

Rapid Molecular Assays for the Diagnosis of Drug-Resistant Tuberculosis

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Abstract: The recognition that drug-resistant tuberculosis (DR-TB) poses a major threat to global tuberculosis (TB) control efforts has catalysed the development of new and urgently needed TB diagnostics. The full beneficial impact of the subsequent flood of new TB diagnostic tests into the market can only be realised if these diagnostic tests are readily accessible to TB programs and contribute to improved patient outcomes. Although phenotypic drug-susceptibility testing remains the gold standard, an improved understanding of the relationship between mutations and different levels of drug resistance coupled with the advantages of molecular diagnostics could result in rapid molecular diagnostic tests replacing phenotypic drug-susceptibility testing. Successful diagnostics need to diagnose all forms of drug-resistant TB prevalent in each geographic region. Given the finite number and often limited availability of effective drugs for DR-TB, the diagnostic test must be able to detect all clinically important types of resistance to available anti-TB drugs. However, less comprehensive resistance profiling may be sufficient in settings where extensively drug-resistant TB (XDR-TB) and pre-XDR are absent. Rapid molecular diagnostic tests for DR-TB detection suitable for DR-TB endemic settings should be accurate, inexpensive, suitable to be performed on an easily accessible sample, detect prevalent circulating drug-resistant strains, and provide results within a short turnaround time to enable timely treatment initiation. In this review, we appraise the wide range of molecular diagnostics for DR-TB endorsed by the World Health Organisation, discuss the challenges in the development and rollout of rapid molecular DR-TB tests in low- and middle-income countries, and highlight user perspectives and cost-effectiveness factors that influence their utility.

Keywords: tuberculosis, drug resistance, molecular assays, point of care, diagnosis

Plain Language Summary

Drug-resistant TB is a major threat to global TB control. Rapid molecular diagnostic tests are crucial for earlier diagnosis, shortened time to treatment initiation, improved retention in care, and improved treatment outcomes. Rapid molecular tests must concord with phenotypic resistance and be capable of diagnosing resistance to a comprehensive list of available drugs for the treatment of DR-TB. In addition, these tests should be accurate, inexpensive, suitable for application on an easily accessible sample, and have a short turnaround time. In this review, we appraise the wide range of molecular diagnostics for DR-TB endorsed by the World Health Organisation, discuss the challenges in the development and rollout of rapid molecular DR-TB tests in low- and middle-income countries, and highlight user perspectives and cost-effectiveness factors that influence their utility.

Introduction

Drug-resistant *Mycobacterium tuberculosis* (*Mtb*) remains a significant challenge to global tuberculosis (TB) care and control efforts. According to the Global TB Report 2021, approximately 10 million people fell ill with TB, including ~50,000 incident cases of drug-resistant TB (DR-TB).¹ Over 1.4 million people died from TB in 2020, with more than 95% of these deaths occurring in low- and middle-income countries.¹ Despite a 20% decrease in TB mortality from 2015 to 2020, the prevalence rates of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) continue to rise among both new and

previously treated TB cases.² To a large extent, the COVID-19 pandemic has reversed years of gains in TB detection, notification, and treatment.¹ More than 40% of the estimated 10 million TB cases in 2020 were not diagnosed, predominantly due to the globally reduced access to TB diagnostic services. Moreover, only 59% of the 4.8 million people diagnosed with TB in 2020 received a bacteriologically confirmed diagnosis, only half of whom were diagnosed by a WHO-endorsed rapid molecular test. The poor access to sensitive diagnostic tests is a major limitation to our ability to detect drug resistance. Recent data suggest that person-to-person transmission of MDR- and XDR-TB is now the driving force of the global DR-TB burden although acquired drug resistance continues to contribute to the evolution of drug resistance in the context of TB treatment mismanagement.^{1,2} In many high-burden settings, delays in access to rapid DR-TB diagnosis and effective treatment perpetuate transmission and fuel the epidemic.

Although several advances have been made in diagnosing DR-TB, no reliable, simple point of care (POC) test exists to diagnose the disease. A POC test that readily detects active TB would reduce diagnostic delays, interrupt transmission with appropriate therapy, and address many current global TB control gaps.³ Improving existing testing means developing highly sensitive and specific assays to diagnose DR-TB and ensuring simplicity, affordability, technical robustness, and the capacity for rapid up-scaling at the most decentralised level by health care workers with minimal training.⁴ The critical advantage of a POC test is its potential ability to enable immediate treatment initiation at the point of presentation, thus circumventing the problem of patient dropout.⁵

Several nucleic acid-based assays that detect TB drug resistance have been developed over the years; however, significant shortcomings need to be addressed for optimal application. In this review, we appraise the wide range of molecular diagnostics for DR-TB endorsed by the World Health Organisation, discuss the challenges in the development and rollout of rapid molecular DR-TB tests in low- and middle-income countries, and highlight user perspectives and cost-effectiveness factors that influence their utility.

