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DIAGNOSTIC PROFILE

A profile on the Truenat assays for the detection of pulmonary tuberculosis and rifampicin resistance

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ABSTRACT

Introduction: Early diagnosis and treatment initiation are essential to prevent ongoing tuberculosis (TB) transmission and reduce the disease burden. Smear microscopy, which is widely used test for diagnosis, has limited sensitivity and does not detect rifampicin resistance. Truenat is a portable, battery-operated, chip-based test that can be placed in peripheral laboratories, serving as a point-of-care test. It also detects rifampicin resistance, which is crucial in the management of TB.

Areas covered: Our review comprehensively covers the functionality and workflow of the Truenat MTB assay, in addition to its cost-effectiveness and diagnostic accuracy, based on recent evidence. We also discuss the merits and drawbacks of alternative diagnostic tests available for detecting TB and rifampicin resistance.

Expert opinion: Truenat assays are rapid molecular tests that revolutionized TB diagnosis, moving toward peripheral testing. Evidence indicates that the Truenat MTB Plus performs better than the original Truenat MTB, with an approximate LOD of 30 CFU/ml. The 2025 WHO guidelines rely exclusively on data from Truenat MTB Plus. We need larger studies on the newer Truenat MTB Ultima to evaluate its performance on tongue swabs. Additionally, data on the accuracy of Truenat MTB in children remains scarce, emphasizing the importance of further research in this group. Evidence regarding its accuracy in detecting rifampicin resistance is also limited, requiring larger studies and technological advancements. Truenat MTB will remain crucial in the fight to eliminate TB in the future.

PLAIN LANGUAGE SUMMARY

Early diagnosis of tuberculosis (TB) is essential to stop its spread, prevent drug resistance, and reduce deaths and disabilities due to TB. Truenat assays are molecular tests that can detect the bacterial DNA and resistance to rifampicin – a key first-line TB drug within few hours. Truenat devices are portable and battery-operated. They use chip-based PCR technology to amplify the bacterial DNA into million folds and target specific genes like nrdB (MTB assay), nrdz, and IS6110 (MTB Plus assay), IS1081 (Truenat MTB Ultima assay) to detect TB and rpoB (MTB-RIF Dx reflex assay) to detect rifampicin resistance. Unlike other molecular tests like Xpert, Truenat can operate in environments up to 40°C and 80% humidity. This makes it ideal for use in rural or resource-limited settings, eliminating the need for transport of samples to air-conditioned labs. Among 100 adults tested with Truenat MTB, it accurately rules out 87 people without TB and detects 86 people with TB. Truenat MTB Plus performs better than Truenat MTB. More research is needed on its use in children and for detecting drug resistance. The World Health Organization (WHO) recommends Truenat as an initial test for TB, expanding its utility to improve TB diagnosis worldwide.

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KEYWORDS
Truenat MTB assays; pulmonary tuberculosis; rifampicin resistance; *rapid molecular test*; mWRD

1. Introduction

Tuberculosis affected around 10.8 million people, leading to 1.25 million TB deaths worldwide in 2023. India accounted for 26% of the global TB burden [1]. There is a commitment from all countries to achieve the targets of the END TB strategy by 2035. In 2023, there were 8.2 million individuals newly diagnosed with TB and notified globally, out of an estimated 10.8 million incident TB cases. Early TB diagnosis and treatment initiation are essential to prevent ongoing TB transmission and reduce the disease burden. TB diagnosis primarily relies on sputum smear microscopy, which is easy to perform in low-resource field settings. However, smear microscopy is limited in its ability to detect TB disease, with a sensitivity of 20% to 80% in paucibacillary conditions, including TB in children, extrapulmonary TB, and in HIV-TB cases [2]. This is because smear microscopy requires an estimated minimum of 5000 bacilli per milliliter of sputum for a positive test. Further, drug resistance testing guides appropriate treatment initiation in terms of effective drugs for improved treatment outcomes. However, culture for drug resistance testing requires a minimum of 6 to 8 weeks. Therefore, a point-of-care rapid diagnostic test that

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Article highlights

- Diagnosis of pulmonary TB and rifampicin resistance is challenging in the resource-limited setting. Upfront rapid detection of rifampicin resistance is crucial in the management of TB
- Truenat is a portable, battery-operated, chip-based test that can be placed in peripheral laboratories, serving as a point-of-care test and eliminating the need for specimen transport.
- The assay involves a chip-based work-time PCR where the target genes involved are a single-copy target of *nrdB* (MTB assay), *nrdz*, and a multicopy target of IS6110 (MTB Plus assay) for MTB detection (MTB Plus assay), IS1081 (Truenat MTB Ultima assay), and *rpoB* (MTB-RIF Dx reflex assay) for rifampicin detection.
- The infrastructure requirement for Truenat MTB testing is minimal, making it suitable for peripheral laboratories and primary health centers.
- Unlike the Xpert machine, Trueprep and Truelab do not require air conditioning and can operate in environments up to 40°C and 80% humidity.
- Truenat is cost-effective when compared to Xpert.
- A Cochrane review included nine eligible articles and reported the summary sensitivity of 87.6% (95% CI: 81.6 to 91.8; high-certainty evidence) and the summary specificity of 86.1% (95% CI 70.1 to 94.3; moderate-certainty evidence) for the diagnosis of PTB using Truenat MTB.
- The Truenat MTB Plus had higher sensitivity (90.6%; high certainty of evidence) and specificity (95.7%; high certainty of evidence) compared to Truenat MTB.
- The recent WHO guidelines included low complexity NAATs (Xpert Ultra and Truenat MTB Plus) and recommended that these tests should be used as initial diagnostic tests for TB among adults and adolescents presumptive TB.
- The next 5 years are expected to witness the wider implementation of Truenat assays in several countries, potentially offering a cost-effective solution for TB diagnosis.

offers both TB diagnosis and drug resistance testing at the same time is ideal to reduce the delay in TB diagnosis and appropriate treatment initiation. In this context, research and development on new TB diagnostics is encouraging with a wide range of products in the TB diagnostics pipeline.

