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[Diagnostic Test Accuracy Review]

Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults and adolescents

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ABSTRACT

Background

Accurate and rapid diagnosis is crucial for ending the tuberculosis epidemic. Truenat assays are World Health Organization (WHO)-recommended rapid molecular diagnostic tests that detect *Mycobacterium tuberculosis* complex and rifampicin resistance.

Objectives

Primary objective

To assess the diagnostic accuracy of Truenat assays (MTB, MTB Plus, and MTB-RIF Dx) for detecting pulmonary tuberculosis and rifampicin resistance in adults and adolescents with presumptive pulmonary tuberculosis.

Secondary objectives

To compare the diagnostic accuracy of Truenat assays and Xpert MTB/RIF Ultra for detecting pulmonary tuberculosis and rifampicin resistance and to investigate potential sources of heterogeneity (e.g. HIV status and smear status).

Search methods

We searched MEDLINE, Embase, Science Citation Index and Biosis previews, Global Index Medicus, SCOPUS, WHO ICTRP, and ClinicalTrials.gov for published articles and trials in progress on 16and 17 October 2023. We searched ProQuest Dissertations & Theses A&I

for dissertations. We contacted tuberculosis experts for ongoing and unpublished studies. A WHO public call for data was made between 30 November 2023 and 15 February 2024.

Selection criteria

We included cross-sectional and cohort studies that evaluated Truenat assays in sputum samples from adolescents and adults (aged 10 years and older). The microbiological reference standard for identifying pulmonary tuberculosis is culture. The reference standard for rifampicin resistance is a culture-based drug susceptibility test. Two review authors independently screened titles and abstracts, and assessed the full texts of potentially eligible articles. A third review author resolved any disagreements.

Data collection and analysis

We tailored and applied the QUADAS-2 and QUADAS-C tools to assess the risk of bias and applicability. Two review authors independently extracted data for each included study, and a third review author resolved any disagreements. We performed meta-analyses to estimate summary sensitivities and specificities using a bivariate model. We assessed the certainty of evidence using the GRADEpro GDT tool.

Main results

Of nine eligible articles, one contributed two distinct participant cohorts, which we considered as separate studies. Thus, we included 10 studies; three assessed Xpert Ultra. Most studies were set in low- and middle-income countries with a high tuberculosis burden. Six studies (4081 participants, 1379 with tuberculosis) assessed Truenat MTB, and four studies (3073 participants, 750 with tuberculosis) assessed Truenat MTB Plus. Two studies (966 participants, 111 with rifampicin resistance) assessed Truenat MTB-RIF Dx. Overall, the risk of bias in the included studies was low. Three of the 10 studies were judged to have high applicability concern in the patient selection domain.

Detection of pulmonary tuberculosis

The summary sensitivity of Truenat MTB was 87.6% (95% confidence interval (CI) 81.6 to 91.8; high-certainty evidence), and the summary specificity was 86.1% (95% CI 70.1 to 94.3; moderate-certainty evidence).

For Truenat MTB Plus, the summary sensitivity was 90.6% (95% CI 83.7 to 94.8; high-certainty evidence), and the summary specificity was 95.7% (95% CI 94.7 to 96.5; high-certainty evidence).

Based on the three comparative studies, the summary sensitivity of Truenat MTB was lower (81.0%, 95% CI 72.8 to 87.2) than that of Xpert Ultra (93.7%, 95% CI 90.4 to 95.9), while the summary specificity of Truenat MTB (97.0%, 95% CI 91.9 to 98.9) was marginally higher than Xpert Ultra (95.3%, 95% CI 90.9 to 97.7).

Detection of rifampicin resistance

The sensitivities from the two studies were 53% and 85% (moderate-certainty evidence) and specificities were both 97% (high-certainty evidence).

Authors' conclusions

Truenat MTB Plus had higher sensitivity and specificity than Truenat MTB. The high false-positive rate for Truenat MTB is a concern. The sensitivity of Xpert Ultra was significantly higher than that of Truenat MTB, while specificity was slightly lower. Evidence on the accuracy of Truenat MTB-RIF Dx was limited.

PLAIN LANGUAGE SUMMARY

How accurate are Truenat assays for detecting pulmonary tuberculosis and rifampicin resistance in adults and adolescents?

Key messages

– Truenat MTB Plus was more accurate than Truenat MTB for detecting pulmonary tuberculosis. Truenat MTB misidentified many people as having tuberculosis when they did not, which raises concern.

– Xpert Ultra was more accurate than Truenat MTB.

– Evidence on the accuracy of Truenat assay for detecting rifampicin was limited.

What is pulmonary tuberculosis?

Pulmonary tuberculosis is a lung disease caused by a bacterium (a germ) that spreads through the air via droplets from an infected person. In early stages, it remains dormant (does not multiply) and presents symptoms like fever, cough, weight loss, and night sweats. When a person coughs and produces sputum (a mix of saliva and mucus) or blood-stained sputum, they are advised to visit a healthcare professional

Drug-resistant tuberculosis is caused by bacteria that are not killed by at least one effective antibacterial medicine (for example, isoniazid or rifampicin) used to treat tuberculosis (called drug resistance). Delay in diagnosis of drug-resistant tuberculosis may increase spread from one person to another, and lead to further drug resistance. Diagnosis relies on demonstrating the presence of the bacteria or its DNA (which carries the genetic material needed for the bacteria to multiply) in a sputum sample. There are several ways of diagnosing tuberculosis. Examining a sputum sample under a microscope is easy and cheap, but it needs the presence of many bacteria so is not useful in early disease. Another way is to grow bacteria in a laboratory, but this takes weeks and is more expensive, particularly for poorer countries. The most-recent way is using a simple, quick, portable, and cost-effective assay to detect the bacteria within hours. These may be useful in poorer countries. While culture is the best way to confirm the disease, early and accurate identification is essential to start treatment and prevent debilitating and fatal illness. Assays would do this.

Why is improving diagnosis important?

According to the World Health Organization (WHO), in 2023, 10.8 million people had tuberculosis, and 1.25 million people died. The number of people with tuberculosis keeps increasing. It is crucial to have a test that accurately determines whether the disease is present (called a true positive) or absent (a true negative) without producing errors (like claiming the disease is present when it is not (false positive), claiming it is not there when it is (false negative), or invalid or inconclusive results). False-positive results cause unnecessary anxiety, and people will be monitored, requiring time and resources. These people may also be started on treatment with severe unwanted effects. False-negative results may miss cases, spreading disease in the general population. People with false-negative results may develop severe forms of tuberculosis with fatal outcomes due to delayed diagnosis and treatment.

What did we want to find out?

We wanted to assess the accuracy of three Truenat assays; two for detecting pulmonary tuberculosis (MTB, MTB Plus) and one for detecting rifampicin resistance (MTB RIF Dx) in adults and adolescents (aged 10 years and older) with suspected pulmonary tuberculosis.

What did we do?

We looked for studies assessing the accuracy of Truenat assays and compared them with another assay recommended by WHO (Xpert Ultra). The results of these tests were verified against culture for detecting pulmonary tuberculosis and tested for resistance to rifampicin, the most common antibiotic used to treat tuberculosis.

What did we find?

We found six studies (4081 people) for Truenat MTB, four studies (3073 people) for Truenat MTB Plus, and two studies (966 people) for Truenat MTB RIF Dx. Three studies also evaluated Xpert Ultra in addition to Truenat.

For the *Truenat MTB assay*, for 1000 people where 100 have tuberculosis confirmed by culture, 214 will be Truenat MTB positive. Of these, the assay will correctly identify 88 people with tuberculosis, but will incorrectly identify 126 people as having tuberculosis when they do not (false positives). Similarly, 786 will be Truenat MTB negative. Of these, the assay will identify 774 people as not having tuberculosis, of whom 12 will actually have tuberculosis (false negatives) and be missed.

For the *Truenat Plus assay*, for 1000 people where 100 have tuberculosis confirmed by culture, 127 will be Truenat MTB Plus positive. Of these, the assay will correctly identify 91 people with tuberculosis, but will incorrectly identify 36 people as having tuberculosis when they do not (false positives). Similarly, 873 will be Truenat MTB Plus negative. Of these, the assay will identify 864 people as not having tuberculosis, of whom nine will actually have tuberculosis (false negatives) and be missed.

For the detection of *rifampicin resistance*, the evidence was limited.

How confident are we in the results of this review?

We are confident of our results. We included a good number of studies and participants. Overall, the included studies were well conducted.

Who do the results of this review apply to?

The results of this review apply to people with symptoms suggestive of pulmonary tuberculosis.

How up to date is this review?

The review is up to date to 16 October 2023

SUMMARY OF FINDINGS

Summary of findings 1. Truenat MTB for the detection of pulmonary tuberculosis

What is the diagnostic accuracy of Truenat MTB for the detection of pulmonary tuberculosis?

Population: adolescents and adults with presumptive pulmonary tuberculosis

Role: as an initial diagnostic test

Index test: Truenat MTB

Threshold for index test: an automated result is provided

Reference standard: solid or liquid culture

Studies: cross-sectional

Setting: primary care facilities and peripheral laboratories

Sensitivity: 0.88 (95% CI 0.82 to 0.92)

Specificity: 0.86 (95% CI 0.70 to 0.94)

Test result	Number of participants with presumptive tuberculosis (studies)	Number of results per 1000 people tested (95% CI)*			Certainty of the evidence (GRADE)
		Prevalence 2.5%	Prevalence 10%	Prevalence 30%	
True positives (participants with pulmonary tuberculosis)	1379 (6 studies)	22 (21 to 23)	88 (82 to 92)	264 (246 to 276)	⊕⊕⊕⊕ **High**
False negatives (participants incorrectly classified as not having pulmonary tuberculosis)		3 (2 to 4)	12 (8 to 18)	36 (24 to 54)	
True negatives (participants without pulmonary tuberculosis)	2702 (6 studies)	839 (683 to 917)	774 (630 to 846)	602 (490 to 658)	⊕⊕⊕⊖ **Moderate**[a]
False positives (participants incorrectly classified as having pulmonary tuberculosis)		136 (58 to 292)	126 (54 to 270)	98 (42 to 210)	

*Prevalence estimates were assumed based on previous Cochrane reviews on Xpert MTB/RIF and Xpert Ultra which are low-complexity automated nucleic acid amplification tests like Truenat assays. These reviews used prevalence estimates suggested by the WHO Global Tuberculosis Programme (Zifodya 2021).

GRADE certainty of the evidence

High: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]The point estimates of individual studies ranged from 60% to 98% and 95% CIs did not overlap for a few studies. For a prevalence value of 2.5%, the very wide 95% CI around true negatives and false positives may lead to different decisions depending on which confidence limits are assumed. We downgraded one level for inconsistency.

Summary of findings 2. Truenat MTB Plus for the detection of pulmonary tuberculosis

What is the diagnostic accuracy of Truenat MTB Plus for the detection of pulmonary tuberculosis?

Population: adolescents and adults with presumptive pulmonary tuberculosis

Role: as an initial diagnostic test

Index test: Truenat MTB Plus

Threshold for index test: an automated result is provided

Reference standard: solid or liquid culture

Studies: cross-sectional

Setting: primary care facilities and peripheral laboratories

Sensitivity: 0.91 (95% CI 0.84 to 0.95)

Specificity: 0.96 (95% CI 0.95 to 0.97)

Test result	Number of participants with presumptive tuberculosis (studies)	Number of results per 1000 people tested (95% CI)*			Certainty of the evidence (GRADE)
		Prevalence 2.5%	Prevalence 10%	Prevalence 30%	
True positives (participants with pulmonary tuberculosis)	750 (4 studies)	23 (21 to 24)	91 (84 to 95)	273 (252 to 285)	⊕⊕⊕⊕ **High**
False negatives (participants incorrectly classified as not having pulmonary tuberculosis)		2 (1 to 4)	9 (5 to 16)	27 (15 to 48)	
True negatives (participants without pulmonary tuberculosis)	2323 (4 studies)	936 (926 to 946)	864 (855 to 873)	672 (665 to 679)	⊕⊕⊕⊕ **High**

False positives (participants incorrectly classified as having pulmonary tuberculosis)	39 (29 to 49)	36 (27 to 45)	28 (21 to 35)

*Prevalence estimates were assumed based on previous Cochrane reviews on Xpert MTB/RIF and Xpert Ultra which are low-complexity automated nucleic acid amplification tests like Truenat assays. These reviews used prevalence estimates suggested by the WHO Global Tuberculosis Programme (Zifodya 2021).

GRADE certainty of the evidence
High: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Summary of findings 3. Truenat MTB-RIF Dx for the detection of rifampicin resistance

What is the diagnostic accuracy of Truenat MTB-RIF Dx in the detection of rifampicin resistance?

Population: adolescents and adults with presumptive pulmonary tuberculosis

Role: as an initial diagnostic test

Index test: Truenat MTB-RIF Dx

Threshold for index test: an automated result is provided

Reference standard: culture drug susceptibility test

Studies: cross-sectional

Setting: primary care facilities and peripheral laboratories

Sensitivity: 0.53 to 0.85 (range)

Specificity: 0.96 to 0.97

Test result	Number of participants with presumptive tuberculosis (studies)	Number of results per 1000 people tested (95% CI)*			Certainty of the evidence (GRADE)
		Prevalence 2%	Prevalence 10%	Prevalence 15%	
True positives (participants with rifampicin resistance)	111 (2 studies)	11 to 17	53 to 85	80 to 128	⊕⊕⊕⊖ **Moderate**[a]
False negatives		3 to 9	15 to 47	22 to 70	

(participants incorrectly classified as not having rifampicin resistance)					
True negatives (participants without rifampicin resistance)	855 (2 studies)	941 to 951	864 to 873	816 to 825	⊕⊕⊕⊕ **High**
False positives (participants incorrectly classified as having rifampicin resistance)		29 to 39	27 to 36	25 to 34	

*Prevalence estimates were assumed based on previous Cochrane reviews on Xpert MTB/RIF and Xpert Ultra, which are low-complexity automated nucleic acid amplification tests like Truenat assays. These reviews used prevalence estimates suggested by the WHO Global Tuberculosis Programme (Zifodya 2021).