Methods

Manuscripts were identified through searches of PubMed using the terms: “Xpert”, “GeneXpert”, “point of care”, “tuberculosis”, “drug-resistant”, “diagnostics”, “line probe assay”, “NAATS”, and “implementation”. We included manuscripts published between 2000 and 2021 and limited the search to English-language publications. We manually searched all references of included articles to identify additional articles not captured by the electronic search. We included all manuscripts reporting on at least one diagnostic test for detection of *Mtb* and resistance to at least one TB drug. We excluded studies focused purely on culture-based techniques for the detection of *Mtb* or drug resistance.

Characteristics of Point of Care for DR-TB Diagnostic Tests

A minimum set of characteristics of future POC DR-TB diagnostic tests has previously been proposed (Table 1). Notably, POC tests require the ability to maintain high levels of sensitivity and specificity, irrespective of the local prevalence of TB, and must be capable of diagnosing TB, irrespective of the site of disease, type of specimen, or severity of the disease.⁶ Moreover, the test should simultaneously detect first- and second-line drug-resistance-conferring mutations. Overall, the main advantage of a POC diagnostic for DR-TB is the ability to detect TB and profile drug resistance without the support of laboratory infrastructure; this increases access to the test, eliminates the need to transport samples, and reduces travel time from weeks (or months) to hours. Same-day results from POC tests will enable single-visit treatment initiation, improved quality of care, and help mitigate pre-treatment loss to follow-up.⁷

An adequate expectorated sputum sample is required at the primary care level to diagnose pulmonary TB.⁸ However, in up to a third of TB cases, an adequate biological sample is not readily available or has a deficient concentration of TB bacilli rendering the sample smear-negative.⁹ The availability of alternative techniques such as sputum induction, bronchoscopy, gastric aspiration and organ biopsy is limited in TB endemic settings. Therefore, on the one hand, attention has been focused on other biological samples, such as exhaled breath, blood and urine, which are easy to collect and process.⁶ On the other hand, improved sensitivity and specificity of existing tests will improve TB detection even in paucibacillary samples. Urine as a biological fluid for diagnostic testing is particularly attractive because it is easy to collect compared to other fluids such as sputum and serum, is readily available, and contains TB-specific proteins and DNA in patients with TB.^{10–15} The collection of exhaled breath condensate is a completely non-invasive method of

Table I Minimum Characteristics of a Future Point of Care DR-TB Diagnostic Test

Test Specification	Requirement
Sensitivity and specificity (adults)	For pulmonary TB only, regardless of HIV status: Sensitivity: 95% for smear-positive and >60% for smear-negative, culture-positive samples Specificity: 95% compared to culture
Sensitivity and specificity (children)	For pulmonary TB only, regardless of HIV status: Sensitivity: 80% compared to culture of any sample type Specificity: 95% compared to culture
Drug resistance	Should be able to screen for resistance to currently available and new generation first- and second-line drugs First line: streptomycin, rifampicin, isoniazid, pyrazinamide and ethambutol Second line: levofloxacin, moxifloxacin, bedaquiline, linezolid, clofazimine, cycloserine, ethambutol, delamanid, pyrazinamide, amikacin, ethionamide, and p-aminosalicylic
Sample type	Adults: urine, saliva, exhaled breath, blood, or sputum Children: urine, saliva, blood Samples should not require refrigeration
Sample processing	Minimal protocol steps (maximum 3), ability to be performed at a bedside or in a BSL I facility, processing that is not time-sensitive
Sample throughput	≥20 tests per day performed by one technical laboratory staff member
Robust	Shelf-life >24 months (including reagents), stable at high temperatures for shorter time periods, can be battery-powered, easy and environmentally friendly waste disposal, minimal maintenance requirement and utilised existing equipment (repurposed technology)
Cost	Affordable and easily accessible in high-burden countries, ideally <\$10/test
Training	Used by health care workers with minimal training and minimum skill level
Time to results	3 hours maximum (patient must receive results the same day),
Result interpretation	Easy-to-read, unambiguous, simple “yes”, “no”, or “invalid” answer

Abbreviations: BSL I, biosafety level I; HIV, human immunodeficiency virus; TB, tuberculosis.

sampling the respiratory tract, which can be performed with minimal patient effort using portable equipment, and which can be repeated several times within short intervals.¹⁶ Breath analysis has predominantly focused on the detection of volatile organic compound with TB-specific signatures.¹⁷ Recent advances have focused on novel exhaled breath aerosol collection and analysis techniques, which may demonstrate the potential to detect *Mtb* proteins and lipids.^{17,18}