The recent molecular WHO-recommended rapid diagnostic tests (mWRD) can detect TB and rifampicin resistance in 2 h. Automated nucleic acid amplification diagnostic tests with low and moderate complexity targeted next-generation sequencing are promising tools for the diagnosis of TB disease including drug resistance testing. The WHO classifies diagnostic tests and groups individual tests with similar characteristics and performance together into a class. The classification is based on 'type of technology (e.g. automated or reverse hybridization nucleic acid amplification tests [NAATs]), the complexity of the test for implementation (e.g. low, moderate, or high – considering the requirements of infrastructure, equipment, and technical skills of laboratory staff), and the target conditions (e.g. diagnosis of tuberculosis, detection of resistance to first-line or second-line drugs)' [3,4].

Xpert MTB/RIF and Xpert MTB/RIF Ultra (Cepheid) or Truenat (Molbio) belong to low-complexity NAATs technology and use real-time polymerase chain reaction (PCR) methods. These automated tests detect TB and rifampicin resistance as an all-in-one solution and are currently widely used [4]. Although these tests provide quick and accurate results, their availability is hindered by factors such as cost, infrastructure, and the need for specialized training. Loop-mediated isothermal amplification (TB-LAMP) belongs to low-complexity manual NAATS and has few advantages over automated NAATS in terms of infrastructure requirements. However, it does not detect drug resistance simultaneously. Line probe assays (LPA), which are ideal for a reference laboratory setting, are DNA strip-based tests that can detect the Mycobacterium tuberculosis complex DNA and identify drug resistance [4]. One of the targets for monitoring the implementation of the END TB strategy is the diagnosis of 100% of the persons with TB with the initial mWRD by 2027 [1]. mWRD was used as the initial test in 48% of the 8.2 million incident TB cases worldwide in 2023 and this ranged from 78% in the European region to 39% in the South -East Asian region [1]. Further, 79% of those with bacteriologically confirmed TB were tested for rifampicin resistance globally in 2023 [1]. Tests for TB available at the peripheral level could contribute to increase in testing and have many advantages in terms of reducing delay in diagnosis and early treatment initiation. The molecular assay Truenat MTB, MTB Plus and MTB-RIF Dx assays developed by Molbio Diagnostics, Goa, India, offer this advantage, unlike Xpert MTB/RIF, which requires temperature management for optimal performance, Truenat is a portable, battery-operated, chip-based test which can be placed in peripheral laboratories and can serve as a point-of-care test, avoiding the need for specimen transport. Truenat is recommended as an initial diagnostic test for the detection of TB and rifampicin resistance by the WHO [4]. While Truenat is primarily validated for sputum, early studies suggest utility in gastric aspirates, BAL, lymph node aspirates, pleural fluid, and CSF, with performance comparable to Xpert in some paucibacillary forms. However, evidence remains limited and further validation is needed before routine use in extrapulmonary TB. A practical limitation is the risk of contamination, as Truenat involves multiple manual handling steps unlike fully closed cartridge systems. Design improvements have reduced this risk, but strict adherence to good laboratory practices and operator training are essential [5]. This review will focus on the profile of the Truenat assay in detecting pulmonary TB and drug resistance to rifampicin.

2. Overview of the Truenat MTB diagnostic device

2.1. Functionality of the Truenat MTB assay

Truenat assay is a novel point-of-care (POC), cost-effective, battery-operated assay with robust performance and could serve as an alternative to Xpert for the detection of *M. tuberculosis* (MTB) and rifampicin (RIF) resistance. The assay involves a chip-based real-time PCR where the target genes involved are a single-copy target of *nrdB* (MTB assay), *nrdz*, and a multicopy target of IS6110 (MTB Plus assay) for MTB detection and *rpoB* (MTB-RIF Dx reflex assay) for RIF detection [6]. The DNA is extracted from 0.5 ml of sputum using an automated cartridge-based Trueprep device in less than 20 min. The extracted DNA elute is then loaded onto the chip-based Truelab micro-PCR device [7]. The PCR device is available in single, double and four module configurations, with the utmost capability of testing four samples concurrently (Figure 1). The National TB Elimination Programme (NTEP) in India recommends Truenat MTB and MTB Plus assay for pulmonary TB and extrapulmonary TB diagnosis,

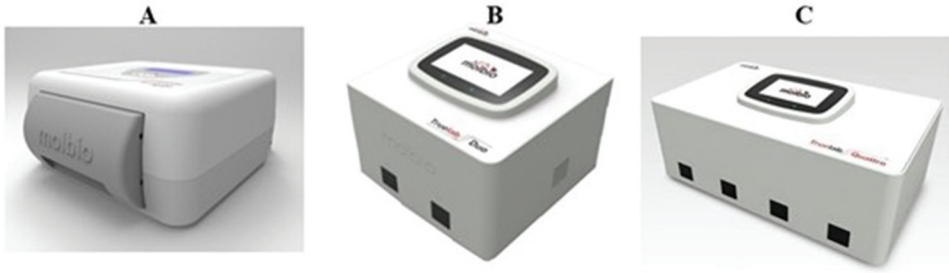

Figure 1. Devices used in Truenat testing. A. Trueprep DNA extraction device. B. Truelab duo PCR device. C. Truelab quattro PCR device.

respectively. While Xpert MTB-XDR offers testing for FQ resistance detection, Truenat Plus assay with drug resistance chips is available for Isoniazid (INH), Fluoroquinolones, and Bedaquiline (BDQ) resistance detection, increasing their clinical utility in peripheral settings for early detection and treatment of MDR, INH-monoresistant, and XDR TB [8].

