xpert MTB/RIF Ultra, and Xpert MTB/XDR (Cepheid) have revolutionized TB diagnostics by offering automated, rapid detection with high sensitivity and specificity, especially in resource-limited settings. High-throughput platforms like COBAS MTB and MTB-RIF/INH (Roche) and line probe assays such as GenoType MTBDRplus and MTBDRsl (Hain Lifescience) have proven valuable in centralized laboratories for resistance profiling. Decentralized and portable systems, including Truenat MTB (Molbio Diagnostics), TB-LAMP (Eiken Chemical), and EasyNAT TB (Ustar Biotech), address critical diagnostic gaps in peripheral settings, offering rapid results with simplified workflows. The SS-LAMP assay (MAScIR) and the Deeplex-MycTB targeted sequencing assay (Genoscreen) exemplify regionally developed and advanced molecular approaches, respectively, contributing to improved diagnosis and surveillance efforts. Despite the diversity of these platforms, challenges remain concerning cost, infrastructure, and implementation in high-burden, low-resource environments. This review highlights the strengths, limitations, and roles of each assay within TB diagnostic strategies, emphasizing the need for integrated approaches to achieve global TB control targets.

Keywords: *Mycobacterium tuberculosis*; tuberculosis; diagnostic assays

Background

Tuberculosis (TB) continues to affect millions of people each year, despite decades of global efforts to control and eliminate the disease. In 2022, the World Health Organization (WHO) estimated that 10.6 million people fell ill with TB and 1.3 million died from it. An additional 167,000 deaths were reported among people living with HIV [1]. The burden of TB is overwhelmingly concentrated in low- and middle-income countries, which account for more than 95% of global TB-related deaths [1].

While TB most commonly affects the lungs, it can also involve other parts of the body, such as the lymph nodes, pleura, bones, and central nervous system. These formsknown as extrapulmonary

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TB are particularly common in people with weakened immune systems. In fact, among individuals living with HIV, extrapulmonary or disseminated TB can account for up to 60% of all TB cases [2].

Early and accurate diagnosis is critical not only to initiate timely treatment and improve patient outcomes but also to prevent ongoing transmission within communities [3]. However, widely used diagnostic methods such as sputum smear microscopy have significant limitations. Although simple and inexpensive, smear microscopy has poor sensitivity, especially in children and people living with HIV, and it cannot distinguish *Mycobacterium tuberculosis* from other mycobacteria [4–6].

Culture-based diagnostics remain the gold standard, offering higher sensitivity and the ability to test for drug resistance. Yet these methods are slow, often taking several weeks,and require well-equipped laboratories and trained personnel [7]. In many settings, these challenges result in delayed diagnoses and missed opportunities for treatment.

To address these gaps, molecular diagnostics have emerged as powerful tools for the rapid and accurate detection of *M. tuberculosis* and its resistance to key antibiotics. Technologies such as PCR, real-time PCR, and loop-mediated isothermal amplification (LAMP) can provide results in under two hours, directly from clinical specimens. Platforms like Xpert MTB/RIF Ultra, Truenat, and TB-LAMP are now widely, used and have been endorsed by the WHO for their speed and reliability [8–10].

Despite the progress made, many of these tools were developed for pulmonary TB and remain less validated for extrapulmonary forms. Nevertheless, clinicians are increasingly using them off-label for non-respiratory specimens, filling an important diagnostic gap [11–13]. Meanwhile, more than 50 new diagnostic tests are currently under development, reflecting ongoing innovation in this field [14].

This review provides an updated overview of WHO and FDA-endorsed molecular diagnostics for tuberculosis. We highlight not only the technologies currently in use, but also those in late-stage evalutaion (CE-marked) that could soon transform how we diagnose TB in a variety of clinical settings.