WHO-Endorsed Molecular Diagnostics for Detection of TB and Drug-Resistance

The WHO recommends that TB Programmes transition from microscopy as the initial TB diagnostic test to rapid molecular diagnostics that allow for the simultaneous detection of *Mtb* and drug resistance.² Sputum smear is one of the oldest front-line diagnostic techniques currently used to detect acid-fast bacilli directly from sputum.¹⁹ This is a simple and inexpensive technique, but its sensitivity is limited, requiring 10000 bacilli per mL of sputum to reliably detect *Mtb*,²⁰ and results are subject to interpretation by the reader, which can result in variability of results.²¹ Moreover, smear microscopy cannot differentiate between *Mtb* and non-tuberculous mycobacteria, viable and nonviable organisms, or drug-susceptible and drug-resistant strains.²² *Mtb* can also be cultured from clinical specimens using various solid and liquid culture media such as (i) Lowenstein – Jensen, (ii) Middlebrook 7H10/11, and (iii) Middlebrook 7H9.²³ The cultivation and susceptibility testing of *Mtb* has evolved from solid culture to automated systems, ie, BACTEC-Mycobacteria Growth Indicator Tube (MGIT) and other liquid media, and represents a significant advance in TB culture-based diagnostics. The BACTEC-MGIT 960 method overcame many drawbacks of the BACTEC 460 TB system, such as the need for radioactive substances and needles.²⁴ However, phenotypic DST still has several limitations,

including costly, time-consuming culture-based methods that generate results 2–8 weeks after specimen collection, increased risk of culture contamination, and the need for complex laboratory infrastructure.²⁵

During the last decade, the WHO has endorsed a range of highly sensitive and specific molecular diagnostic techniques for detecting *Mtb* nucleic acids and drug resistance to overcome these challenges (Table 2). Currently, the molecular diagnostic assays, including Xpert MTB/RIF Ultra and Xpert MTB/XDR (Cepheid, Sunnyvale, CA), Truenat MTB-RIF Dx (Molbio), line probe assays (LPA) (GenoType MTBDRplus, MTBDRsl and GenoScholar NTM+MDRTB II; Hain Lifescience GmbH, Nehren, Germany), and moderate complexity automated NAATS, can rapidly identify a limited number of mutations in first-line and second-line antibiotic resistance-conferring genes.² These assays have significantly improved *Mtb* detection and drug susceptibility testing (DST) and support rapid turnaround times.

Xpert MTB/RIF, Xpert MTB/RIF Ultra and Xpert MTB/XDR Assays

The Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA) uses semi-quantitative real-time polymerase chain reaction (PCR) to rapidly identify *Mtb* complex and detect rifampicin (RIF) resistance, a surrogate marker for MDR-TB, by amplifying a fragment containing the 81-base pair hotspot region of the *rpoB* gene (codons 507–533) that is then hybridised to five molecular beacon probes.^{26–31} Each probe covers a separate sequence and is labeled with a fluorescent dye.³² Several large-scale trials have assessed the accuracy of Xpert MTB/RIF in pulmonary and extrapulmonary TB.^{33–39} Compared to standard culture-based DST, sensitivity and specificity for smear-positive samples can reach 100 and 99%, respectively, and 62.6 and 99% for smear-negative samples.^{6,33,35,40–42} For the detection of RIF resistance in regions with high DR-TB prevalence, the sensitivity and specificity are ~94% and ~97%, respectively.^{2,6} The time to diagnosis of RIF resistance has been significantly reduced from 2 to 8 weeks (culture and DST) to two hours, enabling rapid initiation of DR-TB treatment.³¹ Moreover, Xpert MTB/RIF increases the *Mtb* detection rate (23%) among culture-confirmed cases compared with smear microscopy, with higher accuracy of *Mtb* detection, limiting the misdiagnosis between *Mtb* and non-tuberculous mycobacteria.^{27,35} However, some studies reported false-positive results with Xpert MTB/RIF due to silent mutations [eg, at codon 514 of the *rpoB* gene]²⁹ and false-negative results because of the inability to detect RIF-resistance conferring mutations outside the *rpoB* hotspot region.⁴³ In addition, this test does not detect mutations in genes associated with isoniazid (INH) resistance but uses RIF resistance as a proxy for MDR-TB detection.⁴⁴ Consequently, many INH mono-resistant TB cases are erroneously classified as drug-susceptible.

POC feasibility for Xpert MTB/RIF has been demonstrated in well-resourced clinics primarily because it is semi-automated and requires minimally trained non-technical personnel.^{45–48} Despite these advancements, several limitations are noteworthy, such as suboptimal sensitivity in special populations (HIV-positive adults, children, patients with extrapulmonary TB),^{49,50} the need for an uninterrupted power supply, and dependence on mild ambient conditions (15°C–30°C).⁵¹ Despite being designed for POC use, the Xpert MTB/RIF assay is predominantly implemented in centralised laboratories.⁵² Furthermore, despite reducing the time to TB diagnosis, there is no evidence of Xpert MTB/RIF reducing morbidity and mortality among TB patients in high TB/HIV burden settings.^{53–56}