2.2. Workflow of Truenat testing

The specimen is first pre-treated with Trueprep AUTO MTB sample pre-treatment pack for liquefaction and lysis of the testing sample. After 20 min, the pretreated sample is mixed with Trueprep AUTO v2 Universal Cartridge-based sample prep kit and loaded into the Trueprep AUTO v2 Universal Cartridge-based sample prep device for DNA extraction and purification, which requires 20 min. The extracted purified DNA is mixed with freeze-dried PCR reagent in microtubes and loaded on a Truenat MTB chip and run using Truelab Real-time micro-PCR analyzer (Figure 2). The MTB or MTB Plus assay runs take around 40 min. If MTB is detected, the same DNA elute can be used to run the MTB-RIF Dx reflex assay, which will take another 60 min [9,10].

2.3. Interpretation of the test results

Once the MTB or MTB Plus assay run is completed, the result screen will show the detection, MTB load and validity of the result. The display shows 'DETECTED' or 'NOT DETECTED' for the positive or negative result, respectively. The result screen will also display the MTB load as 'HIGH,' 'MEDIUM,' 'LOW,' or 'VERY LOW' in Truenat MTB Plus and CFU/ml in Truenat MTB (Figure 3). In addition, the validity of the test run is indicated on the screen as 'VALID' or 'INVALID.' The invalid results occur if amplification of the internal positive control either due to poor sample collection or error in DNA extraction.

Truenat RIF (rifampicin resistance) testing results will be displayed as 'Rifampicin Resistance Detected,' 'Rifampicin Resistance Not Detected,' 'Indeterminate,' or 'Error.' The indeterminate result occurs due to low bacilli load or a run error. However, since the presence of MTB is confirmed by an initial Truenat MTB testing, only the RIF testing for indeterminate results is usually repeated at least once using either the same DNA or the sample. If both the test results are indeterminate, additional investigations to assess resistance to RIF need to be considered. The other types of errors which might occur due to the malfunctioning of the different parts of the devices, and troubleshooting for these errors is provided by the manufacturers [5].

2.4. Infrastructure and training requirements

The infrastructure requirement for Truenat MTB testing is minimal, making it suitable for peripheral laboratories and primary health centers. Unlike the Xpert machine, Trueprep and Truelab devices do not need an air-conditioned room and can be operated at the normal setup that can go up to 40°C temperature and 80% relative humidity. The Truenat system uses portable, battery-operated devices and room-temperature-stable reagents, enabling testing in various settings with limited resources [9,10]. Trueprep and Truelab instruments are to be installed on a flat and stable surface. They should be kept out of direct sunlight and away from instruments that produce or radiate heat, cause vibrations or electromagnetic interference [5].

The battery will last for up to 8 h on a single charge. Electric power is required for recharging, and testing can be done simultaneously while charging. The electrical outlets should be grounded, and three sockets are required for charging all the instruments at once [5]. They operate within the 100–240 voltage range, which is the standard voltage used in most countries and hence can be distributed across the world without the need for modifications [5].

The Truenat TB test procedures involve several manual steps and meticulous micro-pipetting. Hence, lab personnel conducting the test would need proper training on all procedures. Background knowledge on principles of Truenat TB testing, operation of testing instruments, standard operating procedures, troubleshooting of invalids and errors, use of test requisition and reporting forms, is essential for the personnel to ensure adherence to good laboratory practices. Competency assessment of trained personnel shall be monitored by checking their efficiency in sample preparation, DNA extraction, and loading onto the chip, evaluating quality controls, and external quality assessment programs. In addition, training on good laboratory practices is also required for proper maintenance of equipment maintenance, storage of reagents, biomedical waste disposal, and following chemical and biological safety measures as per the standards [5]. Training needs for the laboratory personnel in conducting Truenat are considerably less as the testing methodology is simple. Around 2 days of training might suffice for lab personnel who did not have earlier Xpert or molecular testing