GRADE certainty of the evidence
High: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Sensitivity ranged from 53% to 85% and the 95% CIs between the two studies did not overlap. We could not explain the low sensitivity in one study. We downgraded one level for inconsistency.

BACKGROUND

Tuberculosis, the second leading infectious killer after Coronavirus disease 2019 (COVID-19), poses a diagnostic and therapeutic enigma. Globally, an estimated 10.8 million individuals had tuberculosis in 2023, with an increase from 10.7 million in 2022. The 30 countries with the highest number of people with tuberculosis accounted for 87% of all estimated incident cases worldwide, with eight low- and middle-income countries accounting for two-thirds of the total cases. In 2023, 1.25 million people died from tuberculosis, with people living in low- and middle-income countries accounting for nearly 95% of tuberculosis deaths (WHO Global TB Report 2024). The COVID-19 pandemic negatively impacted tuberculosis burden by restricting access to diagnosis and treatment, resulting in a reversal of the global progress achieved until 2019 towards eliminating tuberculosis. The World Health Organization (WHO) estimated that COVID-19 resulted in an increase of 200,000 tuberculosis fatalities between 2019 and 2021 and caused a drop in the yearly notification rate (WHO Global TB Report 2022).

Goal 3 of the United Nations Sustainable Development Goals includes the target of ending the tuberculosis epidemic by 2030 by reducing annual tuberculosis incidence to 80% of the 2015 level (UN 2015). It is estimated that there was a 3.9% increase in the tuberculosis incidence rate between 2020 and 2022 (WHO Global TB Report 2023). Although the cumulative incidence of tuberculosis decreased by 8.7% between 2015 and 2022, this reduction was just halfway to the 2020 goal of the End TB Strategy (WHO Global TB Report 2022). The End TB goals are challenging to attain because of several impediments in diagnosis and treatment, the most significant of which are diagnostic delays and drug resistance.

Treating tuberculosis is extremely challenging if the bacteria that cause the disease are resistant to first-line drugs. If bacteria are resistant to rifampicin, the disease is termed rifampicin-resistant tuberculosis (RR-TB), and if they are also resistant to isoniazid, the disease is termed multiple-drug-resistant tuberculosis (MDR-TB). Treatment for RR-TB and MDR-TB is expensive, requires prolonged duration, and is associated with a high likelihood of adverse events, including mortality (Jang 2020; Soeroto 2021; WHO Global TB Report 2022). In 2022, the incidence of RR-TB among people with newly detected disease was 3.3% and among previously treated individuals was 17%. The percentage of people with bacteriologically confirmed tuberculosis tested for rifampicin resistance rose from 61% (2.2 million/3.6 million population) in 2019 to 73% (2.9 million/4 million population) in 2022. In 2019, the global success rate of RR-TB/MDR-TB treatment was 60% (WHO 2022a). According to mathematical modelling, the prevalence of MDR-TB is likely to increase, reaching 8.9% in India (95% prediction interval 9.4% to 16.2%) and 5.7% in South Africa (95% prediction interval 3.0% to 7.6%) by 2040 (Sharma 2017).

Microbiological confirmation is recommended for diagnosing pulmonary tuberculosis. Traditional sputum smear microscopy, a key diagnostic tool in low- and middle-income countries, is inexpensive, fast, and widely applicable. However, it has limited sensitivity, and a positive result requires the concentration of bacteria to be between 5000 bacilli/mL and 10,000 bacilli/mL (Arora 2020; Steingart 2006). While *Mycobacterium tuberculosis* (*M tuberculosis*) culture has better sensitivity and specificity, it is often unavailable in low-resource peripheral settings. Even in a sophisticated laboratory, this test has a turnaround time of four to eight weeks. Similarly, phenotype-based drug susceptibility testing is expensive and also has a long turnaround time. With increasing drug resistance, detecting resistance to rifampicin is crucial as soon as an individual is diagnosed with tuberculosis so that appropriate treatment can be initiated.

Innovative rapid molecular-based diagnostic tools have revolutionised the diagnosis of tuberculosis and rifampicin resistance. A few molecular-based diagnostic tests are currently recommended by the WHO, including the Xpert MTB/RIF assay (Cepheid Inc. subsidiary of Danaher Corp, Sunnyvale, USA) (Cepheid 2022a; WHO 2013). Xpert MTB/RIF assay uses nested real-time polymerase chain reaction (PCR) for the qualitative detection of *M tuberculosis* complex and rifampicin resistance. The newer version of this test, Xpert MTB/RIF Ultra, uses melting-temperature-based analysis to enhance the accuracy of rifampicin-resistance detection (Cepheid 2022b; WHO 2024). However, both Xpert MTB/RIF and Xpert MTB/RIF Ultra require adequate infrastructures, such as continuous power supply and air conditioning (Gomathi 2020a). As a result, the use of these tests is restricted in low-resource peripheral laboratories. The Truenat assay, which is a nucleic-acid amplification-based test that can detect rifampicin resistance, has also been recommended by the WHO. The test kit is a point-of-care battery-powered, portable device, providing advantages over the Xpert MTB/RIF and Xpert MTB/Ultra for use in low-resource settings (WHO 2024). The test can be performed by unskilled personnel and detects *M tuberculosis* in sputum samples within one hour (Lee 2019).

Target condition being diagnosed

Pulmonary tuberculosis

M tuberculosis is the bacterium that causes tuberculosis, an infectious disease that spreads through the air via respiratory droplets from an infected individual. *M tuberculosis* can cause pulmonary and extrapulmonary tuberculosis. Pulmonary tuberculosis refers to the tuberculosis disease that exclusively affects the lungs. When tuberculosis affects any organs of the body except the lungs, it is referred to as extrapulmonary tuberculosis. Pulmonary tuberculosis is the most prevalent form of tuberculosis. Loss of appetite, loss of weight, lethargy, fever, chills, night sweats, cough, and haemoptysis are common symptoms of pulmonary tuberculosis. Treatment for drug-susceptible pulmonary tuberculosis comprises an initial two months of daily isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by four months of daily isoniazid and rifampicin. In some cases, the WHO recommends a reduced four-month regimen (WHO 2022b).

Rifampicin resistance

Rifampicin is a potent bactericidal drug that has played a significant role as a first-line treatment for tuberculosis. Rifampicin acts on the β subunit of the DNA-dependent ribonucleic acid polymerase encoded by the *rpoB* gene. Mutations in the *rpoB* gene account for more than 95% of rifampicin resistance (Zaw 2018). People with RR-TB or MDR-TB are treated with second-line drugs such as fluoroquinolones, aminoglycosides, bedaquiline, or linezolid. The duration of treatment ranges from six months for a shorter regimen, nine months for an all-oral regimen, and 18 months for a longer regimen (WHO 2022a).

Index test(s)

This review evaluated the Truenat and Xpert MTB/RIF Ultra assays for the detection of pulmonary tuberculosis and rifampicin resistance in adults and adolescents. The WHO categorises tests with similar characteristics and performance into a class (WHO 2021a). Xpert and Truenat assays are considered low-complexity automated nucleic acid amplification tests. Low complexity refers to a circumstance in which no additional infrastructure is required, and basic laboratory capacities are sufficient to execute the test. However, equipment may still be required (Pillay 2022). Truenat assays, developed by Molbio Diagnostics in Bangalore, India, include Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx. The Truenat and Xpert assays can both detect dead and live bacilli in the test sample. One study found that Truenat assays were non-inferior to Xpert assays (Penn-Nicholson 2021). Truenat MTB targets the ribonucleoside-diphosphate reductase B single-copy gene (*nrdB*), and Truenat MTB Plus uses multiple targets, *nrdZ* and IS*6110*, for identifying *M tuberculosis* complex. Truenat MTB is a quantitative test that gives actual colony-forming units (CFUs) per millilitre count, while Truenat MTB Plus is semi-quantitative and gives four grades (high, medium, low, and very low) based on CFUs but does not specify the actual count (Molbio 2019; Molbio 2020). Both assays have a similar run time and shelf life. Truenat MTB-RIF Dx targets the *rpoB* gene (ribonucleic acid polymerase gene's β subunit) for detecting rifampicin resistance (Nikam 2013; Nikam 2014). Both index tests have well-defined thresholds and the machine gives a positive or a negative test result.

As an initial step in Truenat analysis, a fully automated sample preparation device called Trueprep is used for extracting and purifying nucleic acids from a wide range of biological specimens. The Trueprep device uses an automated bead-based technique with a universal cartridge for extracting DNA from the sputum sample. The DNA extracted from a single instance in the Trueprep device can be used across all the Truelab devices for detection of *M tuberculosis* and rifampicin resistance. The time needed for DNA extraction and *M tuberculosis* detection is approximately one hour (Beall 2019). Users of Truenat can deselect rifampicin resistance testing and use the device for tuberculosis detection only, which is not possible when using Xpert assays. Mutations associated with rifampicin resistance are detected by a probe melt curve analysis of the amplified products in real-time PCR. In addition to the time required for *M tuberculosis* detection, rifampicin resistance detection takes approximately one more hour (Gomathi 2020a; Penn-Nicholson 2021). One multicentre trial evaluating the diagnostic accuracy of these assays for pulmonary tuberculosis reported 73% sensitivity (95% confidence interval (CI) 67 to 78) for Truenat MTB and 80% sensitivity (95% CI 75 to 84) for Truenat MTB Plus (Penn-Nicholson 2021). Truenat MTB showed lower sensitivity in smear-negative individuals with 36% (95% CI 27 to 47) for Truenat MTB and 47% (95% CI 37 to 58) for Truenat MTB Plus (Penn-Nicholson 2021).

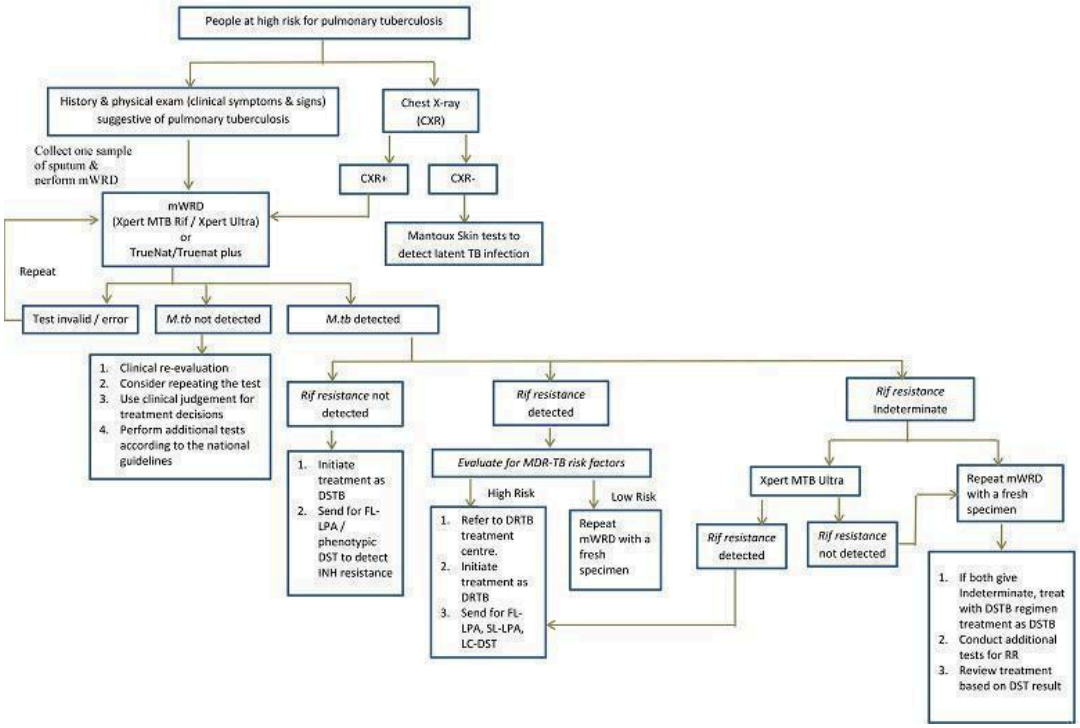
Xpert assays detect the presence of MTB and rifampicin resistance in a single step. Sample processing and the amplification process are combined in a closed system. Xpert MTB/RIF is based on detecting five overlapping 81-bp regions in the *rpoB* gene (i.e. rifampicin resistance-determining region (RRDR)) and uses molecular beacon technology (Cepheid 2022a; Rajendran 2022). The Xpert MTB/RIF Ultra test is based on two multicopy targets, IS*6110* and IS*1081*, for MTB detection and rifampicin resistance, respectively, with improved cartridge design and assay design (Cepheid 2022b; Chakravorty 2017). The test procedure involves mixing the sample reagent with the sputum provided by the manufacturer at a ratio of 2:1 for a direct specimen and 3:1 for processed pellets (Blakemore 2010). After an incubation period of 15 minutes, the mixture is loaded into the cartridge. The steps following sample loading are fully automated.

The total run time for the Xpert MTB/RIF and Xpert MTB/RIF Ultra assays are two hours and one to 1.5 hours, respectively (Chakravorty 2017; Theron 2014). According to one systematic review, Xpert MTB/RIF Ultra showed a higher sensitivity (90.9%, 95% credible interval (CrI) 86.2 to 94.7) compared to Xpert MTB/RIF (84.7%, 95% CrI 78.6 to 89.9), but exhibited lower specificity (95.6%, 95% CrI 93.0 to 97.4 for Xpert MTB/RIF Ultra; 98.4%, 95% CrI 97.0 to 99.3 for Xpert MTB/RIF) (Zifodya 2021). The current WHO recommendation, based on high-certainty evidence, is to use Xpert MTB/RIF Ultra for the initial detection of tuberculosis and rifampicin resistance (WHO 2024).