The next-generation Xpert MTB/RIF Ultra (Cepheid Inc., Sunnyvale, CA, USA) assay has better *Mtb* identification and RIF resistance detection capabilities. It has a larger amplification chamber to increase the amount of sputum and two additional targets (IS1081 and IS6110) to identify *Mtb*.^{57,58} The bacilli limit of detection (LOD) for Xpert MTB/RIF Ultra is four-fold lower compared to Xpert MTB/RIF, at 15.6 CFU/mL vs 112.6 CFU/mL. The increased sensitivity of Xpert MTB/RIF Ultra is due to additional probes capable of detecting very low concentrations or “traces” of TB, making it more appropriate for use among children and people living with HIV/AIDS who often have paucibacillary or disseminated forms of TB.⁵⁷ While the sensitivity of the Xpert MTB/RIF Ultra is 5% higher than that of Xpert MTB/RIF, specificity is 3.2% lower.⁵⁹ Various studies reported a higher incremental sensitivity among paucibacillary forms of TB disease (childhood TB, HIV-associated TB, or extrapulmonary TB).^{60–62} However, specificity is lower in patients with a previous history of TB, generating false-positive results due to the detection of non-viable *Mtb*. When Xpert MTB/RIF Ultra probes detect only “traces” of TB - and not more substantial concentrations of TB bacteria - an inconclusive test result is produced.⁶³ Furthermore, false positivity is associated with the recent previous TB, a high cycle threshold (>30), and a chest radiograph without features of active TB.⁴¹ Therefore, Xpert MTB/RIF Ultra results should be interpreted carefully, together with a comprehensive clinical history and physical examination^{2,4} in patients with recent previous TB.

Table 2 Performance of WHO-Endorsed Molecular Diagnostics for Drug-Resistant TB Diagnosis and Drug-Susceptibility Testing

Assay (Manufacturer) Year	Mtb and Drug Resistance (Sensitivity, Specificity) %	DR-TB Category	Cost Per Test	Time to Result	Specimen Types	Benefits	Limitations
GeneXpert assays							
XPRT MTB/RIF (Cepheid) 2010	Mtb: (85, 98) Drug resistance: RIF (96, 98)	RR-TB	\$10	2 hours	Raw sputum Concentrated sputum sediments	- One- step process- automated - Results available in <2 hours - Requires fewer biosafety measures than culture/LPA, so can be used in lower level laboratories - High sensitivity and specificity - Multi- platform: can be used for HIV, hepatitis C diagnosis and viral load monitoring - Can be used on extrapulmonary TB samples	- Reliant on electricity - Expensive - Cannot be used to track treatment progress - Requires annual calibration
XPRT MTB/RIF Ultra (Cepheid) 2017	Mtb (90, 96) Drug resistance: RIF (94, 98)	RR- TB	\$10	2 hours	Raw sputum Concentrated sputum sediments		
XPRT MTB/XDR (Cepheid) 2021	Mtb: none Drug resistance: INH (94.2, 98); FQ (93.1, 98.3); AMK (86.1, 98.9); ETH (98, 99.7); KAN (98.1, 97); CAP (70, 99.7)	RR- TB; MDR- TB; Pre- XDR- TB;	N/A	1.5 hours	Raw sputum Concentrated sputum sediments		
Truenat assay							
TRUENAT MTB-RIF Dx (Molbio) 2020	Mtb: none Drug resistance: RIF (84, 97)	RR- TB	\$1240	1 hour	Sputum Extrapulmonary body fluids	- Results available in <1 hour - Multi- platform: can be used for hepatitis C, Human Papillomavirus, SARS- CoV2	- Several manual steps that need to be performed by skilled personnel
Moderate complexity automated NAATs							
RealTime MTB RIF (Abbott) 2019	Mtb: none Drug resistance: RIF (94.8, 100); INH (88.3, 94.3)	RR-TB; MDR-TB	N/A	10.5hours	Raw sputum Bronchial alveolar lavage NALC sediment of sputum or bronchial alveolar lavage	-High sample throughput -Each assay has specific multi-platform benefits for HIV-1, HBV, HCV, HPV, SARS-CoV2	-Reliant on electricity -Expensive equipment and specialised training required
BD MAX MDR-TB (Becton Dickson) 2021	Mtb: none Drug resistance: RIF (90, 95); INH (82, 100)	RR-TB; MDR-TB	N/A	4 hours	Raw sputum Concentrated sputum sediments		
Cobas MTB-RIF/INH (Roche) 2021	Mtb: none RIF (97.2, 98.6); INH (96.9, 99.4)	RR-TB; MDR-TB	N/A	4.5 hours	Raw sputum Sputum sediment Bronchoalveolar lavage		
FluoroType MTBDR (Hain) 2021	Mtb: none Drug resistance: RIF (98.9, 100); INH (91.7, 100)	RR-TB; MDR-TB	N/A	3 hours	Decontaminated sputum		

(Continued)

Table 2 (Continued).