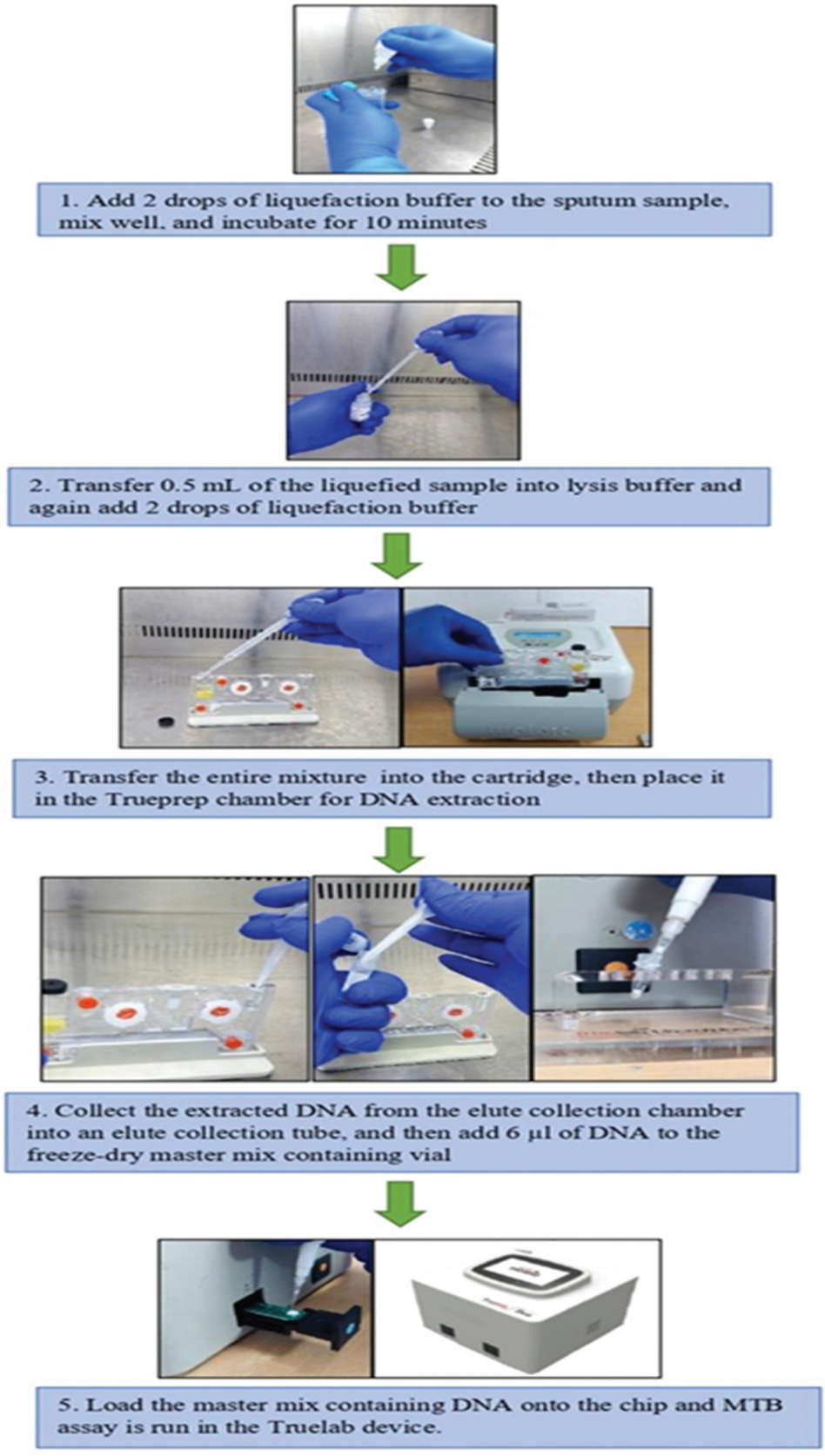

Figure 2. Procedures involved in Truenat MTB testing.

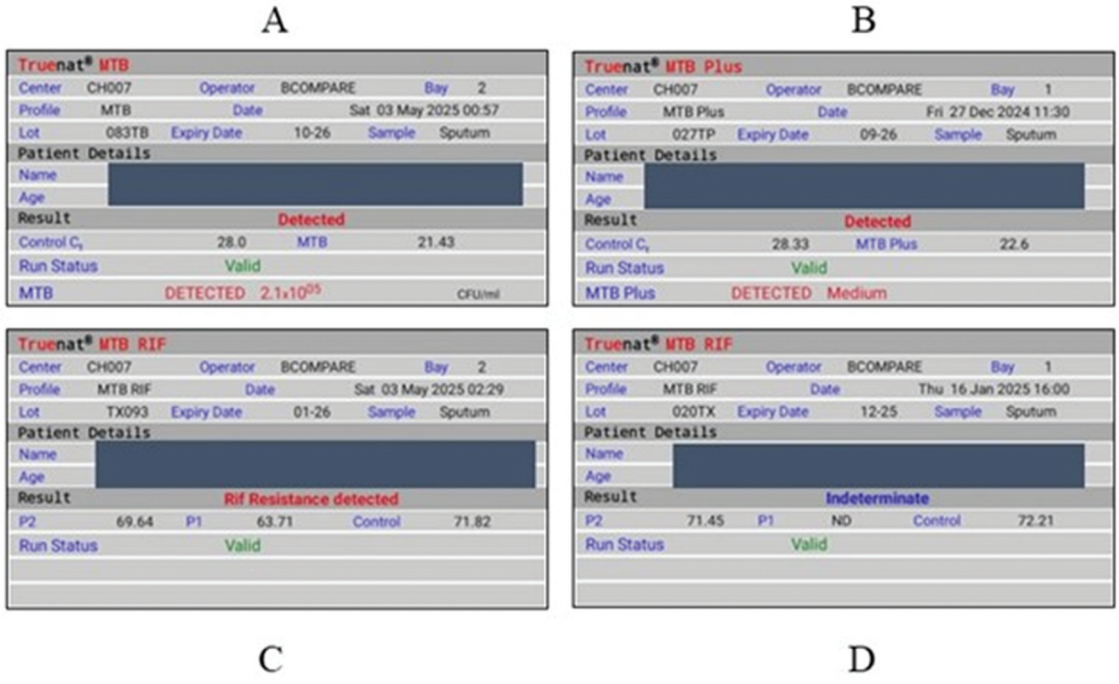

Figure 3. Results interpretation in Truenat testing. A. MTB detected with CFU/ml value in Truenat MTB assay; B. MTB detected with load of bacilli in Truenat MTB Plus assay; C. Detection of rifampicin resistance in Truenat MTB/RIF DX assay; D. Indeterminate result in Truenat MTB/RIF DX assay.

experience [11]. Within 10 days of running the Truenat assay, the laboratory personnel felt comfortable, and the invalid test results decreased over time in India [12].