Clinical pathway

In low- and middle-income countries, molecular WHO-recommended rapid diagnostic tests (mWRDs), such as Truenat MTB and Xpert MTB/RIF Ultra, are recommended for the initial diagnosis of tuberculosis and rifampicin resistance in people with presumptive tuberculosis. Figure 1 describes the clinical pathway and the context in which these tests may be used.

Figure 1. Clinical pathway Abbreviations: CXR+: chest X-ray abnormal findings present; CXR-: normal chest X-ray; DSTB: drug-sensitive tuberculosis; DRTB: drug-resistant tuberculosis; FL-LPA: first-line line probe assay; RR: rifampicin resistance; INH: isoniazid; LC-DST: liquid culture drug susceptibility testing; MDRTB: multiple-drug-resistant tuberculosis; MTB: *Mycobacterium tuberculosis*; mWRD: molecular WHO-recommended rapid diagnostics; RIF: rifampicin; SL-LPA: second-line line probe assay. Adapted from WHO 2024.



Clinical suspicion of tuberculosis is based on symptoms of weight loss, fever, night sweats, cough, and haemoptysis, determined through medical history and physical examination (Heemskerk 2015; Lewinsohn 2017). Individuals with these symptoms should have a chest X-ray (posteroanterior view) in an erect position while holding their breath in full inspiration. Lateral views and lateral decubitus views may be clinically indicated. Individuals with these clinical manifestations, with or without chest X-ray abnormalities, are considered to have presumptive tuberculosis. A sputum sample should be collected and tested with an mWRD for rapid bacteriological confirmation of *M tuberculosis,* with or without additional testing for rifampicin resistance (WHO 2022b).

People with a positive mWRD result should always be followed up with further evaluations to establish a definitive diagnosis of tuberculosis. For people with a history of tuberculosis in the previous five years, a positive result may be due to the detection of DNA of dead bacilli persisting from the earlier tuberculosis episode. Therefore, a positive test in such individuals should be investigated with phenotypic methods to exclude a false-positive result. A negative mWRD test result may be followed up with further clinical evaluation if suspicion of tuberculosis is still high. This could include retesting with the same or another diagnostic method and close follow-up of clinical symptoms, with or without subsequent chest imaging.

If an mWRD for rifampicin resistance is performed and the result is negative, the individual is considered to have drug-sensitive tuberculosis, and should be started on the drug-sensitive tuberculosis regimen. The WHO recommends that all individuals presumed to have tuberculosis should undergo a rapid rifampicin resistance test as a component of universal drug susceptibility testing (WHO 2024). Positive rifampicin resistance detection leads to a diagnosis of drug-resistant tuberculosis and the administration of RR-TB or MDR-TB treatment regimen. If the rapid molecular test result is indeterminate, the test should be repeated with another mWRD or Xpert Ultra. If the result is still indeterminate, a sample is sent for phenotypic drug sensitivity testing to detect rifampicin resistance, and the individual is started on the drug-sensitive tuberculosis regimen.

False-positive results may necessitate additional testing and treatment, resulting in adverse events and potential stigma associated with tuberculosis. In contrast, false-negative reports may result in missed diagnoses, increasing the risk of community transmission. False-negative results can also cause severe forms of disease, leading to fatal outcomes (WHO 2024).

Settings of interest

We were interested in how the index tests were performed in adults and adolescents with presumptive tuberculosis presenting to local hospitals or primary care centres. The index tests can play a significant role in diagnosing pulmonary tuberculosis in peripheral laboratories when used as a point-of-care test in primary care facilities. These tests could mitigate diagnostic delays and increase the tuberculosis detection rate, thus breaking the transmission chain of tuberculosis.

Role of index test(s)

The role of the index tests is as an initial test for detection of pulmonary tuberculosis and rifampicin resistance in primary care facilities and peripheral laboratories.

Alternative test(s)

Phenotypic tests

Smear microscopy

Examination of acid-fast bacilli by sputum smear microscopy is a simple and rapid technique and the most widely used diagnostic tool for pulmonary tuberculosis. Ziehl-Neelsen-stained smears can be examined under light microscopy, while auramine-phenol-stained smears require fluorescence microscopy (Hooja 2011). Despite its utility in low-resource settings and advantages such as fast turnaround time and cost-effectiveness, smear microscopy has the major drawback of reduced sensitivity (50% to 60%). Detection under a microscope requires a high bacterial concentration of 5000 CFU/mL to 10,000 CFU/mL of bacilli (Arora 2020; Steingart 2006), and cannot distinguish between drug-resistant and drug-sensitive pulmonary tuberculosis (Kik 2014). Hence, WHO guidelines recommend replacing smear microscopy with mWRDs such as Xpert or Truenat assays as the initial test for all individuals with presumptive tuberculosis (WHO 2024).

Culture

Sputum culture is considered the reference standard for pulmonary tuberculosis diagnosis, with 10 to 100 viable bacilli being the minimum threshold for detection. Culture can detect 20% to 30% more people with pulmonary tuberculosis than smear microscopy and can also be used for drug susceptibility testing (Acharya 2020). However, solid culture takes four to 12 weeks to become positive for *M tuberculosis* growth. To overcome this limitation, in 2007, the WHO recommended the liquid culture system for *M tuberculosis* detection and drug susceptibility testing; this approach has a faster turnaround time, ranging from 10 to 42 days (WHO 2007). Kumari 2020 reported that liquid culture had higher sensitivity for *M tuberculosis* diagnosis than Lowenstein-Jensen (LJ) solid medium (100% for liquid culture versus 70.7% for LJ medium). Although the introduction of liquid culture has improved the turnaround time for diagnosis of pulmonary tuberculosis, it has a high contamination rate and must be performed by highly trained personnel in specialised laboratories.

Genotypic tests

The genotypic tests for diagnosing *M tuberculosis* include probes and gene amplification techniques; various molecular methods have been developed from these techniques since the early 2010s. In 2016, the WHO approved loop-mediated isothermal amplification technology (Eiken Chemical, Japan) as a diagnostic test for peripheral laboratories (WHO 2016a). The amplification process utilises at least four different sets of primers and is carried out in a single step, comprising a strand displacement reaction at 65 °C for 15 to 60 minutes. Loop-mediated isothermal amplification has been implemented for tuberculosis diagnosis based on the results of operational feasibility studies in peripheral settings of high-burden countries (Boehme 2007; Pandey 2008). The sensitivity of this test in different settings varies from 76% to 80%, and specificity from 97% to 98% (WHO 2016a).

Line probe assays (LPAs) are an alternative method for detecting resistance to drugs other than rifampicin. The technique is based on PCR amplification followed by hybridisation on a strip with a particular oligonucleotide probe (Nathavitharana 2017). GenoType MTBDR*plus* VER 2.0 (Hain Lifesciences, Germany) and INNO-LIPA RIF TB (Innogenetics, Belgium) are commercial LPA-based tests. INNO-LIPA RIF TB detects rifampicin alone, while GenoType MTBDR*plus* VER 2.0 detects both rifampicin and isoniazid from respiratory samples (Crudu 2012; Hain Lifescience 2022). Meta-analysis results from one systematic review evaluating the diagnostic accuracy of all three LPA techniques estimated a summary sensitivity of 96.7% (95% CI 95.6 to 97.5) and a summary specificity of 98.8% (95% CI 98.2 to 99.2) for rifampicin resistance, and a summary sensitivity of 90.2% (95% CI 88.2 to 91.9) and a summary specificity of 99.2% (95% CI 98.7 to 99.5%) for isoniazid resistance among people with smear-positive disease (Nathavitharana 2017). Commercial LPAs can act as initial tests for detecting resistance to isoniazid and rifampicin in the sputum of smear-positive people (direct testing) and culture specimens of both pulmonary and extrapulmonary tuberculosis (indirect testing), as per WHO recommendations (WHO 2016b). GenoType MTBDR*plus* VER 2.0 has the advantage of rapid turnaround time and is used in reference laboratories with established infrastructure and biosafety measures.

Rationale

In 2020, the WHO recommended Truenat MTB for the diagnosis of pulmonary tuberculosis. Since then, India's National Tuberculosis Elimination Programme (NTEP) has incorporated the test into its diagnostic algorithm. However, the WHO's recommendations to use Truenat as an initial diagnostic test for adults with presumptive pulmonary tuberculosis are conditional and based on moderate-certainty evidence from one multicentric prospective clinical evaluation of 1336 people (WHO 2024). The guidelines express serious concerns about the quality of evidence regarding the sensitivity of Truenat MTB and conclude that the certainty of evidence is low for sensitivity but high for specificity for the detection of pulmonary tuberculosis in adults (WHO 2024). The WHO recommendations to use Truenat MTB-RIF Dx for detecting rifampicin resistance are based on an analysis of 186/1336 participants. These participants were from seven reference laboratories across four countries (WHO 2024). The WHO Guideline Development Group expressed concerns about indirectness and inconsistency in sensitivity estimates for the detection of rifampicin resistance due to the small number of participants contributing to the analysis and concluded that the evidence on rifampicin resistance may not be generalisable to all settings (WHO 2024). The guideline contains a conditional recommendation based on low-certainty evidence for the use of Truenat MTB-RIF Dx for detecting rifampicin resistance (WHO 2024). There is also uncertainty regarding the use of this assay in people with HIV (WHO 2024). Therefore, we aimed to perform a systematic review and meta-analysis to synthesise evidence on Truenat assays that may aid the WHO and other agencies in formulating future guidelines and policies on the diagnosis of pulmonary tuberculosis and rifampicin resistance. In addition, given the established role of Xpert assays in the clinical pathway, we aimed to compare the accuracy of Truenat and Xpert by including studies that included a head-to-head comparison of the two assays (i.e. direct comparison). Since Xpert MTB/RIF Ultra has superseded Xpert MTB/RIF, and the

manufacturer discontinued Xpert MTB/RIF in most countries in 2023, we considered only Xpert MTB/RIF Ultra (Kay 2022)

This review was developed as part of the low-complexity automated nucleic acid amplification tests class-based review to inform the WHO Guideline Development Group meeting in May 2024. Prior to submitting this review, we became aware that Molbio Diagnostics will no longer be producing Truenat MTB assay for the international market and Truenat MTB will, therefore, be excluded from the low-complexity automated nucleic acid amplification tests class in WHO guidelines.

OBJECTIVES

To assess the diagnostic accuracy of Truenat assays (MTB, MTB Plus, and MTB-RIF Dx) for detecting pulmonary tuberculosis and rifampicin resistance in adults and adolescents with presumptive pulmonary tuberculosis.

Secondary objectives

To compare the diagnostic accuracy of Truenat assays and Xpert MTB/RIF Ultra for detecting pulmonary tuberculosis and rifampicin resistance and to investigate potential sources of heterogeneity (e.g. HIV status and smear status).

METHODS

Criteria for considering studies for this review

Types of studies

We included cross-sectional and cohort studies that reported the diagnostic accuracy of Truenat. For the comparison of Truenat and Xpert, we included comparative diagnostic accuracy studies in which each participant received both the index tests (paired design) or was randomised to receive one of the index tests (randomised design). We included studies that evaluated the index tests for the detection of pulmonary tuberculosis, rifampicin resistance, or both. We also included studies that performed the tests on sputum samples for confirmation of diagnosis alone. We only included studies that provided the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN), or statistics that enabled their derivation. We excluded studies with a case-control (two-gate) design because they could lead to biased accuracy estimates, especially when they enrol severe cases and healthy controls.

Participants

We included adults and adolescents (aged 10 years and older) with presumptive pulmonary tuberculosis (drug-susceptible tuberculosis, RR-TB, or MDR-TB). The diagnosis of presumptive pulmonary tuberculosis is based on symptoms of pulmonary tuberculosis, which typically include weight loss; loss of appetite; cough for two weeks or more, sometimes with blood-streaked sputum; and fever, especially at night. An individual with presumptive tuberculosis may also have a chest X-ray abnormality. MDR-TB refers to *M tuberculosis* resistance to both rifampicin and isoniazid, the most potent first-line drugs used in the treatment of tuberculosis. People with lung cavities, previously diagnosed tuberculosis, and a history of tuberculosis treatment are at significant risk for MDR-TB (Xi 2022). We included studies that recruited people with HIV, diabetes mellitus, or a history of tuberculosis. We excluded participants who were receiving tuberculosis treatment or had received treatment within the past seven days, as this could interfere with the index test and reference standard results. We included studies from all healthcare settings and peripheral, intermediate, and central laboratories, even though our setting of interest was peripheral laboratories. We also included studies from community and healthcare facilities, irrespective of the burden of tuberculosis in those settings. We placed no restrictions on the sex of participants or geographical location.

Index tests

Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx were the primary index tests. Truenat MTB-RIF Dx can detect rifampicin resistance to *M tuberculosis* in Truenat MTB- and Truenat MTB Plus-positive specimens. We compared the diagnostic accuracy of Truenat assays (MTB, MTB Plus, and MTB-RIF Dx) to Xpert MTB/RIF Ultra. For brevity, we refer to the Truenat assays as Truenat and Xpert MTB/RIF Ultra as Xpert Ultra unless it is necessary to distinguish between different types.

Target conditions

Pulmonary tuberculosis and rifampicin resistance.