Assay (Manufacturer) Year	Mtb and Drug Resistance (Sensitivity, Specificity) %	DR-TB Category	Cost Per Test	Time to Result	Specimen Types	Benefits	Limitations
Line probe assays							
GenoType MTBDR _{plus} (Hain) 2008	<i>Mtb</i> : None Drug resistance: RIF (98.2, 97.8); INH (95.4, 98.8)	RR-TB; MDR-TB	\$7,50	5 hours	Decontaminated sputum Cultured material (solid/liquid medium)	-Can be performed from pulmonary specimen and from culture material -Results are obtained in 5hrs -Fast detection of INH and RIF resistance, allows early, appropriate treatment, which reduces transmission and spread of MDR-TB.	-Cannot fully replace methods like conventional cultures -Not as fast as Xpert -Requires complex laboratory infrastructure and expensive equipment -Requires well-trained staff -Requires BSL3 -High number of uninterpretable results is high -Target coverage is limited to the main mutations
GenoType MTBDR _{sl} (Hain) 2016	<i>Mtb</i> : none Drug resistance: FLQ (100, 98.9); AMK (93.8, 98.5); CAP (86.2, 95.9)	RR-TB; MDR-TB	\$7,50	5 hours	Decontaminated sputum Cultured material (solid/liquid medium)		

Abbreviations: HIV, human immunodeficiency virus; *Mtb*, *Mycobacterium tuberculosis*; TB, tuberculosis; RR-TB, rifampicin-resistant tuberculosis; MDR-TB, multidrug-resistant tuberculosis; N/A, not applicable; RIF, rifampicin; INH, isoniazid; FLQ, fluoroquinolone; AMK, amikacin; KAN, kanamycin; ETH, ethionamide; CAP, capreomycin.

The novel Xpert MTB/XDR assay (Cepheid Inc., Sunnyvale, CA, USA) is also a rapid, cartridge-based assay that is intended to be used as a reflex or follow-up test to any *Mtb* positive result for the detection of first- and second-line drug-resistance in sputum specimens.⁶⁴ In specimens where *Mtb* has been detected, the Xpert MTB/XDR assay can also detect resistance-conferring mutations of INH (target genes: *inhA* promoter, *katG*, *fabG1*, *oxyR-aphC* intergenic region), fluoroquinolones (FQs) (*gyrA* and *gyrB*), ethionamide (ETH) (*inhA* promoter), and second-line injectable drugs (*rrs* and *eis* promoter).⁶⁵ The Xpert MTB/XDR assay is a fully integrated and automated system utilising the exact sample processing techniques as the Xpert MTB/RIF and Ultra. Additionally, this assay can be run on existing GeneXpert platforms equipped with 10-colour modules. The rapid profiling of extended drug resistance is a fundamental benefit of the Xpert MTB/XDR assay.⁶⁴ It also has minimal biosafety requirements, offers quick turnaround times and can be operated by health care professionals with limited training. Traditional culture-based DSTs typically take several weeks compared to the Xpert MTB/XDR assay, which produces results in less than 90 minutes.⁶⁴

Several studies have also reported this assay's high performance and specificity for detecting resistance to INH and second-line injectable drugs.^{57,65–67} The first prospective study that assessed the clinical diagnostic accuracy of the Xpert MTB/XDR assay for INH, FQ, ETH, and second-line TB drug resistance conducted in Africa, Europe, and South-East Asia reported a resistance detection sensitivity of 94% for INH, 95% for FQs, 54% for ETH, 73% for amikacin, 86% for kanamycin, and 61% for capreomycin. Specificity for all drugs was greater than 98%.⁶⁸

While significant specificity and sensitivity data describing the Xpert MTB/XDR assay's ability to detect DR-TB rapidly exists, shortcomings associated with this assay need to be acknowledged. The assay detects mutations only in "hot spots" of target resistance-conferring genes. Moreover, it cannot detect resistance against newer/repurposed drugs now included in the WHO-approved DR-TB treatment regimen (bedaquiline, delamanid, linezolid, clofazimine, carba-penems, and pyrazinamide). The 18-month cartridge shelf-life and the requirement for annual refitting and recalibration⁶⁴ of the GeneXpert 10-colour multiplex technology for both new and pre-existing Cepheid GeneXpert Instrument Systems are significant additional limitations, especially in low- and middle-income countries. While the assay is attractive for decentralisation, its optimal placement in different locations needs to be guided by local diagnostic algorithms and local feasibility and cost-effectiveness. Moreover, POC implementation will be limited in high-burden countries due to cost, logistical constraints and poor infrastructure.⁶⁹