2.5. Cost-effectiveness of Truenat testing

A cost-effectiveness study evaluated Truenat using a microsimulation model with the projections involving life expectancy, costs, incremental cost-effective ratio (ICER), and the 5-year budget effect of deploying Truenat in the public sector under programmatic conditions in India. The study concluded that Truenat will be able to increase life expectancy, improve link to patient care, and be cost-effective compared with smear microscopy and Xpert with an increase in life expectancy of 0.39 years and 0.08 years, and be cost-effective with ICER $210/years of life saved (YLS) and $120/YLS, respectively [13]. This conclusion was supported by a systematic review aimed at evaluating the cost-effectiveness of Truenat in pediatric tuberculosis. The findings indicated that Truenat is more cost-effective than Xpert, as it identified an additional 13,260 cases for an added cost of $14.36 per case detected, resulting in an ICER of $20.01 per life year gain [14]. The cost of each test for Truenat MTB is 7.8 $, Truenat MTB Plus 9.45$ and Truenat RIF 6.7$ and for Xpert MTB RIFvaries from 12$ in LMIC to 21$ in other countries. Even though the cost of Truenat MTB and MTB RIF approximates the cost of Xpert MTB, RIF resistance assay in Truenat is done only when TB is detected, thus potentially reducing the number of tests conducted, which is not possible in Xpert as it is a single assay for the detection of TB and RIF resistance [13]. The cost of diagnosis to treatment initiation was similar or lower for Truenat than Xpert, suggesting comparable or better cost-effectiveness under program conditions, which improved with higher testing volumes [15]. Compared to Xpert, the need for reduced infrastructure, ease of access, and portability makes the utility of the Truenat device extra feasible as a point of care (POC) test. This added advantage proves to be cost-effective in resource-limited settings, offering a faster turnaround time (TAT) of test results and treatment initiation, which in turn could result in potential cost savings due to reduced referrals [13,16].

Truenat's portability, battery operation, and tolerance to variable temperature and humidity make it suitable not only for peripheral labs and primary health centers but also for non-clinical settings like health camps, mobile units, and outreach in hard-to-reach areas. This enables on-site molecular confirmation, reducing sputum transport needs and pre-treatment loss to follow-up [9,10]. However, effective use depends on uninterrupted supply of consumables, safe sample handling, and reliable patient follow-up systems. Robust maintenance, reliable supply chains, and accessible local technical support are essential to prevent downtime and ensure continuity. Thus, Truenat functions best as a 'near-patient' test, flexible for diverse settings but requiring integration into referral and treatment pathways for maximum impact.

2.6. Diagnostic accuracy of Truenat

Due to multiple amplification targets, the limit of detection (LOD) for Truenat MTB Plus is 30 CFU/ml, lower than 100CFU/ml for Truenat MTB. Hence, the performance of Truenat MTB Plus is superior to that of Truenat MTB in detecting pulmonary TB [17].

In 2020, a multicentric study conducted in India demonstrated the sensitivities of Truenat and Xpert as 88.3 and 79.7%, respectively, in comparison with culture [17]. When the Truenat MTB Plus assay was compared with Xpert testing

in culture-positive pulmonary samples, the sensitivity was found to be similar between Truenat MTB Plus (91%) and Xpert (90%) [18]. Another study conducted in the pediatric Indian population also demonstrated higher sensitivity of Truenat (58.7%) compared to Xpert (56%) with culture as the reference standard [19]. However, the data is limited regarding the diagnostic accuracy of Truenat assays among children and in detecting Rifampicin resistance.

Our Cochrane review that informed the recent WHO guidelines on molecular diagnosis of TB included nine eligible articles and reported the summary sensitivity of 87.6% (95% CI: 81.6 to 91.8; high-certainty evidence) and the summary specificity of 86.1% (95% CI 70.1 to 94.3; moderate-certainty evidence) for the diagnosis of PTB using Truenat MTB. The Truenat MTB Plus had higher sensitivity (90.6%; high certainty of evidence) and specificity (95.7%; high certainty of evidence) compared to Truenat MTB. The review included three comparative studies and demonstrated that the summary sensitivity of Truenat MTB was lower (81% vs 93.7%) compared to Xpert Ultra; the summary specificity was higher (97% vs 93.7%) than that of Xpert Ultra [20].

Penn Nicholson et al. reported a 23% rate of indeterminate rifampicin resistance results, and of the tests repeated, 73% remained unresolved. Non-determinate results of Rifampicin resistance may depend on the bacillary load and M. tb detection, as evidenced by the lower rate of non-determinate Truenat MTB-RIF Dx results (6.7%) from Truenat MTB-positive specimens and the higher rate (72%) from specimens that were Truenat MTB-negative but Truenat MTB Plus-positive [6]. Ssengooba et al. reported 17.4% as indeterminate results for rifampicin resistance for Truenat MTB-RIF DX assays [11]. In a multisite retrospective study across national TB program sites in India, the proportion of indeterminate test results was 15.3% from Truenat MTB-RIF DX assays. Some of the reasons identified for higher rates of indeterminate results are lower bacillary load in the sputum samples, improper storage of the test chips and reagents, non-adherence to the testing algorithms and protocol, inadequate training and lack of proficiency of the laboratory technician [21].

Jeyashree et al. estimated that testing the sputum of presumptive TB patients using Truenat instead of sputum smear microscopy could lead to an additional yield of 18 new TB positives/100000 [12]. Lee et al. in a modeling study showed that Truenat point of care (POC) tests improve life expectancy by 0.39 years and 0.08 years when compared to sputum smear microscopy and Xpert, respectively, in addition to cost-effectiveness [13].