Reference standards

The reference standard for identifying pulmonary tuberculosis is automated liquid culture, solid culture, or a combination of solid and liquid culture methods. The most commonly used solid culture medium is LJ, and liquid culture methods are the BACTEC 460 system (BD, USA) and the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 automated system (BD, USA). We considered any commercially available culture method as the primary reference standard. We also considered a composite reference standard. We accepted either a study-specific definition (i.e. a standardised definition of tuberculosis defined by the primary study authors) or a widely accepted standard definition for a composite reference standard to diagnose pulmonary tuberculosis. This composite reference standard may include symptoms and radiographic findings suggestive of pulmonary tuberculosis. A culture positive for *M tuberculosis* or a positive composite reference standard was considered pulmonary tuberculosis positive. Culture negative for *M tuberculosis* or a negative composite reference standard indicated the absence of pulmonary tuberculosis. The reference standard for rifampicin resistance was culture-based drug susceptibility testing. A positive culture-based result of drug resistance suggests the presence of rifampicin resistance, and a negative result indicates the absence of rifampicin resistance.

Search methods for identification of studies

Electronic searches

The Cochrane Infectious Diseases Group (CIDG) Information Specialist performed the search on 16 and 17 October 2023 using terms and strategies described in Appendix 1, without applying any language or date restrictions. We searched the following databases: MEDLINE (Ovid; 1946 to 16 October 2023), Embase (Ovid; 1947 to 16 October 2023), Science Citation Index (ISI Web of Knowledge, 1900 to 16 October 2023), Biosis previews (ISI Web of Knowledge, 1926 to 16 October 2023), Global Index Medicus, and SCOPUS (Elsevier, 1970 to 17 October 2023). We also searched the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/clinical-trials-registry-platform) and ClinicalTrials.gov (clinicaltrials.gov)

on 17 October 2023 to identify any ongoing trials. A WHO public call for data was made between 30 November 2023 and 15 February 2024 for ongoing and unpublished studies from manufacturers and researchers. We also contacted the authors of the studies for additional information.

Searching other resources

We performed bibliography mining of included studies manually. We searched tuberculosis conference proceedings to identify relevant conference abstracts and searched ProQuest Dissertations & Theses A&I for dissertations using terms for tuberculosis and Truenat. We also searched for reviews and guidelines and searched their respective reference lists. We contacted researchers at the New Diagnostic Working Group of the Stop TB Partnership, FIND (the global alliance for diagnostics), and other experts working on tuberculosis diagnostics for any ongoing and unpublished studies. We contacted the test manufacturers (Molbio Diagnostics, India) for unpublished studies. There were no language restrictions.

Data collection and analysis

Selection of studies

Four review authors (VA, MKS, AB, JD) independently screened titles and abstracts for eligibility using Rayyan software. Two review authors (VA and MKS) obtained and individually assessed potentially relevant publications. A third review author (LR) resolved any disagreements. We checked the reference lists of shortlisted articles for potentially relevant records not retrieved in the computerised searches. We listed reasons for exclusion of records at the full-text stage in the Characteristics of excluded studies table.

Data extraction and management

Four review authors (VA, MKS, AB, JD) independently extracted data using a piloted data extraction form (Appendix 2). We extracted the following information from the included studies.

- Study details: first author; publication year; country; World Bank economic classification of country (World Bank 2022); study setting (community; outpatient area of peripheral clinics; outpatient area of tertiary care hospitals; inpatients; peripheral, intermediate, and central referral laboratories); study design; method of participant allocation; number of participants screened, enroled, and excluded; study funding.
- Study participants: history of pulmonary tuberculosis, comorbidity status (diabetes, HIV, acid-fast bacilli smear).
- Target conditions: pulmonary tuberculosis, rifampicin resistance, or both.
- Reference standards: solid culture (LJ) or automated liquid culture (MGIT), drug susceptibility testing, manufacturer, cross-contamination of the culture media.
- Index tests: Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx. In addition, for comparative studies, details of Xpert Ultra.
- Sputum collection: type (such as expectorated sputum, induced sputum, bronchoalveolar lavage), condition (fresh or frozen), and smear status (positive or negative).
- Results: number of true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), and the number of missing or unavailable test results. We recorded the time of treatment initiation since the sputum collection date and the time to diagnose pulmonary tuberculosis after running the Truenat assay.
- Non-determinate and indeterminate index test results: Truenat MTB and MTB Plus can also yield test results such as invalid, error, or no result. We defined non-determinate results as a combination of operator and equipment errors, failures, or invalid and indeterminate results. The result is invalid in *M tuberculosis* testing if the internal positive control did not amplify, which could indicate poor sample collection or extraction error. The result is indeterminate in Truenat MTB-RIF Dx rifampicin resistance testing due to low bacilli load or a run error. There are different types of errors depending on the parts of the device that malfunction. We extracted the proportion of non-determinate (pulmonary tuberculosis) results and indeterminate (rifampicin resistance) results. We considered a trace Xpert Ultra result as a positive result for *M tuberculosis* (WHO 2017).

For the studies which did not have relevant data, we contacted the primary authors for further details. We used Microsoft Word for data extraction and entered the data directly into Review Manager (RevMan 2024).

Assessment of methodological quality

Two review authors (VA and MKS) assessed the methodological quality of the included studies using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (Whiting 2011). For comparative accuracy studies of Truenat and Xpert Ultra, we used the Quality Assessment of Diagnostic Accuracy Studies-Comparative (QUADAS-C) tool to assess the risk of bias (Yang 2021). We tailored the QUADAS-2 and the QUADAS-C tools to our review question, and seven review authors (LR, JD, PR, AB, VA, MKS, and MM) piloted and refined both tools (see Appendix 3). We summarised the results of the QUADAS-2 and QUADAS-C assessments graphically and narratively in the review text.

Statistical analysis and data synthesis

For both Truenat and Xpert, we categorised the results of *M tuberculosis* detection and rifampicin resistance as follows.

- *M tuberculosis* detected, rifampicin resistance not detected.
- *M tuberculosis* detected, rifampicin resistance detected.
- *M tuberculosis* not detected, rifampicin resistance not detected.
- *M tuberculosis* detected, rifampicin resistance indeterminate.

The unit of analysis was the participant rather than the specimen.

We summarised key study characteristics in Table 1 and the Characteristics of included studies table. We presented individual study estimates of sensitivity and specificity graphically in forest plots and in receiver operating characteristics (ROC) space using Review Manager (RevMan 2024). We performed meta-analysis to estimate summary sensitivities and specificities using a bivariate model. Where we were unable to fit a bivariate model due to sparse data, few studies, or limited variability in specificity, we simplified the model to a univariate random-effects model and synthesised sensitivity and specificity separately (Kay 2020; Takwoingi 2017). To compare index tests, we performed meta-regression by adding a covariate for test type to univariate models due to the limited data. We calculated absolute differences in sensitivity and specificity

using the model parameters. Meta-analyses were performed using the meqrlogit command in Stata 18.0.

Approach to non-determinate and indeterminate index test results

We reported the proportion of non-determinate results but did not perform meta-analysis for repeat tests in people with non-determinate test results due to insufficient data.

Note: for sensitivity and specificity, we rounded some numbers up when presenting percentages rather than raw data.

Investigations of heterogeneity

We examined individual study estimates of the sensitivity and specificity of Truenat using forest and summary ROC (SROC) plots to visually investigate heterogeneity. We investigated the effect of smear status, HIV status, and history of tuberculosis. We also planned to investigate other sources of potential heterogeneity such as setting, burden of tuberculosis, and blinding of reference standards. However, we were unable to do so due to the paucity of data.

Sensitivity analyses

We did not perform any of the prespecified sensitivity analyses using QUADAS-2 signalling questions due to insufficient data.

Assessment of reporting bias

We did not formally investigate reporting bias due to a lack of a well-developed methodology for test accuracy reviews (Takwoingi 2023). We contacted the study authors for relevant information that was missing. Our search strategy involved contacting experts and relevant organisations for unpublished and ongoing studies to minimise the risk of publication bias.

Assessment of the certainty of evidence

We assessed the certainty of the evidence using the GRADE approach for diagnostic studies (Balshem 2011; Schünemann 2008; Schünemann 2016). The evaluation of the certainty of evidence was largely based on our confidence in the estimates of sensitivity and specificity. We rated the certainty of the evidence as high (not downgraded), moderate (downgraded one level), low (downgraded two levels), or very low (downgraded more than two levels) for each of the five domains (risk of bias, indirectness, inconsistency, imprecision, and publication bias).

If there were high-quality cross-sectional or cohort studies that enroled participants with diagnostic uncertainty, we assessed the certainty of the evidence as high for both sensitivity and specificity. If there was a reason for downgrading, we used our judgement to determine whether the reason was serious (which would result in a one-level reduction) or very serious (which would result in a two-level reduction). Five review authors (LR, JD, MKS, VA, AB) discussed the judgements of certainty of the evidence and applied GRADE in the following format (GRADEpro GDT; Schünemann 2020a; Schünemann 2020b). We used the GRADEpro GDT online tool to create summary of findings tables for each target condition.

- **Risk of bias:** we used the QUADAS-2 and QUADAS-C tools to assess the risk of bias.
- **Indirectness:** we assessed indirectness in relation to the target population (including disease spectrum), setting, index tests, reference standards, and accuracy outcomes.
- **Inconsistency:** GRADE recommends downgrading for unexplained inconsistency in sensitivity and specificity estimates.
- **Imprecision:** we judged a precise estimate to be one that would enable a clinically meaningful decision. We considered the width of 95% CIs. We determined projected ranges for true positives (TP), false negatives (FN), true negatives (TN), and false positives (FP) for a given prevalence of tuberculosis and made judgements on imprecision based on these calculations.
- **Publication bias:** we considered the thoroughness of the literature search, outreach to tuberculosis researchers, the presence of studies that produce precise estimates with high accuracy despite a small sample size, and knowledge of studies that were conducted but not published.

RESULTS

Results of the search

We identified 1175 research articles from searches of electronic sources. After deduplication, we screened the titles and abstracts of 651 unique articles. We identified three studies through the WHO open call for data and identified one study by citation searching. Of the 655 articles, we excluded 617 based on titles and abstracts. We performed full-text screening of 38 articles and excluded 24 for various reasons (Figure 2 and Characteristics of excluded studies table). We included nine articles that met the eligibility criteria (Characteristics of included studies table). We identified five ongoing studies (Characteristics of ongoing studies table). No trials are awaiting classification.

Figure 2. Study flow diagram. #One study with two cohorts (Gomathi 2020a; Gomathi 2020b).

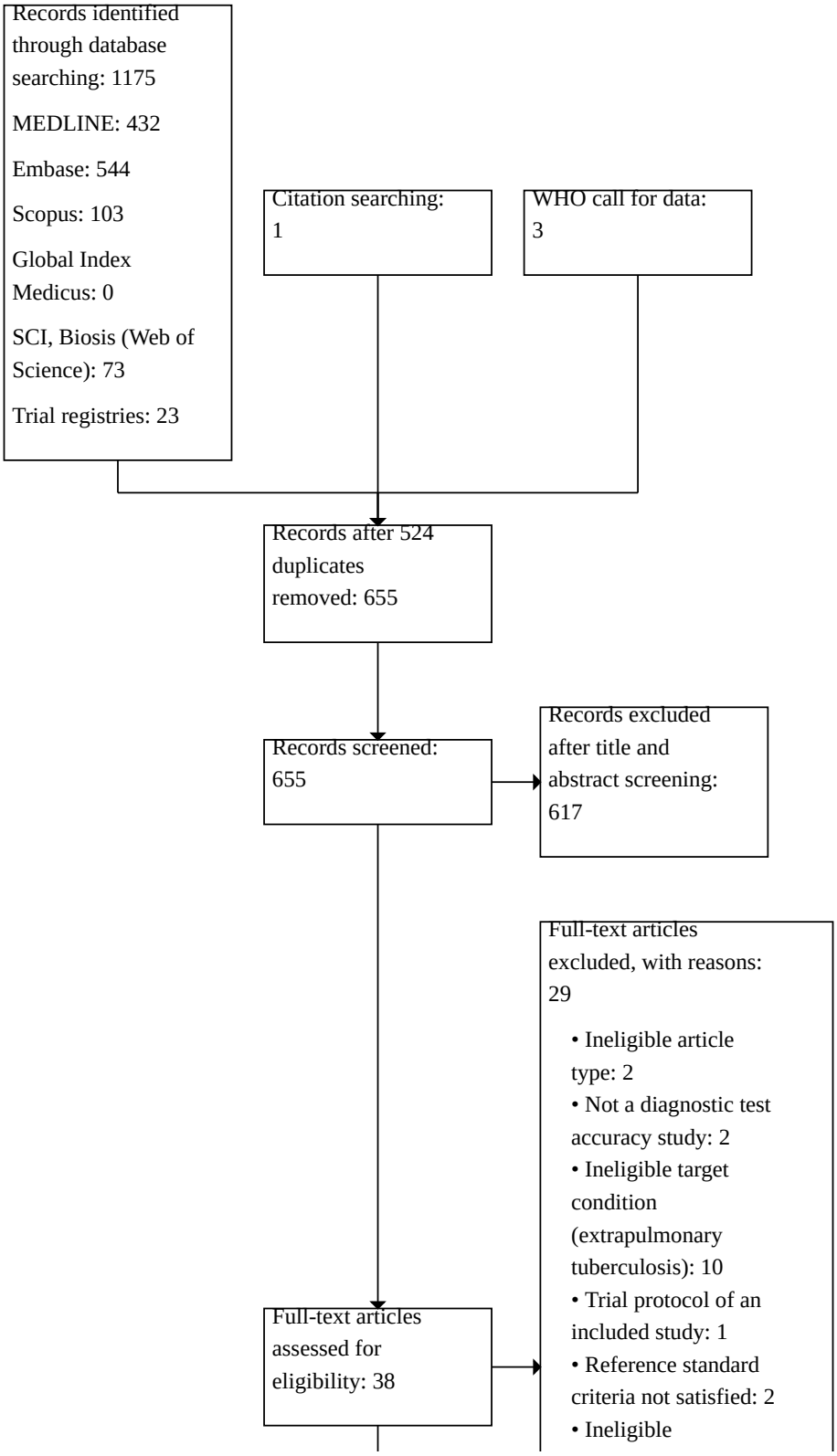
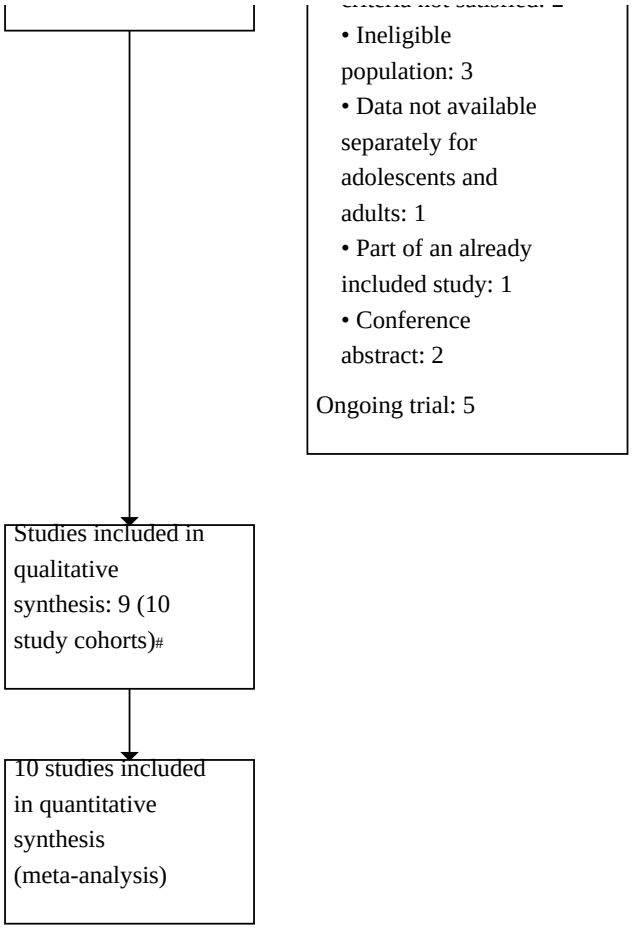