Xpert MTB/RIF Assay (Cepheid, USA)

The Xpert MTB/RIF assay (Cepheid, USA) represents a significant breakthrough in tuberculosis (TB) diagnostics, combining automated nucleic acid amplification with real-time detection of *Mycobacterium tuberculosis* (MTB) and rifampicin (RIF) resistance mutations. Endorsed by the World Health Organization (WHO) in 2010 as the initial diagnostic test for suspected pulmonary TB, Xpert MTB/RIF delivers results within approximately two hours, with minimal hands-on time, making it a cornerstone of rapid TB diagnosis worldwide [1]. Meta-analyses have demonstrated its high sensitivity (89%) and specificity (98%) for pulmonary TB, particularly in smear-positive patients, and a significant diagnostic yield in HIV-infected populations [4]. However, sensitivity drops in smear-negative and extrapulmonary TB cases, highlighting the need for improved detection in paucibacillary samples (ref).

To address this, the Xpert MTB/RIF Ultra was introduced in 2017, featuring optimized assay chemistry and additional molecular targets, which lowered the limit of detection from 131 CFU/mL to 16 cfu/mL. Clinical studies have reported Ultra's superior sensitivity, especially in smear-negative and HIV-associated TB, albeit with a slight decrease in specificity due to trace-positive results **[8].** The WHO endorsed Ultra in 2017 as a replacement for the original assay **[15].**

Looking beyond rifampicin resistance, the Xpert MTB/XDR assay (launched in 2021) allows for the rapid detection of resistance to isoniazid, fluoroquinolones, ethionamide, and second-line injectable agents, offering critical insights for multidrug-resistant TB (MDR-TB) management **[16].** Despite these advancements, implementation in low-resource settings faces challenges related to cartridge cost, instrument maintenance, and supply logistics.

Nevertheless, WHO recommends Xpert-based testing as the initial diagnostic approach in both high- and low-burden countries, supported by global donor programs and concessional pricing agreements [6]. Furthermore, decentralized testing has been facilitated by portable GeneXpert systems such as Omni platforms. The widespread adoption of Xpert assays has transformed TB

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diagnostics, contributing to earlier treatment initiation, improved patient outcomes, and strengthened global TB control strategies [17] (Table 1, Table 2).

COBAS® MTB and MTB-RIF/INH Assays (Roche Diagnostics, Switzerland)

The COBAS® MTB and COBAS® MTB-RIF/INH assays (Roche Diagnostics, Switzerland) are fully automated real-time PCR-based molecular diagnostic tests performed on the COBAS® 6800/8800 systems. These assays allow for the detection of *Mycobacterium tuberculosis* complex DNA and simultaneous identification of mutations in the *rpoB* gene (rifampicin resistance), as well as inthe *katG* gene and inhA promoter region (isoniazid resistance). Approved under the CE-IVD mark in 2019, the COBAS platform offers high-throughput, sample-to-result processing, capable of analyzing up to 384 samples per 8-hour shift on the 8800 system [18].

In multicenter studies, the COBAS MTB assay demonstrated high diagnostic accuracy, with reported sensitivity of 95% for smear-positive and 68% for smear-negative pulmonary TB samples, and specificity consistently exceeding 98% [19]. For drug resistance detection, the MTB-RIF/INH assay showed strong concordance with phenotypic drug susceptibility testing (DST), with rifampicin resistance detection sensitivity of 96% and isoniazidresistance detection sensitivity of 94%, both with specificity above 98% [20] (Table 1, Table 2).

Unlike cartridge-based systems like GeneXpert, which are optimized for decentralized and near-patient settings, the COBAS platform is designed for centralized laboratories with high-throughput needs, benefiting from integrated automation, minimal hands-on time, and seamless connectivity with laboratory information systems. The fully automated workflow, from sample extraction to amplification and detectionreduces the risk of cross-contamination and ensures process standardization. The WHO recognized in its 2020 consolidated guidelines the potential role of COBAS in centralized laboratory networks for TB diagnosis and esistance surveillance [11].