Truenat Assays

In 2020, the WHO also recommended Truenat MTB, MTB Plus, and the RIF-resistance detection reflex assays (Truenat MTB-RIF Dx) (Molbio Diagnostics, India) as initial tests for the detection of TB and RIF resistance in all people being evaluated for pulmonary TB.² Truenat uses real-time micro-PCR to detect the presence of major mutations (single nucleotide polymorphisms) in the *Mtb* genome that are known to cause resistance to RIF.⁷⁰ A large multicentre clinical trial assessed the diagnostic accuracy of the Truenat assays MTB and MTB Plus to diagnose pulmonary TB and the MTB-RIF Dx assay to diagnose RIF resistance in symptomatic adults in India, 24% of whom were sputum culture-positive. Sensitivity and specificity for each assay were as follows: Truenat MTB: 73% (95% CI 67–78%) and 97% (95% CI 96–98); Truenat MTB Plus: 79% (95% CI 74–84) and 96% (95% CI 95–97) and Truenat MTB-RIF Dx: 84% (95% CI 62–94) and 94% (95% CI 90–97).⁷¹

The semi-automated Truenat tests are performed using the Trueprep sample preparation device that extracts DNA and the Truelab PCR device that produces a result in under one hour. Unlike the fully automated Xpert tests that simultaneously test for TB and RIF resistance, the Truenat test series requires several manual steps to be performed by trained technical laboratory staff. Trueprep and Truelab instruments are battery-powered, run-up to a full eight-hour day on a single battery charge, and may be operated without air conditioning at temperatures $\leq 40^{\circ}\text{C}$, enabling them to be positioned in microscopy centres.⁷⁰

Moderate Complexity Automated NAATs

High-throughput molecular tests highlighted in this section are automated, highly sensitive and specific and use the same PCR technology as rapid molecular tests such as Xpert and Truenat. Several manufacturers have developed moderate complexity automated NAATs for detecting TB and resistance to RIF and INH on high throughput platforms in

laboratories.⁷² Currently available high-throughput molecular tests for resistance to RIF and INH include Abbott's RealTime MTB RIF/INH; BD MAX[®] MDR-TB, Roche's Cobas MTB-RIF/INH, and Fluoro-Type (FT) MTBDR.² Tests belonging to this class are faster and less complex to perform than phenotypic culture-based DSTs and LPA and have the advantage of being automated after the initial sample preparation step. Moderate complexity automated NAATs may be used as an initial test for the detection of TB and resistance to both first-line TB drugs simultaneously (RIF and INH). They offer the potential for the rapid provision of accurate results (important to patients) and for testing efficiency where high volumes of tests are required daily. Hence, these technologies are suited to areas with a high population density and rapid sample referral systems.

Abbott Molecular has two NAATs for TB, one for the detection of *Mtb* (RealTime MTB-96 samples per run) that targets both the IS6110 genetic element and the *PstSI* (*Rv0934*) gene and the other for the detection of both RIF and INH resistance (RealTime MTB RIF/INH). The RIF and INH resistance tests use eight dye-labelled probes to detect variants in the RIF resistance determining region of the *rpoB* gene and four probes to detect INH resistance, with two probes each for the *katG* and *inhA* genes.⁶ The sensitivity and specificity of the Abbott RealTime MTB and INH/RIF assays for detection of *Mtb* and detection of INH resistance were 92.4% (95% CI 83.6–96.9) and 95.4% (95% CI 91.1–97.7) and 84.2% (95% CI 60.4–96.6) and 100% (95% CI 89.7–100), respectively. In the case of RIF resistance, no discrepant results were observed,⁷³ a finding supported by several other studies.^{74–77}

The multiplexed real-time PCR (MAX MDR-TB) (Becton Dickinson) NAAT platform using five-colour detection enables direct detection of *Mtb* in raw sputum or concentrated sputum sediments. This test satisfies the WHO consolidated guidelines for *Mtb* diagnostics by providing rapid TB detection using multicopy genomic elements IS6110 and IS1081 together or singly.² To detect resistance to RIF, the test targets codons 426–452 of the *rpoB* gene; for detecting resistance to INH, the test targets both the *inhA* promoter region and the 315 codons of the *katG* gene.⁷⁸ A recent diagnostic accuracy study using the BD MAX MDR-TB test found sensitivity and specificity of 90.6% and 98.5%, and 82.5% and 98.9% for detection of *Mtb* in pulmonary and extrapulmonary samples, respectively. For detecting INH and RIF resistance, sensitivity and specificity were 71.4% and 96.8%, and 100% and 93.9%, respectively.⁷⁹ Similar reports of the diagnostic accuracy of the BD MAX MDR-TB assay have been reported from a diversity of geographic and TB prevalence settings.^{79–84} Overall, the BD MAX[™] MDR-TB assay is automated and integrated and requires a stable source of electricity; 24 specimens can be tested in one run and the turnaround time from the start of testing to the result is four hours. This platform is expected to be most suitable for use in high throughput central laboratories where minimal operator hands-on time is desirable.