Figure 2. (Continued)



Description of included studies

Nine reports included 10 study cohorts (Gomathi 2020a; Gomathi 2020b; Gomathi 2020c; Jose 2024; Mangayarkarasi 2019; Meena 2023; Ngangue 2022; Penn-Nicholson 2021; Ssengooba 2024; Theron 2024). Gomathi 2020a and Gomathi 2020b were conducted at four sites across India. Of the four sites, two used single sputum specimens (unpooled) per participant, while the other two pooled multiple sputum specimens per participant. Since these were two different participant cohorts, we considered them separate studies. Thus, we included 10 studies. Penn-Nicholson 2021 asked participants enroled at primary healthcare centre clinics to provide four sputum specimens over two consecutive days. Two sputum specimens were collected on day one and sent to a centralised reference laboratory, where they were homogenised, pooled, and processed for culture, Xpert MTB/RIF or Ultra, Truenat, and smear testing. On day two, two sputum specimens were collected, of which one was sent to the reference laboratory for culture, while the other remained at a microscopy centre for Truenat assay testing. We included data from day one to maintain consistency with the analysis that informed the conditional recommendation by WHO in the 2020 guideline. Gomathi 2020c included participants at risk for drug-resistant tuberculosis from four sites across India.

We contacted all study authors for additional information and data except Ngangue 2022, as the published paper had adequate data and information. Four studies provided additional data and information (Gomathi 2020a; Gomathi 2020b; Gomathi 2020c; Theron 2024), and we obtained individual participant data for three studies (Jose 2024; Penn-Nicholson 2021; Ssengooba 2024).

Eight studies were conducted in low- and middle-income countries with high tuberculosis burden (Gomathi 2020a; Gomathi 2020b; Gomathi 2020c; Jose 2024; Mangayarkarasi 2019; Meena 2023; Ngangue 2022; Penn-Nicholson 2021); five studies were exclusively conducted in India (Gomathi 2020a; Gomathi 2020b; Gomathi 2020c; Jose 2024; Mangayarkarasi 2019); one in Cameroon (Ngangue 2022); one in South Africa (Theron 2024); one in Uganda (Ssengooba 2024); and one was in multiple countries (Penn-Nicholson 2021). Four studies included people with HIV (prevalence 2.7% to 54%) (Ngangue 2022; Penn-Nicholson 2021; Ssengooba 2024; Theron 2024). Mangayarkarasi 2019 used only solid culture. Gomathi 2020c, Meena 2023, and Theron 2024 used only liquid culture as the reference standard. All other studies used either solid or liquid culture. None of the studies used a composite reference standard. Two studies reported analysis of Truenat MTB Plus in addition to Truenat MTB (Ngangue 2022; Penn-Nicholson 2021). Jose 2024 and Theron 2024 evaluated only Truenat MTB Plus. One multiple-country study performed Xpert Ultra at a single

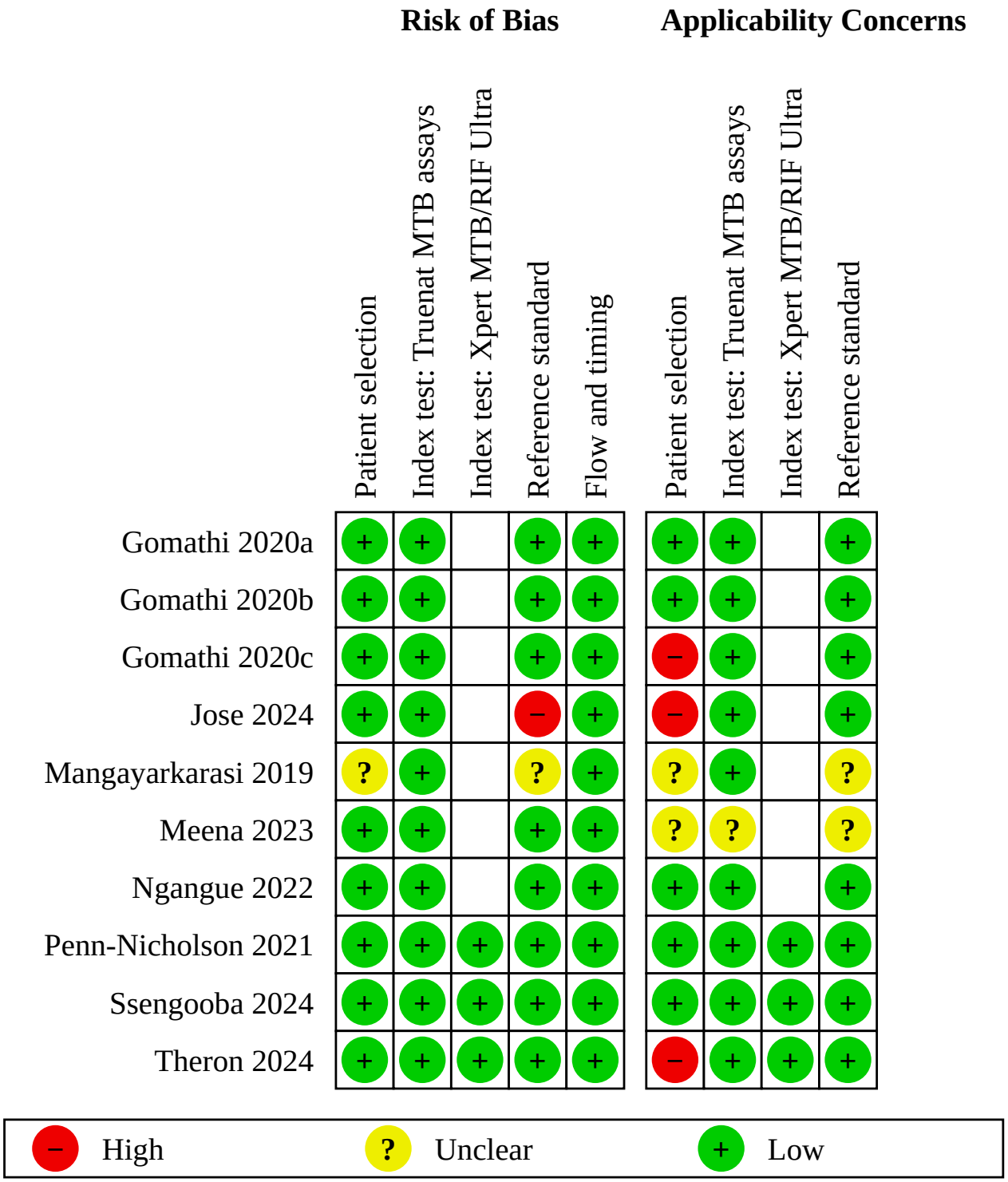
site in Peru (Penn-Nicholson 2021). Theron 2024 and Ssengooba 2024 also evaluated Xpert Ultra in addition to Truenat assays. Key characteristics of the included studies are described in Table 1, and full details in the Characteristics of included studies table.

Methodological quality of included studies

Truenat MTB for diagnosing pulmonary tuberculosis

Figure 3 summarises the results of the risk of bias and applicability assessment.

Figure 3. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study.



Patient selection

All studies except Mangayarkarasi 2019 were at low risk of bias in this domain. Mangayarkarasi 2019 did not report how the study participants were enroled. The risk of bias was also judged low for QUADAS-C for the studies, which included Xpert Ultra (Penn-Nicholson 2021; Ssengooba 2024; Theron 2024). Half of the studies had low applicability concern. Three studies were judged to have high applicability concern in the patient selection domain (Gomathi 2020c; Jose 2024; Theron 2024). Both Gomathi 2020c and Theron 2024 used frozen sputum specimens, and Jose 2024 recruited participants from the inpatient setting of a tertiary care hospital. Two studies recruited participants from a tertiary care hospital (Mangayarkarasi 2019; Meena 2023), while Meena 2023 included participants from inpatient and outpatient settings, and Mangayarkarasi 2019 did not report the setting. We judged these two studies to have unclear applicability concern.

Index tests

All studies were at low risk of bias since test results were machine-generated and followed prespecified manufacturer-recommended methods. All studies except Meena 2023 had low applicability concern since it was unclear whether the index test was performed according to the manufacturer's instructions in Meena 2023. The risk of bias was low for the QUADAS-C index test domain for the studies that evaluated Xpert Ultra (Penn-Nicholson 2021; Ssengooba 2024; Theron 2024).

Reference standard

Eight studies (80%) were at low risk of bias. In all of these studies, study personnel were blinded when interpreting the reference standard and all used standard culture methods. However, Jose 2024 did not blind the assessor and was at high risk of bias in this domain. Similarly, Mangayarkarasi 2019 did not mention blinding and was at unclear risk of bias. The reference standard domain was at low risk of bias for QUADAS-C for the studies with the Xpert Ultra (Penn-Nicholson 2021; Ssengooba 2024; Theron 2024). Eight studies (80%) were rated as having low applicability concern since all studies performed mycobacterium speciation and sensitivity of the culture isolate. We were unsure whether Mangayarkarasi 2019 and Meena 2023 performed speciation of the culture isolates and judged them to have unclear applicability concern.

Flow and timing

We judged all studies at low risk of bias in this domain. The risk of bias was also low for QUADAS-C for the studies with Xpert Ultra (Penn-Nicholson 2021; Ssengooba 2024; Theron 2024)

Truenat MTB-RIF Dx for detection of rifampicin resistance

The two studies that evaluated rifampicin resistance were at low risk of bias in all the domains (Gomathi 2020c; Penn-Nicholson 2021). Gomathi 2020c had high applicability concern in the patient selection domain as the tests were performed using frozen sputum specimens.

Findings

1. Detection of pulmonary tuberculosis

Truenat MTB for pulmonary tuberculosis detection

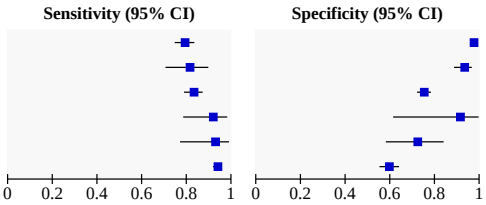
Six studies (4081 participants, 1379 with tuberculosis) assessed the diagnostic accuracy of Truenat MTB (Gomathi 2020a; Gomathi 2020b; Mangayarkarasi 2019; Meena 2023; Penn-Nicholson 2021; Ssengooba 2024) (Summary of findings 1). The median sample size was 657 (interquartile range 72 to 1208).

The prevalence of pulmonary tuberculosis ranged from 29% to 76%. The sensitivity of Truenat MTB for the detection of pulmonary tuberculosis ranged from 79% to 94%, and specificity ranged from 60% to 98% (Figure 4). The summary sensitivity of Truenat MTB was 87.6% (95% CI 81.6 to 91.8; high-certainty evidence), and the summary specificity was 86.1% (95% CI 70.1 to 94.3; moderate-certainty evidence) (Table 2; Figure 5).

Figure 4. Forest plot of Truenat MTB for pulmonary tuberculosis (including subgroups). The studies are sorted on the plot by sensitivity. FN: false negative; FP: false positive; TB: tuberculosis; TN: true negative; TP: true positive.