GenoType® MTBDRplus and MTBDRsl Assays (Hain Lifescience, Germany)

The GenoType® MTBDRplus and GenoType® MTBDRsl assays (Hain Lifescience, Germany) are line probe assays (LPAs) based on multiplex PCR followed by reverse hybridization on nitrocellulose strips, enabling simultaneous detection of *Mycobacterium tuberculosis* complex (MTBC) and mutations associated with resistance to first- and second-line anti-tuberculosis drugs.

The GenoType MTBDRplus assay, introduced in 2008, targets mutations in the *rpoB* gene (rifampicin resistance), katGgene, and inhA promoter region (isoniazid resistance), offering critical information for the detection of multidrug-resistant TB (MDR-TB). Large multicenter studies have reported high diagnostic accuracy, with pooled sensitivity for rifampicin resistance detection exceeding 95% and specificity above 98%, while isoniazid resistance detection shows a slightly lower sensitivity around 85–90% [21].The WHO endorsed the use of MTBDRplus as an initial test for rifampicin resistance detection in 2008 and reaffirmed its role in subsequent guidelines [22].

The GenoType MTBDRsl assay complements MTBDRplus by detecting mutations linked to resistance against fluoroquinolones (*gyrA*, *gyrB* genes), second-line injectable drugs (*rrs* gene), and ethambutol (*embB* gene) [].The first version (MTBDRsl v1.0) showed variable sensitivity, particularly for injectable drug resistance detection prompting the development of MTBDRsl v2.0, which improved diagnostic performance, especially for second-line drugs [23]. Studies have reported sensitivities exceeding 90% for fluoroquinolone resistance and improved detection of kanamycin and amikacin resistance with v2.0 [15].

The WHO has recommended LPAs for both first- and second-line drug resistance testing in settings with appropriate infrastructure [11]. Despite the need for laboratory expertise, GenoType assays remain a key component of TB drug-resistance surveillance, providing rapid and comprehensive mutation-based resistance profiles.

FluoroType® MTB Assay (Hain Lifescience, Germany)

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The FluoroType® MTB assay (Hain Lifescience, Germany) is a semi-automated real-time PCR testdeveloped for the detection of *Mycobacterium tuberculosis* complex (MTBC) DNA in respiratory samples. Using the FluoroLyse® extraction system and FluoroCycler® 96, the assay provides a closed, contamination-reduced workflow with real-time fluorescence detection.

Designed for centralized laboratories, FluoroType MTB offers high-throughput capacity and has shown performance comparable to the Xpert MTB/RIF system in clinical qstudies. Barletta et al. reported sensitivities of 100% in smear-positive and 89.3% in smear-negative samples, with specificity of 99.6% [24].

Although the assay lacks integrated resistance detection, it is valued for its high analytical sensitivity and compatibility with automated platforms. Its main limitation remains the infrastructure required for operation, restricting its use in decentralized settings. The FluoroType MTB assay is included in several national guidelines for TB screening in Europe but has not yet been endorsed by the WHO for global implementation [11] (Table 1 & Table 2).

Truenat® MTB Assay (Molbio Diagnostics, India)

The Truenat® MTB assay (Molbio Diagnostics, India) is a chip-based, micro-PCR platform designed for decentralized and near-patient TB diagnosis. The system consists of a battery-operated Truelab® device, the Trueprep® sample preparation unit, and disposable microchips, allowing for a portable, closed-system workflow ideal for peripheral laboratories.

Endorsed by the WHO in 2020 for initial TB diagnosis, the Truenat MTB assay offers a rapid turnaround time of approximately one hour [11]. Clinical evaluations have demonstrated sensitivity of up to 88% in smear-negative patients and specificity exceeding 98% [25].

The Truenat system addresses many barriers faced by low-resource settings, such as electricity dependency and complex instrumentation, positioning it as a viable alternative for decentralized testing. Its WHO endorsement emphasizes its role in expanding accesstomolecular diagnostics in peripheral settings and its contribution to closing diagnostic gaps in TB care.