2.7. Performance standards

India was the first country to roll out Truenat under the NTEP after the WHO's recommendation on the use of this technology for MTB diagnosis. Although there is enough evidence on diagnostic accuracy of Truenat, its operational feasibility and impact on the program after deployment are less explored. A multicenter validation study by Gomathi et al. highlighted the operational advantages of Truenat, supporting its use as an initial molecular diagnostic test [17]. In addition, a study by Jeyashree et al. also endorsed the operational feasibility of Truenat by showing an increase in case detection when deployed as a POC test [12]. However, both these studies were conducted in a few sites with appropriate training of lab personnel, a controlled system, and a restricted sample size. In contrast, in 2023, when the Truenat test results were analyzed in a real working setup, the invalid and error rates of MTB testing were found to be 5.2% and 2.5%, respectively, and the indeterminate and error rates of RIF testing were found to be 15.3% and 1.6%, respectively [21]. Several factors contributing to this deprived performance include a lack of adherence to the standard operating procedure (SOP), delay in machine maintenance, improper kit storage conditions, lack of quality control, and inadequate training. Unlike Xpert MTB, Truenat involves additional manual steps for DNA extraction, and hands-on training is very important. Truenat was implemented under the restricted conditions of COVID-19 in 2020. Although the NTEP trained the trainers, further in-person training of the laboratory staff was not done due to the COVID-19 pandemic. The virtual training sessions conducted were found to be ineffective compared to the traditional onsite hands-on training. This could also be the reason for the poor performance of Truenat in the past years. The root cause analysis conducted during the study and recommendations drawn were consolidated into technical guidance documents and videos by NTEP for all the TB laboratories to improve Truenat MTB-RIF testing performance. WHO has released 'Target product profile for tuberculosis diagnosis and drug resistance testing' in 2024, which defines the ideal and minimal criteria for TB diagnostic tests to enhance detection and treatment, especially in low-resource settings. It emphasizes rapid, accurate detection with high sensitivity and specificity, ability to use multiple specimen types, operation by minimally trained staff, portability, affordability, and drug resistance detection. Both Truenat and Xpert MTB/RIF Ultra largely align with the WHO TPP. Truenat excels in portability and battery operation suited for decentralized settings, while Xpert Ultra offers higher sensitivity and robustness. Both meet key sensitivity, specificity, and rifampicin resistance detection goals but cost reduction to be done to be used as a universal test [22]. We have described real-world challenges, limitations and possible solutions in Table 1.

2.8. Biomedical waste management

The Truenat assay generates a considerable volume of plastic waste matter in the form of Truenat chips, micro tube, micro tube cap, transfer pipette, pipette tips, etc., and should be discarded properly, adhering to the standard medical waste disposal guidelines. The manufacturer recommends submerging the used plastic consumables in a freshly prepared 0.5% sodium hypochlorite solution for 30 min before disposal. Autoclaving is not recommended for the consumables soaked in sodium hypochlorite solution [5]. The uncontaminated pouches of cartridges, chips, desiccants, and wrappers of the transfer pipette can be discarded as general waste [5].

Table 1. Real-world Challenges and solutions in implementing Truenat assays.

Limitations & real-world challenges	Solutions/Recommendations
Multi-step workflow and low throughput (sequential chip loading, max four samples per 40 min run, increases patient waiting time)	• Streamline workflow through automation • Optimize patient scheduling • Use additional Truelab units in high-burden sites • Prioritize same-day results for presumptive TB patients
Infrastructure and maintenance requirements (periodic calibration, electricity/battery dependence, temperature stability)	• Strengthen local biomedical engineering support • Provide solar/battery backup • Integrate calibration into national maintenance programs • Ensure dedicated space for stable operations
Supply chain disruptions (chips, extraction reagents, consumables)	• Develop centralized procurement hubs • Maintain buffer stocks at district level • Adopt digital inventory tracking • Establish vendor accountability through service-level agreements
Indeterminate rifampicin results (low bacillary load, borderline probe signals, reagent/storage issues)	• Standardized storage/transport of reagents • Provide refresher staff training • Implement re-testing protocols • Encourage R&D to improve assay chemistry and probe design
Cost considerations (multiple chips per patient for MTB + resistance, maintenance, training)	• Leverage pooled procurement to reduce unit cost • Integrate Truenat into national subsidy schemes • Conduct cost-effectiveness studies to guide scale-up • Explore inclusion in donor-funded diagnostic packages.
Equity and access gaps (rural/tribal settings face staff shortages, service delays, weak logistics)	• Strengthen training and retention of local lab staff • Deploy portable Truelab devices in outreach camps • Establish efficient referral linkages • Ensure regular servicing through regional hubs.

3. Alternative diagnostics for the detection of pulmonary TB and rifampicin resistance

There are several alternate diagnostics available for the Truenat assay, and their performance, turnaround time, advantages and limitations are described in Table 2.

4. Policy recommendations

WHO endorsed Truenat MTB assays in 2020 as an initial molecular test for the diagnosis of pulmonary tuberculosis. Subsequently, these assays were implemented by the National TB programs of many of the low- and middle-income countries. The recent WHO guidelines recommended that Low complexity NAATs (Xpert Ultra and Truenat MTB Plus) should be used as initial diagnostic tests in adolescents and adults having clinical features of TB or those screened positive for pulmonary TB. The guideline also recommended them as initial tests for the detection of rifampicin resistance after bacteriological confirmation of TB. WHO acknowledged the limitation of data on Truenat MTB Plus and MTB-RIF Dx for the detection of both pulmonary TB and rifampicin resistance compared to Xpert Ultra, necessitating more research studies on the diagnostic accuracy of these tests.

5. Conclusion

Truenat assays are semi-quantitative, point-of-care, real-time, micro-PCR tests designed for diagnosing *Mycobacterium tuberculosis* (MTB). The performance of Truenat MTB and MTB Plus assays in diagnosing pulmonary TB is comparable to that of Xpert assays. Truenat diagnostics are battery-powered and necessitate minimal biosafety measures and infrastructure compared to Xpert platforms. Consequently, Truenat assays are suitable for deployment in remote or resource-limited settings. Since their endorsement, the Truenat assays have incorporated new technological advancements to enhance test quality. These assays are among the promising rapid molecular tests that will support the global efforts toward TB elimination in the near future.