Roche Cobas MTB-RIF/INH is an automated, qualitative real-time PCR test designed as a reflex test together with cobas[®] MTB to detect RIF resistance-associated mutations of the *rpoB* gene and INH resistance-associated mutations in the *katG* and *inhA* genes. The test is intended for use on raw sputum, decontaminated sputum, or bronchial alveolar lavage samples, irrespective of smear status.^{1,2} The LOD for this test is 7.6–8.8 CFU/mL.⁶ Sensitivity and specificity for RIF vs INH resistance detection was 88.4% and 97.6% vs 76.6% and 100.0%, respectively.⁸⁵ Similar findings were noted in other comparative studies assessing Cobas MTB-RIF/INH.^{86,87}

The FluoroType MTBDR (FT-MTBDR; Hain Lifescience) is a new in vitro automated molecular-based assay that simultaneously detects *Mtb* mutations associated with RIF and INH in a single reaction without the need for reflexing.^{88,89} Decontaminated sputum and culture isolates can be used as sample material. The FluoroType MTBDR test uses asymmetric excess PCR and light on/off probes. The LOD is 20 CFU/mL for the FluoroType MTBDR assay (FluoroType[®] MDRTB, 2019, Unitaid, 2017). Manual (FluoroLyse) and automated (GenoXtract) options are available for DNA extraction. The FluoroCycler XT for the MTBDR assay is used for amplification and detection. The diagnostic accuracy of FluoroType MTB and MTBDR VER 2.0 assays was assessed in smear-positive and -negative specimens. Sensitivity for detecting *Mtb* complex in smear-positive and smear-negative specimens were 97.9% and 91.8%, respectively. Sensitivity and specificity for detecting RIF versus INH resistance from smear-positive specimens were 96.9% and 97% versus 98.3% and 97.3%, respectively.⁹⁰ Other studies also noted similar findings.^{88,91,92}

While accuracy, speed, and capacity to concurrently test large sample volumes are clear advantages of high-throughput molecular tests, their centralised placement is a clear disadvantage due to reduced patient accessibility and shortened result

turnaround time. Furthermore, test costs are yet to be made public. Hence, affordability to endemic resource-limited settings remains unknown.⁹³

Line Probe Assays

In 2008, the WHO recommended using LPAs as initial DST for first- and second-line TB drugs instead of liquid culture. LPAs are highly accurate and produce results within five hours compared with the two- to six-week turnaround time required for culture-based DST.^{1,2} LPA technology is based on reverse hybridisation of DNA on a visually read test strip, targeting DNA sequences associated with resistance to first-line (RIF and INH) and some second-line TB drugs (fluoroquinolones and second-line injectable drugs).⁹⁴ Current WHO recommended LPAs for initial drug-resistance testing on sputum smear-positive samples include GenoType MTBDRplus and GenoType MTBDRsl (Hain LifeScience GmbH, Germany).²

The GenoType MTBDRplus (First-line Line probe assay; FL-LPA) assay detects *Mtb* complex and drug resistance by targeting specific mutations in the rifampicin resistance determining region of the *rpoB* gene (codon 505 to 533), as well as mutations in the isoniazid resistance determining *inhA* promoter (−16 to −8 nucleotides upstream of *inhA*) and *katG* (codon 315), from direct sputum smear-positive samples or indirect culture isolates.^{95–98} In sputum smear-positive samples, sensitivity and specificity for detecting RIF and INH resistance were 96.7% and 98.8%, and 90.2% and 99.2%, respectively.^{99–103} The sensitivity and specificity of MTBDRplus to detect RIF on culture isolates were 95.1% (95% CI 92.2% to 98.1%) and 100%, respectively. The sensitivity and specificity to detect INH resistance were 96.1% (93.5% to 98.7%) and 96.1% (90.8% to 100%), respectively.¹⁰⁴ The GenoType MTBDRplus enables a rapid result from sputum smear-positive specimens, thereby enabling appropriate treatment; however, the dependence on culture isolates from respiratory tract samples in the case of smear-negative specimens is a major shortcoming of this assay. Furthermore, the MTBDRplus assay, which produces amplicons in an open-tube format, requires an experienced operator, strict adherence to standard operating procedures with a minimum of three separate rooms for DNA extraction, and multiple procedures to minimise the risk of amplicon cross-contamination. These assays also require experienced readers to interpret the hybridised bands, although this can be semi-automated at additional expense.⁹¹

The GenoType[®] MTBDRsl assay (Second Line –Line probe assay; SL-LPA) version 2.0, released in 2015, detects mutations associated with FQ and SLID resistance. The MTBDRsl assay includes the quinolone resistance-determining regions of the *gyrA* gene (codon 85 to 96) and the *gyrB* (codon 536 to 541) gene, as well as the SLID resistance-determining regions of the *rrs* gene (position 1401, 1402 and 1484) and the *eis* gene (from −37 to −2 nucleotides upstream). The assay procedure can be performed directly using a processed sputum sample or indirectly using DNA isolated and amplified from *Mtb* culture. MTBDRsl has a pooled sensitivity and specificity of 86.2% and 98.6%, respectively, for detecting FQ resistance by direct testing, and pooled sensitivity and specificity of 87.0% and 99.5%, respectively, for the detection of SLID resistance.¹⁰⁵ Several other studies assessed the diagnostic accuracy of the GenoType[®] MTBDRsl assay and reported similar findings.^{104,106–108}

In summary, LPAs are rapid, simple, easy to perform and straightforward to interpret (generated manually or automatically). Limitations to using LPAs include the need for complex laboratory infrastructure, including expensive equipment ordinarily available in reference laboratories only.² Furthermore, the high number of indeterminate results and restriction of LPA target coverage to common high-confidence mutations only present additional limitations.