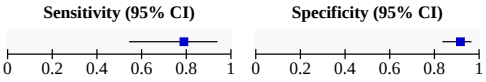
Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	275	27	71	1168	0.79 [0.75, 0.84]	0.98 [0.97, 0.99]
Ssengooba 2024	58	11	13	160	0.82 [0.71, 0.90]	0.94 [0.89, 0.97]
Gomathi 2020a	273	189	54	581	0.83 [0.79, 0.87]	0.75 [0.72, 0.78]
Meena 2023	35	1	3	11	0.92 [0.79, 0.98]	0.92 [0.62, 1.00]
Mangayarkarasi 2019	27	14	2	37	0.93 [0.77, 0.99]	0.73 [0.58, 0.84]
Gomathi 2020b	535	202	33	301	0.94 [0.92, 0.96]	0.60 [0.55, 0.64]



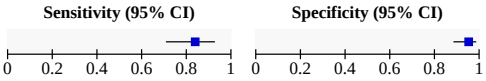
HIV-positive, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ssengooba 2024	15	7	4	77	0.79 [0.54, 0.94]	0.92 [0.84, 0.97]



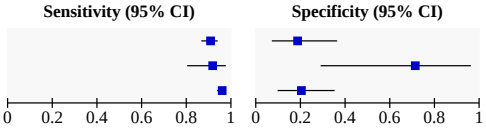
HIV-negative, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ssengooba 2024	42	4	8	82	0.84 [0.71, 0.93]	0.95 [0.89, 0.99]



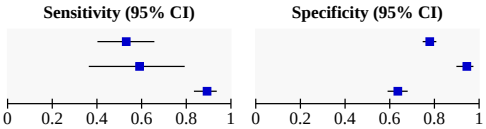
Smear-positive, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Gomathi 2020a	239	26	24	6	0.91 [0.87, 0.94]	0.19 [0.07, 0.36]
Ssengooba 2024	45	2	4	5	0.92 [0.80, 0.98]	0.71 [0.29, 0.96]
Gomathi 2020b	393	35	16	9	0.96 [0.94, 0.98]	0.20 [0.10, 0.35]



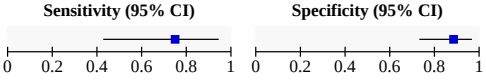
Smear negative, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Gomathi 2020a	34	163	30	575	0.53 [0.40, 0.66]	0.78 [0.75, 0.81]
Ssengooba 2024	13	9	9	155	0.59 [0.36, 0.79]	0.95 [0.90, 0.97]
Gomathi 2020b	142	167	17	292	0.89 [0.83, 0.94]	0.64 [0.59, 0.68]



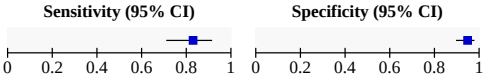
History of tuberculosis, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ssengooba 2024	9	4	3	31	0.75 [0.43, 0.95]	0.89 [0.73, 0.97]



No history of tuberculosis, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ssengooba 2024	49	7	10	129	0.83 [0.71, 0.92]	0.95 [0.90, 0.98]



Truenat MTB for pulmonary tuberculosis in central laboratories

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	275	27	71	1168	0.79 [0.75, 0.84]	0.98 [0.97, 0.99]
Ssengooba 2024	58	11	13	160	0.82 [0.71, 0.90]	0.94 [0.89, 0.97]
Meena 2023	35	1	3	11	0.92 [0.79, 0.98]	0.92 [0.62, 1.00]
Mangayarkarasi 2019	27	14	2	37	0.93 [0.77, 0.99]	0.73 [0.58, 0.84]

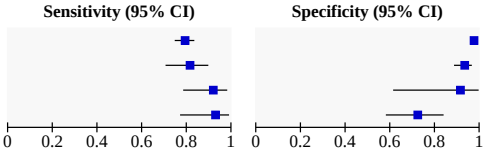
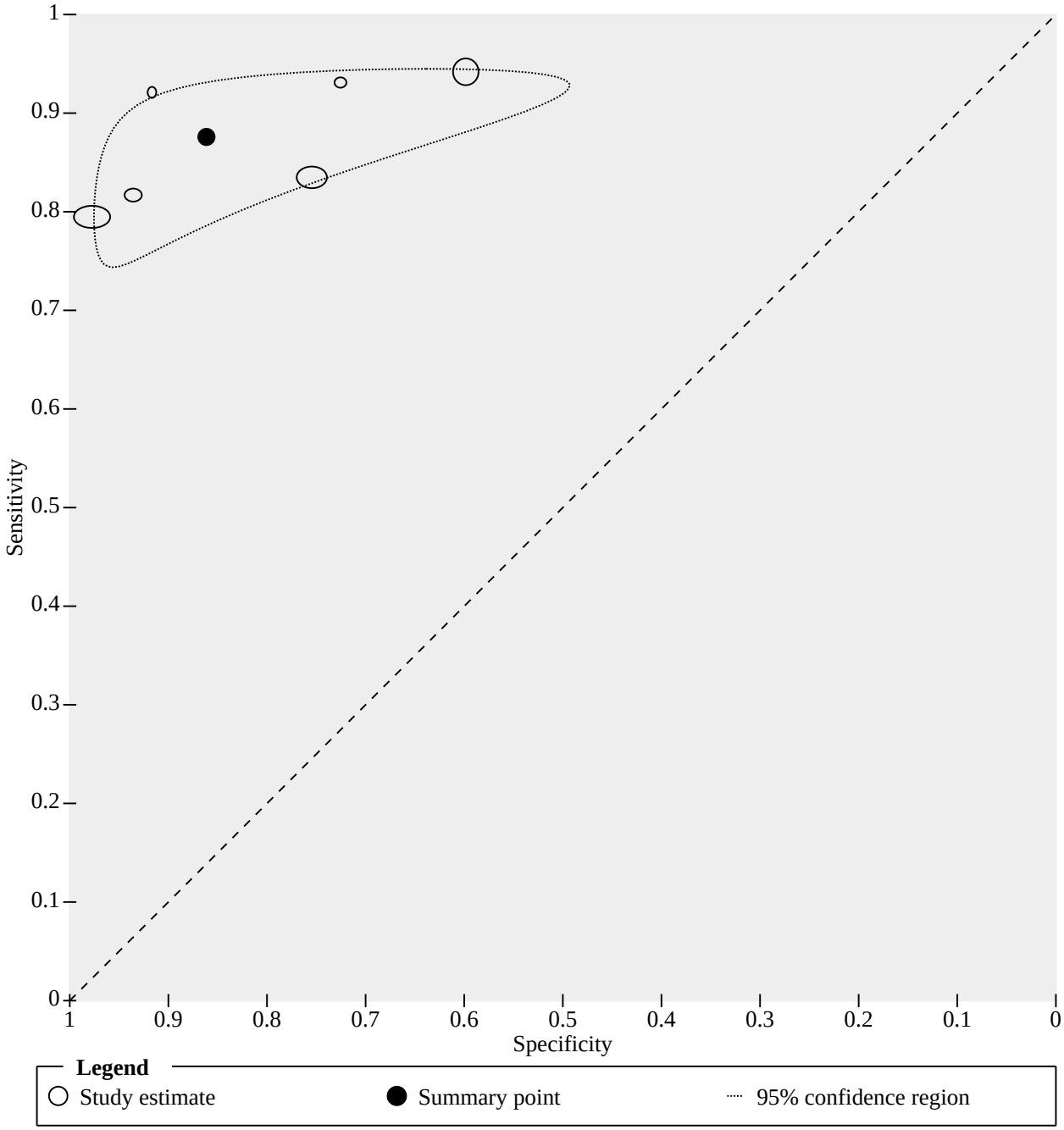


Figure 5. Summary ROC plot of Truenat MTB for pulmonary tuberculosis. The hollow circles/ovals are study points indicating the estimates of sensitivity and specificity. The width and height of each study point is proportional to the sample size for cases and non-cases, respectively. The solid black circle is the summary point (summary estimates of sensitivity and specificity). The dotted region around the summary point is the 95% confidence region, illustrating the uncertainty around the summary point.



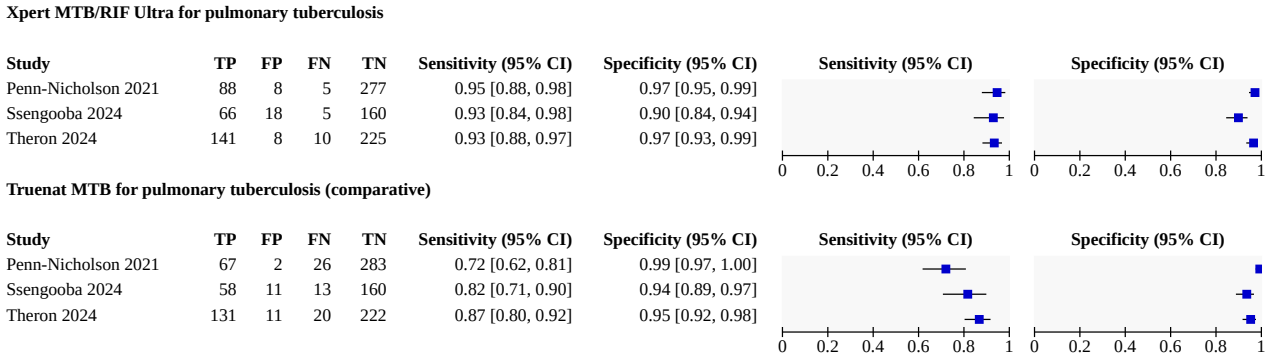
Truenat MTB versus Xpert Ultra for pulmonary tuberculosis detection

Two studies (Ssengooba 2024; Theron 2024), and a single site within a multiple-country study (Penn-Nicholson 2021), assessed Truenat MTB and Xpert Ultra. The studies included 315 people with tuberculosis amongst 1004 participants for Truenat MTB and 1011 for Xpert Ultra.

The summary sensitivity of Truenat MTB (81.0%, 95% CI 72.8 to 87.2) was lower than that of Xpert Ultra (93.7%, 95% CI 90.4 to 95.9), with an absolute difference of −12.7% (95% CI −20.3 to −5.0;

P = 0.001). The summary specificity of Truenat MTB was marginally higher (97.0%, 95% CI 91.9 to 98.9) than that of Xpert Ultra (95.3%, 95% CI 90.9 to 97.7), with an absolute difference of 1.64 (95% CI −2.79 to 6.06; P = 0.47) (Table 2; Figure 6).

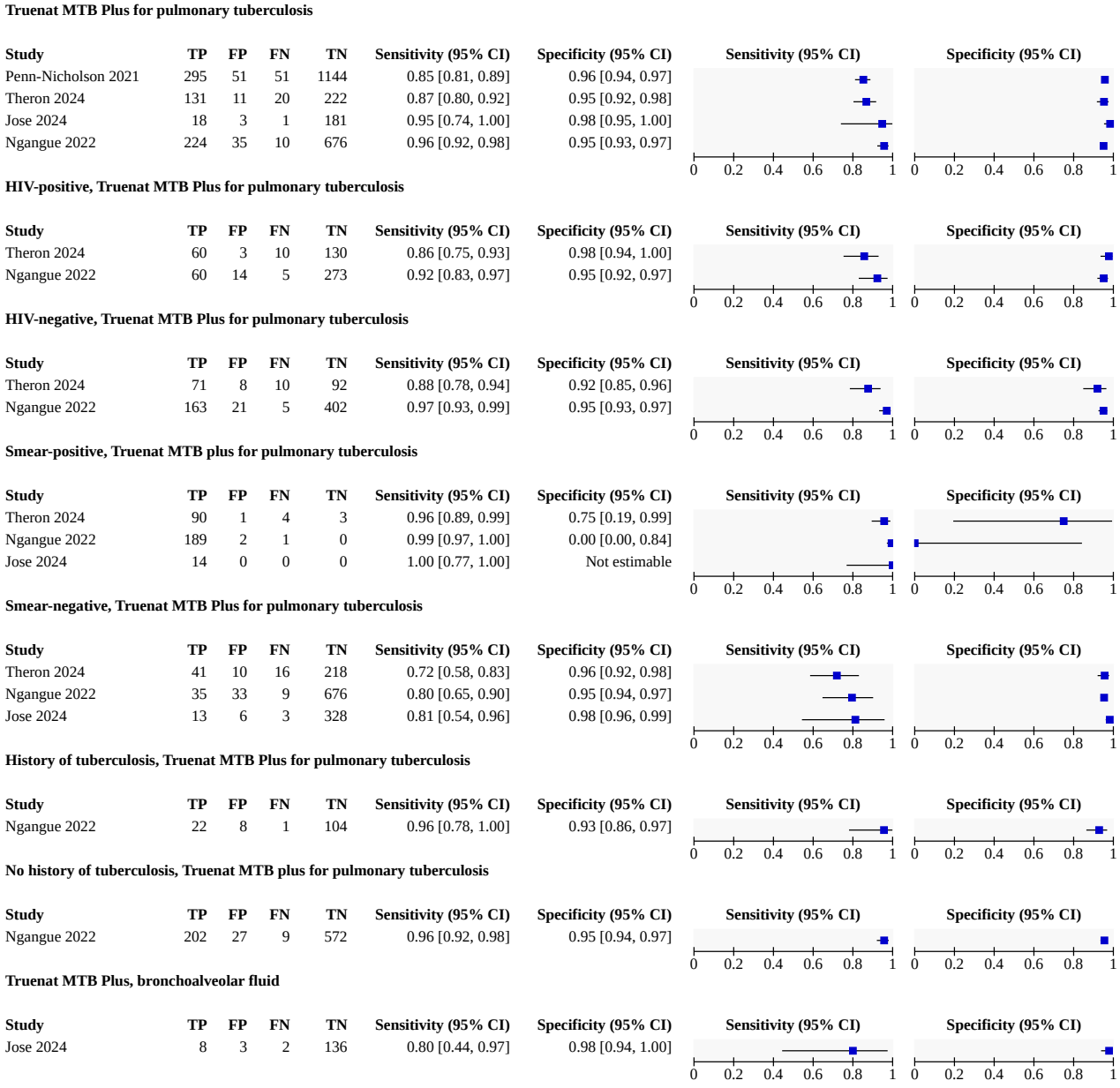
Figure 6. Forest plot of Truenat MTB for pulmonary tuberculosis. FN: false negative; FP: false positive; TB: tuberculosis; TN: true negative; TP: true positive.