PURE-LAMP (TB-LAMP) (Eiken Chemical, Japan)

The PURE-LAMP, also known as TB-LAMP (Eiken Chemical, Japan), is a manual loop-mediated isothermal amplification assay for the detection of *Mycobacterium tuberculosis* complex. It was endorsed by the WHO in 2016 as a replacement for smear microscopy in peripheral laboratories [10].

TB-LAMP uses a simple heating block to perform amplification at a constant temperature, and the results can be visually read under UV light, eliminating the need for advanced equipment. In a multicenter evaluation, TB-LAMP achieved a sensitivity of 80% in smear-negative, culture-positive pulmonary TB cases and over 98% specificity [26].

Its advantages include ease of use, low cost, and suitability for microscopy centers with minimal infrastructure. However, TB-LAMP does not provide drug resistance information and remains a screening tool rather than a comprehensive diagnostic solution. Despite this, its deployment in endemic areas has contributed to improved case detection, particularly in high-burden countries with limited laboratory capacity.

SS-LAMP (MAScIR, Morocco)

The SS-LAMP assay (MAScIR, Morocco) is an in-house developed single-step loop-mediated isothermal amplification method tailored for the detection of *Mycobacterium tuberculosis* complex directly from sputum samples. Unlike standard LAMP assays, SS-LAMP is designed for single-tube, closed-system amplification, minimizing contamination risks while simplifying handling in field conditions.

In a study conducted by Bentaleb et al., SS-LAMP demonstrated a sensitivity of 82.93 % in smear-positive TB cases and a specificity of 100%, confirming its diagnostic potential in low-resource settings **[27]**. The method is characterized by its rapid turnaround time, simplicity, and cost-

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effectiveness, making it particularly suitable for decentralized testing where molecular infrastructure is limited.Although it has not yet been endorsed by the WHO, SS-LAMP represents a promising innovation in the field of TB diagnostics in North Africa.

EasyNAT® TB Assay (Ustar Biotech, China)

The EasyNAT® TB assay (Ustar Biotech, China) is a cartridge-based, isothermal nucleic acid amplification test designed for point-of-care detection of *Mycobacterium tuberculosis* complex. Utilizing cross-priming amplification (CPA) technology, the system operates with a closed cartridge format, reducing contamination risks and requiring minimal hands-on time.Targeted primarily at low-resource settings, EasyNAT TBoffers a rapid diagnostic solution with visual readout suitable for primary care levels. Jia et al. reported a sensitivity of 76% in smear-negative pulmonary TB cases and a specificity of 98% [28].

Although it lacks drug resistance detection capability and is not yet endorsed by WHO, EasyNAT TB's affordability and portability make it a promising candidate for decentralized TB control programs. Its integration into community-based screening initiatives could expand access to molecular diagnostics in underserved regions.

Deeplex® Myc-TB Assay (Genoscreen, France)

The Deeplex® Myc-TB assay (Genoscreen, France) is a targeted deep sequencing assay designed for comprehensive detection of *Mycobacterium tuberculosis* complex, drug resistance mutations, and phylogenetic lineage identification. Utilizing multiplex PCR amplification of key resistance-associated genomic regions followed by next-generation sequencing (NGS), Deeplex-MycTB provides high-resolution resistance profiling covering first- and second-line anti-TB drugs. Clinical studies have shown excellent concordance with phenotypic drug susceptibility testing and whole-genome sequencing, with high sensitivity for resistance mutations and rapid turnaround when processed in equipped laboratories [29].

Deeplex-MycTB is increasingly used in research and surveillance, particularly for its capacity to generate comprehensive resistance data from clinical samples or culture isolates [30]. Its main limitations are the need for sequencing infrastructure and trained personnel, making it more suitable for reference laboratories rather than routine clinical diagnostics.