Impediments to the Rollout of Rapid Molecular Assays

The WHO recommends universal access to new rapid molecular TB diagnostic technologies to reduce the high TB morbidity and mortality rates. Underdiagnosis of TB remains a problem, with only two-thirds of those who develop TB currently being detected and only half of these cases being bacteriologically confirmed.² The main reasons for these gaps are inadequate diagnostic capacity and an overreliance on chest radiography and/or sputum smear microscopy as diagnostic tools.²² Patients with HIV-associated TB, sputum smear-negative TB, and drug-resistant TB are the most disadvantaged by the failure of microscopy as a primary diagnostic tool.¹⁰⁹ The diagnosis of HIV-associated and drug-resistant TB is expensive, complex, slow and technically challenging, relying on conventional culture and drug susceptibility testing.¹¹⁰ The long delay (up to several weeks) required to obtain results has devastating consequences for patients who go undiagnosed (and therefore untreated or suboptimally treated), are diagnosed too late, or are lost to follow-up before treatment can be initiated.¹¹¹

Therefore, detecting more TB cases early and rapidly identifying drug resistance is essential for improving individual patient care and interrupting transmission in the community. This requires universal access to modern tools and innovative strategies.^{2,111,112} However, significant economic challenges impede the development and implementation of new diagnostics.

Developer, Policy, and Programmatic Perspectives

The lack of well-defined target product profiles (TPP) remains an ongoing challenge from the test developer's perspective.¹¹³ Prioritisation of test features may differ significantly based on the setting and local disease burden. Considerations in each setting may include the level of automation or integration with multiplexed platforms, test throughput, result reporting requirements, position within a diagnostic algorithm (eg, screening of asymptomatic vs symptomatic patients), location within health systems (POC vs peripheral or centralised laboratory), target populations (HIV-infected, inpatients vs outpatients, adult vs paediatric), target conditions (eg, latent TB vs sub-clinical TB vs active TB; pulmonary vs extrapulmonary TB), drug-resistance profiles (which drugs should be evaluated in a changing treatment landscape) and the availability of well-characterised biological samples.^{113,114} The absence of a validated global biomarker that detects active TB disease poses another challenge, impeding the development of a "one size fits all" TB test.¹¹⁵

Many TB endemic settings incorporate molecular tests in their TB diagnostic algorithm. A significant challenge for incorporating rapid molecular assays from a policy perspective is the lack of data assessing the impact of new TB diagnostic tools on patient outcomes.¹¹⁶ To produce estimates of effectiveness for economic evaluation, studies must also assess clinical outcomes through longitudinal follow-up, which increases study costs and duration, and is rarely measured as part of diagnostic test accuracy studies.¹¹⁴ Furthermore, the performance and impact of implementing new tests within existing diagnostic algorithms are impeded by limited data, and cost-effectiveness studies may be highly context-dependent and may not translate into an "affordable" test.¹¹⁴

There is uncertainty if TB diagnostic programmes can afford recurring investments as multiple TB diagnostic tools emerge into the market. Furthermore, studies assessing TB diagnostic accuracy may only provide limited data on various programmatic considerations such as simplicity, affordability, ability to be used at the bedside, personnel training, required infrastructure and quality assurance.¹¹⁴ To improve existing TB diagnostic tools or to incorporate new adjunctive diagnostics, policymakers and programme implementers need to consider the following favourable characteristics: more efficient, lower cost, reduced sample processing time and reagent costs, shorter time to a result, minimum training and skill-level requirements, while offering the same or better diagnostic accuracy for detection of prevalent DR-TB resistance profiles as the existing programmatic test/s.

Conclusion

The global rollout of WHO-endorsed molecular assays has improved the detection of DR-TB cases. Despite this progress, there is no reliable and rapid POC test for diagnosing DR-TB. To bridge this gap, rapid molecular diagnostic tests for DR-TB detection should be accurate, inexpensive, performed on an easily accessible sample, detect prevalent circulating drug-resistant strains and provide results within a short turnaround time to enable timely treatment initiation, reduce pre-treatment loss to follow-up, and shorten the time to an optimal treatment regimen. Each of the assays included in this review demonstrates their own strengths and weaknesses, and their selection for programmatic implementation will depend heavily on the available skills and resources, the capacity and distribution of laboratory infrastructure, the positioning of the assay within the programmatic algorithm, and the epidemiology and evolution of the local DR-TB epidemic.

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