6. Expert opinion

The development of Truenat assays marked a significant step in providing equitable access to molecular diagnostics for tuberculosis (TB). The simplicity and portability of Truenat assays make it particularly suited for peripheral laboratories, district hospitals, and outreach programs where uninterrupted electricity and air conditioning are often lacking. In high-burden countries where TB elimination strategies rely on early and accurate case detection, Truenat could substantially reshape diagnostic pathways and patient outcomes.

6.1. Impact on real-world outcomes

Truenat can influence TB care across multiple dimensions in low- and middle-income countries (LMICs). The system is

Table 2. Alternative tests for the diagnosis of Tuberculosis.

Test	Sensitivity	Specificity	Limit of Detection (LOD)	Turnaround Time	Drug Resistance Detection	Key Advantages	Key Limitations	References
Smear Microscopy (AFB smear)	50–60% (children: 7–40%)	>90%	~5,000– 10,000 bacilli/mL	Few hours	Not detected	• Inexpensive • Rapid • No need for sophisticated equipment or air-conditioning • Widely available.	• Low sensitivity (misses ~50% of cases) so requires multiple samples for higher yield • Poor performance in HIV/children due to paucibacillary samples • Subjective interpretation • Cannot differentiate MTBC vs NTM, live vs dead bacilli • Cannot detect drug resistance	[23–25]
Culture (Solid)	80–85%	98–100%	10–100 CFU/mL (solid)	1–4 weeks	All drugs (phenotypic DST)	• Reference standard for TB diagnosis • Detects viable bacilli • Enables full DST	• Slow turn-around time • Requires biosafety & skilled staff • Contamination risk • Low sensitivity in children	[26–32]
Culture (Liquid)	90–95%	98–100%	~100–1000 CFU/mL	2–8 weeks	All drugs (phenotypic DST)	• Reference standard for TB diagnosis • Detects viable bacilli • Enables full DST	• Slow turn-around time • Requires biosafety & skilled staff • Contamination risk • Low sensitivity in children	[26–32]
Xpert MTB/RIF	78.6–89.9%	97–99.3%	~113 CFU/mL	~2 hours	Rifampicin only	• High sensitivity/specificity, automated, simultaneous TB & RIF detection, closed system.	• High cost, requires AC & stable electricity, annual calibration, cannot distinguish live vs dead bacilli.	[24,25,33]
Xpert MTB/RIF Ultra	86.2–94.7%	93–97.4%	~15–20 CFU/mL	1–1.5 hours	Rifampicin only	• More sensitive in paucibacillary/ HIV/children, low LOD, rapid.	• Slightly lower specificity than Xpert, same infra/ cost barriers, cannot differentiate live vs dead bacilli.	[24,25,33]
TB-LAMP	76–80%	97–98%	~10^3 CFU/mL	~1 hour	Not detected	• Rapid, inexpensive vs Xpert, no AC required, simple equipment	• Operator-dependent interpretation, contamination risk, limited EPTB evidence, cannot differentiate live vs dead bacilli.	[34–36]
Line Probe Assay (LPA)	95–99% (smear+); lower in smear–	98–100%	Lower than smear (often needs culture isolates)	24–48 hours	Rifampicin, INH, FQ, injectables (known mutations only)	• Detects MDR/pre-XDR/XDR, covers multiple drugs, high accuracy in smear+	• Requires advanced lab & skilled staff, complex workflow, misses rare/novel mutations, costly	[37,38]
LAM (LF-LAM, FujiLAM)	32% (LF-LAM), 51% (FujiLAM)	~90–95%	Not applicable	25–30 minutes	Not detected	• Non-invasive (urine), useful in HIV+ and children, POC friendly	• Very low sensitivity in HIV–, false positives in NTM/renal disease, no drug resistance	[39,40]
Truenat MTB	~85–90% (smear+), lower in smear	~97–99%	~100 CFU/mL	~35–40 min/test	Not detected	• Portable, battery-operable, works in high temp/humidity, suitable for peripheral labs	• Requires separate chip for RIF testing, multi-step workflow	[5,41–43]
Truenat MTB Plus	~90–92%	~97–99%	~50–80 CFU/mL	~35–40 min/test	Not detected	• Improved sensitivity (detects nrdZ/ IS6110 targets), better for paucibacillary TB	• Still requires separate cartridge for resistance	[44–46]
Truenat MTB Ultima	~92–95%	~97–99%	~10–20 CFU/mL	~35–40 min/test	Not detected	• Lowest LOD among Truenat assays, comparable to Xpert Ultra, useful for children/HIV+	• Same limitations as Plus (no resistance unless combined with TruRif)	
Truenat TruRif	~95–97% for RIF resistance	>98%	Depends on bacterial load	Additional ~40 min after MTB detection	Rifampicin only (newer chips in development for INH, FQ, BDQ, LZD)	• Enables MTB + RIF sequential testing, high accuracy vs sequencing	• Requires two-step process (MTB chip + TruRif chip), indeterminate results possible, cost considerations	[47–49]

designed to be highly portable and battery-operated, with options for solar power, making it ideal for use in resource-limited settings with minimal infrastructure. This has been further enhanced by the use of mobile diagnostic vehicles, such as 'Lab on Wheels,' in certain regions, including Timor-Leste and the Democratic Republic of the Congo. From a diagnostic perspective, it decentralizes molecular testing, reducing delays that would otherwise occur when samples must be transported to reference laboratories. Early diagnosis leads to earlier treatment, reduced transmission, and improved outcomes. At a programmatic level, same-day molecular confirmation at primary health centers could improve case-finding in hard-to-reach populations. Economically, although initial establishment costs are comparable to Xpert and higher than smear microscopy, Truenat may lower overall expenditure by reducing loss-to-follow-up and unnecessary empiric treatments. On a policy level, wide deployment across nations would accelerate the transition from symptom-based algorithms to universal molecular testing, aligning with WHO's End TB strategy.