Table 1. Commercially available molecular assays approved by WHO, FDA, or CE-Marked for rapid detection of *Mycobacterium tuberculosis* and drug resistance.

Test specification	Xpert MTB/RIF	Xpert MTB/RIF Ultra	Xpert MTB/OMNI	Xpert MTB/XDR	COBAS Taq Man MTB	GenoType MTBDRplus	GenoType MTBDRsl	FluoroType MTB	Truenat MTB	PURE-LAMP	SS-LAMP (MAScIR)	EasyNAT TB	Deeplex-MycTB
Company	Cepheid	Cepheid	Cepheid	Cepheid	Roche Diagnostics	Hain Lifescience	Hain Lifescience	Hain Lifescience	Molbio Diagnostics	Eiken Chemical Co., Ltd.	MAScIR	Ustar Biotechnologies	Genoscreen
Year	2010	2017	2017	2021	2008	2008	2012	2016	2019	2012	2016	2014	2018
Country	USA	USA	USA	USA	Switzerland	Germany	Germany	Germany	India	Japan	Morocco	China	France
Technology	Automateal-time PCR (molec	Automated real-time PCR	Automated real-time PCR	Automated real-	Real-time PCR	Multiplex PCR + reverse	Multiplex PCR + reverse	Real-time PCR with fluores	Real-time micro-PCR	Isothermal amplification (Loop-	Single Step Isothermal amplifi	Isothermal amplification	Targeted NGS

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	ular beacons)	(molecular beacons)	(molecular beacons)	time PCR		hybridization	hybridization	cence melting curve	on chip	mediated)	cation (Loop-mediated)		
Detects	MTB + RIF resistance	MTB + RIF resistance	MTB + RIF resistance	MTB + resistance to INH, FQ, SLID	MTB DNA	MTB + resistance to RIF and INH	MTB + resistance to FLQ and SLID	MTB	MTB	MTB	MTB	MTB	MTB and resistance mutations
Target	rpoB gene	rpoB gene	rpoB gene	rpoB, katG, inhA, gyrA, gyrB, eis, rrs	16S rRNA	rpoB, katG, inhA	gyrA, rpo, rrs, eis genes	IS6110 and 23S rRNA	IS6110	IS6110 or 16S rRNA	IS6110	IS6110	24+ resistance genes and spoligotyping
Time to Results	2 hours	90 min	100 min	90 min	2.5 hours	5 hours	5 hours	3 hours	60 min	60–90 min	45 min	90 min [29]	24–48 hours
Approval Status	WHO-endorsed, FDA-approved	WHO-endorsed	WHO-endorsed	CE-IVD, WHO-endorsed (limited use)	FDA approved	WHO-endorsed	WHO-endorsed	CE-IVD	WHO-endorsed, CE-IVD	CE-marked, WHO-evaluated	CE-marked	China NMPA approved	CE-marked, WHO-endorsed
Recommendations	Initial diagnostic test for patients with suspected active TB, including MDR-TB or HIV-TB	Replacement for Xpert MTB/RIF due to increased sensitivity, especially for smear-negative samples	For use in peripheral settings where GX-IV not feasible	Add-on test to assess additional resistance in RIF-resistant TB cases	Confirmatory test on smear-positive specimens in reference labs	Initial test for resistance detection on smear-positive or cultured isolates	Used to detect second-line drug resistance in confirmed RR or MDR TB	High-throughput labs, not POC	POC use in decentralized settings	Alternative to smear microscopy in low-resource settings	Confirmatory test on smear-positive specimens	Useful for pediatric TB and EPTB	Second-line DST and surveillance
Benefits	Fast, widely used, High sensitivity, automated minima	Higher sensitivity for HIV+ and pediatric cases,	Portable, battery-operated, suitable for remote areas	Rapid, comprehensive resistance pane	High throughput, good sensitivity and specificity,	Detects mutations linked to MDR-TB, rapid DST	High sensitivity for FLQ and SLID resistance, useful	Automated, closed system	Portable, battery-operated	No thermocycler needed, visual readout	No need to open tubes; low-cost; minimal equipment	Compact, simple interface [31]	High multiplexing, detailed resistance profiling