Truenat MTB Plus for pulmonary tuberculosis detection

Four studies with 3073 participants (750 with tuberculosis) assessed Truenat MTB Plus for detecting pulmonary tuberculosis (Jose 2024; Ngangue 2022; Penn-Nicholson 2021; Theron 2024) (Summary of findings 2). Figure 7 shows the forest plots for all available data for Truenat MTB Plus.

Figure 7. Forest plot of Truenat MTB Plus for pulmonary tuberculosis (including subgroups). The studies are sorted on the plot by sensitivity. FN: false negative; FP: false positive; TB: tuberculosis; TN: true negative; TP: true positive.



The summary sensitivity was 90.6% (95% CI 83.7 to 94.8; high-certainty evidence), and the summary specificity was 95.7% (95% CI 94.7 to 96.5; high-certainty evidence) (Table 2).

Investigations of heterogeneity

There were limited data for investigations of heterogeneity. Table 2 summarises the available data for subgroups according to HIV status, smear status, history of tuberculosis, and laboratory setting. Data were available for subgroup analyses by smear status for both Truenat MTB and Truenat MTB Plus.

For Truenat MTB, three studies provided data for people with smear-positive tuberculosis (804 participants, 721 with tuberculosis) and smear-negative disease (3212 participants, 245 with tuberculosis) (Gomathi 2020a; Gomathi 2020b; Ssengooba 2024) (Figure 4). For smear-positive participants, summary sensitivity was 93.7% (95% CI 89.7 to 96.2) and specificity was 29.1% (95% CI 12.1 to 54.9). For smear-negative participants, summary sensitivity was 71.3% (95% CI 46.5 to 87.6) and specificity was 82.1% (95% CI 61.2 to 93.0).

For Truenat MTB Plus, three studies provided data for tuberculosis detection by smear status (Jose 2024; Ngangue 2022; Theron 2024) (Figure 7). Meta-analysis was not performed for smear-positive participants as specificity was not estimable in one study, 0% for one study, and 75% for the third study. In smear-negative participants (1388 participants, 117 with tuberculosis),

the summary sensitivity was 76.1% (95% CI 67.5 to 82.9), and the summary specificity was 96.4% (95% CI 94.4 to 97.7).

Non-determinate Truenat MTB and Truenat MTB Plus results

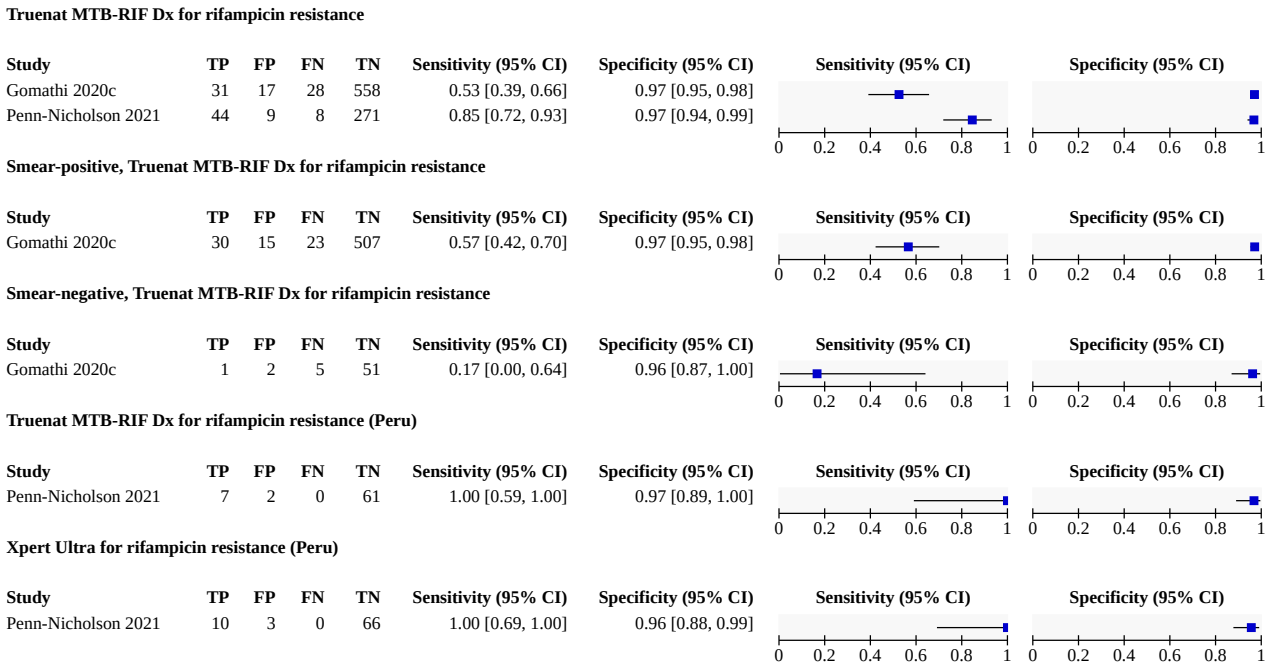
Three studies reported the proportion of non-determinate results with Truenat MTB, which ranged from 1.5% to 19.7% (Gomathi 2020a; Gomathi 2020b; Penn-Nicholson 2021). Ngangue 2022 and Theron 2024 reported non-determinate results of 10% and 17.1% for Truenat MTB Plus. Due to limited data, we did not perform a meta-analysis for repeat testing of people with non-determinate test results.

2. Detection of rifampicin resistance

Truenat MTB-RIF Dx

Two studies (966 participants, including 111 with rifampicin resistance) assessed the performance of Truenat MTB-RIF Dx for detecting rifampicin resistance (Gomathi 2020c; Penn-Nicholson 2021) (Summary of findings 3). The sensitivities were 53% and 85% (moderate-certainty evidence), and specificities were both 97% (high-certainty evidence) (Figure 8).

Figure 8. Forest plot of Truenat MTB-RIF Dx and Xpert Ultra for detection of rifampicin resistance. FN: false negative; FP: false positive; TN: true negative; TP: true positive.



Truenat MTB-RIF Dx versus Xpert Ultra for detection of rifampicin resistance

One study from a single site within a multiple-country study compared Truenat MTB-RIF Dx versus Xpert Ultra (Penn-Nicholson 2021) (Figure 8). The study comprised 70 participants, seven with rifampicin resistance for Truenat MTB-RIF Dx, and 79 participants, 10 with rifampicin resistance for Xpert Ultra.

The sensitivity of Truenat MTB-RIF Dx was 100% (95% CI 59 to 100) and Xpert Ultra was 100% (95% CI 69 to 100). The specificity of Truenat MTB-RIF Dx was 97% (95% CI 89 to 100) and Xpert Ultra was 96% (95% CI 88 to 99).

Truenat MTB-RIF Dx for detection of rifampicin resistance by smear status

Gomathi 2020c (575 participants, including 53 with rifampicin resistance) reported the performance of Truenat MTB for detecting rifampicin resistance in smear-positive and smear-negative people (Figure 8). In smear-positive participants, sensitivity was 57% (95% CI 42 to 70) and specificity was 95% (95% CI 95 to 98). In smear-negative participants, sensitivity was 17% (95% CI 0 to 64) and specificity was 96% (95% CI 87 to 100).

DISCUSSION

Summary of main results

We included nine studies for the detection of pulmonary tuberculosis and two studies for the detection of rifampicin resistance. We summarised the main results in Summary of findings 1; Summary of findings 2; and Summary of findings 3.

- For Truenat MTB (6 studies, 4081 participants), summary sensitivity was 87.6% (95% CI 81.6 to 91.8; high-certainty evidence), and summary specificity was 86.1% (95% CI 70.1 to 94.3; moderate-certainty evidence).
- For Truenat MTB Plus (4 studies, 3073 participants), summary sensitivity was 90.6% (95% CI 83.7 to 94.8; high-certainty evidence), and the summary specificity was 95.7% (95% CI 94.7 to 96.5; high-certainty evidence).
- Based on three comparative studies, the summary sensitivity of Xpert Ultra (93.7%, 95% CI 90.4 to 95.9) was significantly

higher than that of Truenat MTB (81.0%, 95% CI 72.8 to 87.2). In contrast, the summary specificity of Xpert Ultra was slightly lower (95.3%, 95% CI 90.9 to 97.7) than Truenat (97.0%, 95% CI 91.9 to 98.9).

- For the detection of rifampicin resistance, based on two studies, the sensitivities were 53% and 85% (moderate-certainty evidence), and specificities were both 97% (high-certainty evidence).

Truenat MTB assays for pulmonary tuberculosis detection

Our results indicate that in a hypothetical population of 1000 with a 10% prevalence of tuberculosis based on culture (100/1000), 214 would be Truenat MTB positive (21.4% test positive), with 88 (41.1% true positive) having tuberculosis and 126 (58.9% false positive) not having tuberculosis. Similarly, 786 would be Truenat MTB negative (78.6% test negative), with 774 (98.5% true negative) not having tuberculosis but 12 (1.5%) having tuberculosis (false negative) and be missed. Before submitting this review, we became aware that Molbio Diagnostics will no longer be producing Truenat MTB assay for the international market. Truenat MTB will, therefore, be excluded from the low-complexity automated nucleic acid amplification tests class in future WHO guidelines.

For Truenat MTB Plus, in a hypothetical population of 1000 with a 10% tuberculosis prevalence based on culture (100/1000), 127 would be Truenat MTB Plus positive (12.7% test positive), with 91 (71.6% true positive) having tuberculosis and 36 (28.4% false positive) not having tuberculosis. Similarly, 873 would be Truenat MTB Plus negative (87.3% test negative), with 864 (98.9% true negative) not having tuberculosis but 9 (1.1%) having tuberculosis (false negative) and be missed.

Strengths and weaknesses of the review

Completeness of evidence

Our review used a comprehensive search strategy, and we searched several databases. We also performed a grey literature search, handsearching of included studies, and contacted tuberculosis experts for studies missing from the electronic search. In addition, we obtained studies through the WHO public call for data. We contacted study authors for additional information before excluding the studies. We also contacted the authors of nine of the 10 included studies. We obtained additional information and data for four studies and individual participant data for three studies. We believe the chance that we may have missed relevant studies is minimal.

Accuracy of the reference standards used

In this review, we considered culture as the reference standard for detection of pulmonary tuberculosis, as culture is generally regarded as the best method for diagnosing active tuberculosis by detecting live *M tuberculosis* organisms. Since liquid culture is considered more sensitive than solid culture (Kumari 2020), we extracted the type of culture used. Of the 10 studies, three studies exclusively used liquid culture (Gomathi 2020c; Meena 2023; Theron 2024), and six studies used a combination of liquid or solid culture (Gomathi 2020a; Gomathi 2020b; Jose 2024; Ngangue 2022; Penn-Nicholson 2021; Ssengooba 2024). Mangayarkarasi 2019 used only solid culture as a reference standard. The WHO recommends phenotypic culture and drug susceptibility testing as one of the reference standards for detecting rifampicin resistance (WHO 2022a). The WHO also lowered the critical concentration for rifampicin resistance testing in 2021 to reduce false positives (WHO 2021b; WHO 2024). All included studies used drug susceptibility testing as a reference standard for rifampicin resistance.

Quality assessment and quality of reporting of the included studies

The risk of bias was unclear in the patient selection domain for only one study, as it did not report the method of participant enrolment. The risk of bias was low for all studies for the index test and flow and timing domains. One study did not blind the reference standard (Jose 2024), while another study did not describe blinding with respect to the reference standard interpretation (Mangayarkarasi 2019), while all the other studies blinded the reference standard. Based on the published manuscript, it was difficult to understand certain aspects of the study. Hence, we contacted the authors for clarification and obtained original datasets from the study authors but could not differentiate between published and unpublished data as the datasets were anonymised.

Comparison with other systematic reviews

We are unaware of any other systematic review on Truenat MTB for pulmonary tuberculosis or rifampicin resistance. The WHO 2024 guidelines refer to a single unpublished study with 1336 participants for which certainty of evidence was low for sensitivity but high for specificity for pulmonary tuberculosis. We included the published version of that study in our review (Penn-Nicholson 2021). Zifodya 2021 performed a systematic review by including seven studies that evaluated Xpert Ultra and reported summary sensitivity of 90.9% (95% Crl 86.2 to 94.7) and summary specificity of 95.6% (95% Crl 93.0 to 97.4). Our review included three studies of Xpert Ultra with a summary sensitivity of 93.7% (95% CI 90.4 to 95.9) and summary specificity of 95.3% (95% CI 90.9 to 97.7).

Applicability of findings to the review question

Diagnosis of tuberculosis

In the patient selection domain, we judged two studies to have high concerns, as one used stored sputum specimens (Theron 2024), and one recruited participants from an inpatient setting in a tertiary care hospital (Jose 2024). Two more studies were judged to have unclear concerns, as the setting was not clear. Three studies contributed a significant number of participants for the analysis of pulmonary tuberculosis in this review (Gomathi 2020a; Gomathi 2020b; Penn-Nicholson 2021). While all three studies recruited participants in peripheral centres, the samples were processed in central laboratories. One study did not describe if the index test was performed per manufacturer instructions (Meena 2023), and two studies did not describe speciation of the isolates being done based on the reference standard (Mangayarkarasi 2019; Meena 2023). Hence, we marked unclear applicability concerns for these domains. Overall, the setting of the included studies was aligned with the intended setting of the review question.

Detection of rifampicin resistance

Both studies that evaluated rifampicin resistance were at low risk of bias. One study had a high applicability concern because the index test was performed using frozen sputum specimens (Gomathi 2020c).