6.2. Barriers to adoption in clinical practice

Several barriers limit the scaling up of Truenat assays. It has modest throughput, with each Truelab module processing only four samples in 40 min – insufficient for high-volume sites unless multiple units are installed and more staff are running the samples throughout the day, which significantly increases the establishment costs. Rifampicin resistance detection remains hampered by indeterminate results, undermining confidence and necessitating repeat testing. There is a strong correlation between the load of the bacilli and rifampicin. Indeterminate results. Few studies have shown the indeterminate results up to 25%. Additionally, earlier studies have shown that lower MTB load samples have yielded mostly indeterminate results. The manufacturer needs to upgrade the IFU to exclude Rifampicin testing in the isolates which has a lower load of bacilli. Another point on the load of testing is the claim by the company regarding LOD to be 100 CFU/ml, but sometimes (more frequently in extrapulmonary samples) shows results of MTB detection below the LOD level, which could be a false positive, this could be corrected to include only reports above the LOD, this also further reduce indeterminates in Rifampicin resistance levels.

Supply chain fragility, especially for chips, reagents, and calibration services, can interrupt services. Although per-test costs are lower than GeneXpert, the need for multiple chips due to repeated tests (MTB Plus resistance) reduces the advantage. Finally, workflow complexity demands greater training and supervision compared with cartridge-based systems.

A primary concern regarding testing in the periphery is the training. Since the test is a two-step method, there is a strong need to train operators before they perform the test. A country or laboratory needs to conduct training for its personnel before introducing the test; additionally, regular QC and EQA must be performed to verify the test's accuracy. Lastly, there is also a requirement of swab testing (at least monthly) in the Truenat laboratories, which keeps a check on the false positive results.

6.3. Weaknesses and challenges in the field

The key weakness is the gap between analytical performance and operational practicality. Molecular test accuracy is good, but throughput, indeterminate results, and logistics remain major bottlenecks. Current assays mainly detect rifampicin resistance, leaving gaps in identifying isoniazid, fluoroquinolone, and newer drug resistance. Furthermore, the inability to distinguish viable from non-viable bacilli, leading to potential false positives after treatment. Evidence on Truenat's performance in children, HIV-positive individuals, and extrapulmonary TB remains limited. With the rollout of the newer drugs and regimen, wherever possible, the chips for the rapid drug testing need to be developed by the manufacturer and include it as a reflex test. This can be added to the cost per test.

6.4. Potential of further research

Future research can address many of these challenges. Development of expanded resistance panels – for fluoroquinolones, bedaquiline, and linezolid – would allow Truenat to function as a comprehensive MDR/XDR diagnostic tool. Workflow innovations such as closed-cartridge or multiplex formats could reduce contamination risk and increase throughput. Operational research should clarify cost-effectiveness, scalability, and patient-centered outcomes, including digital connectivity for real-time reporting and integration into national TB programs. In addition, biomarker-based adjuncts such as combining Truenat with urine LAM or host-response assays could improve case detection in populations with poor sputum quality. Treunat is also looking to manufacture and validate the ability to other drugs, especially INH and fluoroquinolones. This will be a good addition, but then again, to control over the price, since the numbers required will be less, can be provided as a reflex test. As all positives are tested for Rif INH can also be tested and if there is any resistance to INH or RIF we can offer FLQ testing. A recommendation for the company to consider is the development of testing for newer drugs and NTM testing as well.

6.5. Ultimate goals and future directions

The overarching goal is universal, equitable access to rapid molecular testing for all individuals with presumptive TB. This requires not only assay refinement but also systemic improvements in financing, infrastructure, workforce, and global partnerships. Truenat offers a bridge toward this vision but must be embedded within a broader diagnostics ecosystem where every presumptive case is tested with a high-sensitivity molecular tool at first contact.

6.6. Comparisons and competing technologies

Truenat will coexist with other emerging diagnostics. Cartridge-based systems such as Xpert continue to evolve toward expanded resistance detection. Sequencing-based technologies (tNGS, WGS) are moving from research into reference laboratories, providing comprehensive resistance profiling. Non-sputum diagnostics, including next-

generation urine LAM assays and blood-based host biomarkers, may emerge as valuable triage tools for HIV-positive and pediatric populations. Truenat is well positioned as a decentralized molecular platform, but it must adapt and integrate with these complementary approaches.

6.7. Five- to ten-year outlook

In the next 5 years, Truenat and similar NAAT platforms are likely to replace in peripheral centers, with expanded resistance panels enabling point-of-care MDR-TB detection. Within 10 years, diagnostic ecosystems may evolve toward integrated multiplex platforms capable of detecting TB, HIV, and other pathogens from diverse samples, linked to digital networks for real-time reporting. Truenat's long-term role will depend on adaptability: the ability to incorporate new resistance targets, improve throughput, and integrate with broader diagnostic systems. While the simplicity of smear microscopy may be lost, the field will gain earlier detection, better management of drug-resistant TB, and reduced transmission.

6.8. Conclusion of expert opinion

Truenat assays signify both the progress and the challenges of TB diagnostics. It represents a pragmatic step toward universal molecular testing but will succeed only if operational realities – throughput, supply chains, training, and cost – are addressed alongside technological refinement. If scaled effectively, it could advance global TB elimination goals. If not, it risks remaining a niche tool confined to pilot projects. The coming decade will determine whether Truenat becomes a cornerstone of TB programs or a missed opportunity.

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extraction using disposable cartridge-based kits. It accommodates a wide range of clinical specimens—like sputum, blood, CSF, and tissue—and simplifies processing through pre-programmed steps involving chemical and thermal lysis, matrix binding, washing, and elution. The device operates on mains or rechargeable battery and can perform up to 16 extractions per charge. It features minimal hands-on interaction, making it suitable for decentralized settings with limited technical expertise.

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