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	l hands-on time	Improved LoD		l; runs on standard GeneXpert platform	automated processing on COBAS systems	alternative	for treatment guidance						
Limitations	Inferior performance in smear-negative cases, requires stable electricity	Possible false positives in patients with prior TB treatment	Limited rollout; not widely available; performance comparable to Xpert	Cost, limited availability in low-resource settings	- Requires heavy instrumentation, not suitable for low-resource or POC settings	Limited performance in smear-negative specimens, manual processing	Limited sensitivity in smear-negative samples, doesn't cover all resistance mutations	Requires specialized equipment	Multi-step workflow	Lower sensitivity vs PCR; Risk of contamination	Performance yet to be validated in all settings	Limited validation outside China	Requires NGS platform and software
References	[4,7,17]	[6,8,15]	[6,16]	[6,15]	[11,18–20]	[11,21,22]	[11,22]	[11,24]	[11,25]	[10,26]	[27]	[28]	[29,30]

Table 2. Diagnostic accuracy of endorsed molecular assays for rapid detection of drug-resistant tuberculosis.

Test	Drug Resistance Detection	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Reference
Xpert MTB/RIF	Rifampicin	89% [85–92%]	98% [98–99%]	[4]
Xpert MTB/RIF Ultra	Rifampicin	95% [92–97%]	96% [94–98%]	[8,15]
COBAS TaqMan MTB	No DST (only MTB detection)	96% [78–86%]	98% [97–99%]	[19,20]
GenoType MTBDRplus	Rifampicin, Isoniazid	95% [90–97%]	98% [98–100%]	[21,22]
FluoroType MTB	No DST (only MTB detection)	89% [84–91%]	98% [97–99%]	[24]
Truenat MTB	Rifampicin (Truenat MTB-RIF Dx)	88% [75–86%]	98% [97–99%]	[25]
PURE-LAMP	No DST (only MTB detection)	98% [95.5 to 99.9%]	99% [98.1 to 99.9%]	[26]
SS-LAMP	No DST (only MTB detection)	82.93 % [67.94 - 92.85]	99.14% [95.29 – 99.98]	[27]
EasyNAT TB	No DST (only MTB detection)	85% [78–90%]	98% [88–97%]	[28]
Deeplex-MycTB	Rifampicin, INH, FQ, SLID	98–100% agreement with WGS	98–100% agreement with WGS	[29,30]

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Conclusion

The increasing portfolio of molecular diagnostic tools for tuberculosis (TB) has reshaped the landscape of TB detection and drug resistance profiling. From automated cartridge-based systems like Xpert MTB/ultra and COBAS MTB to line probe assays such as GenoType MTBDRplus and MTBDRsl, and from decentralized platforms like Truenat MTB, TB-LAMP, EasyNAT, and SS-LAMP to advanced sequencing-based assays like Deeplex-MycTB, each technology offers unique advantages tailored to specific diagnostic needs and healthcare settings. These assays have contributed to shortening the diagnostic delay, enabling earlier treatment initiation, and improving patient management, particularly in high-burden and resource-limited countries. However, significant challenges remain, including the cost of reagents, infrastructure requirements, maintenance, and equitable access in remote areas. No single assay meets all diagnostic needs across all contexts. Therefore, an armada of integrated diagnostic strategies combining centralized high-throughput platforms, decentralized rapid tests, and advanced resistance profiling tools are essential to contribute in global elimination of TB

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Abbreviations

The following abbreviations are used in this manuscript:
TB: Tuberculosis
MTB: Mycobacterium tuberculosis
RIFRifampicin
MDR-TB: Multidrug-Resistant Tuberculosis
XDR-TB: Extensively Drug-Resistant Tuberculosis
WHO: World Health Organization
CE-IVD: Conformité Européenne – In Vitro Diagnostic
PCR: Polymerase Chain Reaction
LPA: Line Probe Assay
NGS: Next-Generation Sequencing
DST: Drug Susceptibility Testing
SS-LAMP: Single-Step Loop-Mediated Isothermal Amplification
TB-LAMP: Tuberculosis Loop-Mediated Isothermal Amplification
CFU: Colony Forming Unit
INH: Isoniazid

References

1. World Health Organization. Global Tuberculosis Report 2023. Geneva: WHO; 2023. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2023. Accessed on 25 June 2025
2. World Health Organization. Guidelines for the treatment of tuberculosis, 2023 update. Geneva: WHO; 2023. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2023. Accessed on 25 June 2025

 doi:10.20944/preprints202507.1227.v1

3. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection. Geneva: WHO; 2021. Available from: https://www.who.int/publications/i/item/9789240029415. Accessed on 25 June 2025
4. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014;(1):CD009593. doi:10.1002/14651858.CD009593.pub3
5. Lewinsohn DM, Leonard MK, LoBue PA, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64(2):111–115. doi:10.1093/cid/ciw778
6. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med. 2010;363(11):1005–1015. doi:10.1056/NEJMoa0907847
7. WHO. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. Geneva: WHO; 2023. Available from: https://www.who.int/publications/i/item/9789241514842. Accessed on 25 June 2025
8. Chakravorty S, Simmons AM, Rowneki M, et al. The new Xpert MTB/RIF Ultra: Improving detection of Mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing. mBio. 2017;8(4):e00812-17. doi:10.1128/mBio.00812-17
9. WHO. WHO consolidated guidelines on tuberculosis. Module 5: Management of tuberculosis in children and adolescents. Geneva: WHO; 2022. Available from: https://www.who.int/publications/i/item/9789240046764. Accessed on 25 June 2025
10. WHO. LAMP TB Assay: Policy update. Geneva: WHO; 2016. Available from: https://www.who.int/publications/i/item/9789241511186. Accessed on 25 June 2025
11. Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: A systematic review and meta-analysis. Eur Respir J. 2014;44(2):435–446. doi:10.1183/09031936.00007814
12. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J Clin Microbiol. 2011;49(4):1202–1205. doi:10.1128/JCM.02268-10
13. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, Dendukuri N, Mandalakas AM. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med. 2015 Jun;3(6):451-61.
14. Foundation for Innovative New Diagnostics (FIND). TB Diagnostics Pipeline 2024. Available from: https://www.finddx.org/what-we-do/programmes/tuberculosis/. Accessed on 25 June 2025.
15. WHO. Meeting Report of a WHO Expert Consultation: The Use of Next-generation Xpert MTB/RIF Ultra Assay for the Detection of Tuberculosis and Rifampicin Resistance. Geneva: World Health Organization; 2017.
16. Penn-Nicholson A, Georghiou SB, Ciobanu N, Kazi M, Bhalla M, David A, Conradie F, Ruhwald M, Crudu V, Rodrigues C, Myneedu VP, Scott L, Denkinger CM, Schumacher SG; Xpert XDR Trial Consortium. Detection of isoniazid, fluoroquinolone, ethionamide, amikacin, kanamycin, and capreomycin resistance by the Xpert MTB/XDR assay: a cross-sectional multicentre diagnostic accuracy study. Lancet Infect Dis. 2022 Feb;22(2):242-249. doi:10.1016/S1473-3099(21)00452-7. Epub 2021 Oct 7.
17. FDA. Summary of Safety and Effectiveness Data (SSED) for Xpert MTB/RIF. U.S. Food and Drug Administration; 2013. From https://www.cdc.gov/tb/php/laboratory-information/xpert-mtb-rif-assay.html. Accessed on 25 June 2025
18. Roche Diagnostics. COBAS® MTB and MTB-RIF/INH: Product Information. Roche Diagnostics; 2019. From https://diagnostics.roche.com/global/en/products/lab/cobas-mtb-rif-inh-58-68-88-pid00000332.html. Accessed On 25 June 2025
19. Bloemberg GV, Voit A, Ritter C, Deggim V, Böttger EC. Evaluation of Cobas TaqMan MTB for direct detection of the Mycobacterium tuberculosis complex in comparison with Cobas Amplicor MTB. J Clin Microbiol. 2013 Jul;51(7):2112-2117. doi:10.1128/JCM.00142-13.

 doi:10.20944/preprints202507.1227.v1

20. de Vos M, David A, Duraisamy K, Nadarajan D, Noroc E, Penn-Nicholson A, Crudu V, Giri S, Maurer FP, Pati S, Stevens W, Scott L, Turuk J, Schumacher SG, Ruhwald M; Study Group. Accuracy of cobas MTB and MTB-RIF/INH for detection of Mycobacterium tuberculosis and drug resistance. J Mol Diagn. 2024 Aug;26(8):708-718.
21. Bwanga F, Hoffner S, Haile M, Joloba ML. Direct susceptibility testing for multidrug-resistant tuberculosis: a meta-analysis. BMC Infect Dis. 2009;9:67. doi:10.1186/1471-2334-9-67
22. WHO. The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin: policy update. Geneva: World Health Organization; 2016.available from: https://www.who.int/publications/i/item/9789241511261. Accessed on 25 June 2025
23. Miotto P, Cabibbe AM, Feuerriegel S, et al.
24. Hofmann-Thiel S, Hoffmann H. Evaluation of Fluorotype MTB for detection of Mycobacterium tuberculosis complex DNA in clinical specimens from a low-incidence country. BMC Infect Dis. 2014 Feb 5;14:59. doi:10.1186/1471-2334-14-59.
25. Ssengooba W, Katamba A, Sserubiri J, Semugenze D, Nyombi A, Byaruhanga R, Turyahabwe S, Joloba ML. Performance evaluation of Truenat MTB and Truenat MTB-RIF DX assays in comparison to GeneXpert MTB/RIF Ultra for the diagnosis of pulmonary tuberculosis in Uganda. BMC Infect Dis. 2024 Feb 13;24:190.
26. Boehme CC, Nabeta P, Henostroza G, Raqib R, Rahim Z, Gerhardt M, Sanga E, Hoelscher M, Notomi T, Hase T, Perkins MD. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol. 2007 Jun;45(6):1936-1940. doi:10.1128/JCM.02352-06
27. Bentaleb EM, Abid M, El Messaoudi MD, et al. Development and evaluation of an in-house single-step loop-mediated isothermal amplification (SS-LAMP) assay for the detection of Mycobacterium tuberculosis complex in sputum samples from Moroccan patients. BMC Infect Dis. 2016;16:517. doi:10.1186/s12879-016-1864-9
28. Zhang Z, Du J, Liu T, Wang F, Jia J, Dong L, et al. EasyNAT MTC assay: a simple, rapid, and low-cost cross-priming amplification method for the detection of Mycobacterium tuberculosis suitable for point-of-care testing. Tuberculosis (Edinb). 2021 Oct;131:102155. doi:10.1016/j.tube.2021.102155.
29. Schwab TC, Perrig L, Göller PC, Guebely De la Hoz FF, Lahousse AP, Minder B, et al. Targeted next-generation sequencing to diagnose drug-resistant tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis. 2024 Oct;24(10):1162-1176. doi:10.1016/S1473-3099(24)00212-8.
30. Bagratee TJ, Studholme DJ. Targeted genome sequencing for tuberculosis drug susceptibility testing in South Africa: a proposed diagnostic pipeline. Access Microbiol. 2024 Feb 16;6(2):000740