AUTHORS' CONCLUSIONS

Implications for practice

Truenat MTB Plus had higher sensitivity and specificity than Truenat MTB (high-certainty evidence for both sensitivity and specificity). The high false-positive rate for Truenat MTB is a concern. The sensitivity of Xpert Ultra was significantly higher than that of Truenat MTB, while specificity was slightly lower. Evidence on the accuracy of Truenat MTB-RIF Dx was limited.

Implications for research

There is an urgent need for primary studies to evaluate the diagnostic accuracy of Truenat MTB-RIF Dx for rifampicin resistance in primary care settings and as a point-of-care test.

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We want to thank all the study authors who provided additional information and data. We thank all authors of the included studies for answering our questions and providing additional data, including Dr NS Gomathi and Mr Kannan (Gomathi 2020a; Gomathi 2020b; Gomathi 2020c); Dr Adam Penn-Nicholson (Penn-Nicholson 2021); Dr Grant Theron (Theron 2024); Dr Reena Jose (Jose 2024); and Dr Willy Ssengooba (Ssengooba 2024).

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Editorial and peer-reviewer contributions

The following people conducted the editorial process for this article.

- Sign-off Editors (final editorial decision): Karen R Steingart, MD, MPH, Cochrane Infectious Diseases, Honorary Research Fellow, Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK; Mariska MG Leeflang, Amsterdam UMC
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  - Mia Schmidt-Hansen, Cardiff University (methods review)
  - Marta Roque, Hospital de la Santa Creu i Sant Pau (statistical review)
  - April Coombe, University of Birmingham (search review)
  - One additional peer reviewer provided consumer peer review but chose not to be publicly acknowledged

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References to other published versions of this review

Inbaraj 2023

Inbaraj LR, Daniel J, Rajendran P, Bhaskar A, Srinivasalu VA, Narayanan MK, et al. Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database of Systematic Reviews* 2023, Issue 1. Art. No: CD015543. [DOI: 10.1002/14651858.CD015543]

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Gomathi 2020a

Study characteristics	
Patient Sampling	Adults with presumptive pulmonary TB enroled consecutively 2419 adults with presumptive TB after screening 2465 patients
Patient characteristics and setting	Excluded: people who had received ≥ 1 doses of anti-TB medication in the 60 days before screening. The blinded, cross-sectional, multicentre study was conducted at 4 sites in India: ICMR – National Institute for Research in Tuberculosis, Chennai; National Institute of Tuberculosis and Respiratory Diseases, Delhi; All India Institute of Medical Sciences, Delhi; and JALMA National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra. While all sites were tertiary centres, the study did not clearly mention the location where participants were recruited and samples collected. Study design: cross-sectional study Presenting signs and symptoms: persistent productive cough for ≥ 2 weeks Age: ≥ 18 years Sex: not reported HIV infection: not reported History of TB: not reported Clinical setting: not reported Laboratory level: central Country: India World Bank income classification: lower middle High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB/HIV burden country: yes
Index tests	Truenat MTB
Target condition and reference standard(s)	Pulmonary TB. Reference standards were either liquid culture (MGIT960) or solid culture (LJ). All tests were performed in the central laboratory.
Flow and timing	Quote: "Samples were transported to the laboratories and processed on the same day except on holidays when the samples were stored at 4 to 10°C in the laboratories."
Comparative	Xpert MTB/RIF as a comparator index test. This was not 1 of the index tests in our review.
Notes	Out of 4 sites, ICMR-NIRT and NITRD used single sputum specimens (unpooled) while AIIMS and JALMA used pooled sputum specimens for

Gomathi 2020a *(Continued)*

analysis. Gomathi 2020a described data from the former sites (unpooled) and Gomathi 2020b described the data from the later sites (pooled).

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Yes		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?	Yes		
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	

Gomathi 2020a *(Continued)*

Are there concerns that the target condition as defined by the reference standard does not match the question?		Low concern
DOMAIN 4: Flow and timing		
Was there an appropriate interval between index test and reference standard?	Yes	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		Low risk

Gomathi 2020b

Study characteristics	
Patient Sampling	Adults with presumptive pulmonary TB enroled consecutively 2419 adults with presumptive TB after screening 2465 people
Patient characteristics and setting	Included: adults aged ≥ 18 years with clinical suspicion of pulmonary TB and persistent productive cough for ≥ 2 weeks. Excluded: people who had received ≥ 1 doses of anti-TB medication in the 60 days before screening. The blinded, cross-sectional, multicentre study was conducted at 4 sites in India: ICMR – National Institute for Research in Tuberculosis, Chennai; National Institute of TB and Respiratory Diseases, Delhi; All India Institute of Medical Sciences, Delhi; and JALMA National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra. While all 4 sites are tertiary centres, the study did not clearly mention the location where participants were recruited and samples collected. Study design: cross-sectional study Presenting signs and symptoms: persistent productive cough for ≥ 2 weeks Age: ≥ 18 years Sex: not reported HIV infection: not reported History of TB: not reported Clinical setting: not reported Laboratory level: central Country: India World Bank income classification: lower middle High TB burden country: yes High multiple-drug-resistant-TB burden country: yes

Gomathi 2020b *(Continued)*

	High TB/HIV burden country: yes
Index tests	Truenat MTB
Target condition and reference standard(s)	Pulmonary TB. Reference standards were culture either by liquid culture (MGIT960) or solid culture (LJ). All the tests were performed in the central laboratory.
Flow and timing	"Samples were transported to the laboratories and processed on the same day except on holidays when the samples were stored at 4–10°C in the laboratories"
Comparative	The study used Xpert MTB/RIF as a comparative index test. This was not of our test of interest.
Notes	Out of 4 sites, ICMR-NIRT and NITRD used single sputum specimens (unpooled) while AIIMS and JALMA used pooled sputum specimens for analysis. Gomathi 2020a describes data from the former sites (unpooled) and Gomathi 2020b describes the data from the later sites (pooled).

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
DOMAIN 3: Reference standard			

Gomathi 2020b *(Continued)*

Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Yes		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?	Yes		
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Gomathi 2020c

Study characteristics	
Patient Sampling	Adults aged 18–65 years with presumptive drug-resistant pulmonary TB enroled consecutively Quote: "A total of 2586 presumptive MDR-TB patients met the inclusion criteria. Sputum specimens from the presumptive pulmonary MDR-TB patients under National Tuberculosis Elimination Program treatment were included in the study. The patient samples were collected individually on two consecutive days and two sputum samples were collected i.e. one on the spot and one on the next morning. Pooled samples were coded by the statistician at NIRT, Chennai, before subjecting to Truenat tests. The standard diagnostic tests i.e. sputum smear, culture, and DST [drug susceptibility testing], GeneXpert MTB/RIF, and Truenat MTB-RIF were done."
Patient characteristics and setting	Included: consecutive adults aged 18–65 years with presumptive drug-resistant pulmonary TB, attending National TB Elimination Programme (NTEP) clinics under 4 national institutes: AIIMS (All India Institute of Medical Sciences, New Delhi), NITRD (National Institute of Tuberculosis and Respiratory Diseases, New Delhi), NIRT (National Institute for Research in Tuberculosis, Chennai), and ICMR-National JALMA Institute for Leprosy and other Mycobacterial Diseases, Agra.

Gomathi 2020c *(Continued)*

	Exclusion criteria: inability of the patient to produce 2 sputum samples of > 4 mL, receiving anti-TB medication in the 60 days prior to testing, and TB treatment started > 48 hours before sampling. Study design: cross-sectional study Presenting signs and symptoms: not reported Age: 18–65 years Sex: not reported HIV infection: not reported History of TB: not reported Clinical setting: National Tuberculosis Elimination program clinics Laboratory level: central Country: India World Bank income classification: Lower middle High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB or HIV burden country: yes
Index tests	Truenat MTB and Truenat MTB-RIF Dx
Target condition and reference standard(s)	Pulmonary TB and rifampicin resistance. Reference standard was liquid culture (MGIT960) with drug susceptibility testing. All the tests performed in the central laboratory.
Flow and timing	Samples collected individually on 2 consecutive days and 2 sputum samples collected (i.e. 1 on the spot and 1 the next morning). The samples were subsequently pooled in the laboratory.
Comparative	Xpert MTB/RIF as comparator index test, which is not a test of interest in this review.
Notes	Quote: "Study population for evaluation of modified version," paragraph, Lines 8–17: "The manufacturers incorporated changes to include a control probe and came out with Version 2.0. This retrospective study on Version 2.0 was done at NIRT, Chennai, and was approved by the institutional ethics committee. Leftover de-identified sputum samples from 1201 consecutive presumptive MDR-TB patients attending National Tuberculosis Elimination program clinics of Chennai and Kanchipuram districts, Tamil Nadu, India, that were stored in deep freezer (−80°C) were included in the study."

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		

Gomathi 2020c *(Continued)*

Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Yes		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?	Yes		
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and timing			

Gomathi 2020c *(Continued)*

Was there an appropriate interval between index test and reference standard?	Unclear	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		Low risk

Jose 2024

Study characteristics	
Patient Sampling	Used sputum samples stored in the microbiology laboratory for TB testing
Patient characteristics and setting	Included: adolescents and adults aged 11–90 years whose samples were received in the microbiology laboratory for TB testing. Excluded: people receiving anti-TB treatment All samples tested with sputum smear, culture either solid (LJ) or liquid (BACTEC MGIT) Study design: retrospective cross-sectional study Presenting signs and symptoms: not reported Age: 11–90 years Sex: female (44.4%) HIV infection: not reported History of TB: not reported Clinical setting: inpatients from tertiary care hospitals Laboratory level: central Country: India World Bank income classification: lower middle High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB or HIV burden country: yes
Index tests	Truenat MTB Plus
Target condition and reference standard(s)	Pulmonary TB. Reference standard was solid (LJ) or liquid culture (Bactec MGIT). All the tests were performed in central laboratory.

Jose 2024 *(Continued)*

Flow and timing	Clinical specimens collected as per standard procedures and transported in 2 sterile screw-capped containers to the microbiology laboratory.
Comparative	None
Notes	Study included clinical samples to test for both pulmonary TB and extrapulmonary TB.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	No		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?	No		
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)			
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?	Yes		

Jose 2024 *(Continued)*

Could the reference standard, its conduct, or its interpretation have introduced bias?		High risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Mangayarkarasi 2019

Study characteristics	
Patient Sampling	2 sputum samples (spot and overnight) collected from 80 people with presumptive pulmonary TB. Samples were tested with sputum smear, solid culture (LJ), and Truenat MTB.
Patient characteristics and setting	Included: people with symptoms suggestive of pulmonary and extrapulmonary TB referred to a tertiary care hospital, where samples were collected for the diagnosis of TB. Study design: cross-sectional study Presenting signs and symptoms: not reported Age: not reported Sex: not reported HIV infection: not reported History of TB: not reported Clinical setting: tertiary care hospital Laboratory level: central Country: India World Bank income classification: lower middle High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB or HIV burden country: yes
Index tests	Truenat MTB
Target condition and reference standard(s)	Pulmonary TB. Reference standard was solid culture (LJ). All the tests were performed in the central laboratory.

Mangayarkarasi 2019 *(Continued)*

Flow and timing	Spot and overnight sputum were pooled and aliquoted for laboratory testing.
Comparative	None
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Unclear		
Could the selection of patients have introduced bias?		Unclear risk	
Are there concerns that the included patients and setting do not match the review question?			Unclear
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Unclear		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?			
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?			
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	

Mangayarkarasi 2019 *(Continued)*

Are there concerns that the target condition as defined by the reference standard does not match the question?		Unclear
DOMAIN 4: Flow and timing		
Was there an appropriate interval between index test and reference standard?	Unclear	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		Low risk

Meena 2023

Study characteristics	
Patient Sampling	People with presumptive pulmonary TB. Samples tested with liquid culture (MGIT 960) and Truenat MTB assay.
Patient characteristics and setting	Included: people aged > 15 years with clinical suspicion of pulmonary TB including symptoms of cough with expectoration for > 2 weeks, fever for > 2 weeks, weight loss, loss of appetite and haemoptysis, any abnormality in chest X-ray and immunocompromised individuals Excluded: people with confirmed pulmonary TB, critically ill, current anti-TB treatment Conducted in both outpatients and inpatients of the Department of Pulmonary Medicine in a tertiary care hospital. Study design: cross-sectional study Presenting signs and symptoms: not reported Age: not reported Sex: not reported HIV infection: not reported History of TB: not reported Clinical setting: outpatients and inpatients from pulmonary medicine department in a tertiary hospital Laboratory level: central Country: India World Bank income classification: lower middle High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB or HIV burden country: yes

Meena 2023 *(Continued)*

Index tests	Truenat MTB		
Target condition and reference standard(s)	Pulmonary TB. Reference standard was liquid culture (MGIT960) with drug susceptibility testing. All tests were performed in the central laboratory.		
Flow and timing	Fresh sputum samples collected and subsequently pooled in the laboratory.		
Comparative	None		
Notes			

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Unclear
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Unclear
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Yes		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?			
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		

Meena 2023 *(Continued)*

Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?			
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Unclear
DOMAIN 4: Flow and timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Ngangue 2022

Study characteristics	
Patient Sampling	People with presumptive TB enroled consecutively 1030 participants recruited, out of whom the data from 945 participants were included in final analysis. 52 participants did not provide 2 specimens or specimens with sufficient volume (early exclusions). 29 participants with ≥ 1 results missing, and 4 participants with smear-positive, culture-negative results were excluded.
Patient characteristics and setting	Included: consecutive people who were referred for TB testing to the laboratories of any of 4 study sites. Excluded: currently receiving TB treatment or reported having taken any TB treatment within previous 6 months. Presenting signs and symptoms: prolonged cough of ≥ 2 weeks and ≥1 of fever, night sweats, and weight loss Study design: prospective cohort study Age: ≥ 15 years Sex: 494 females (52%) HIV infection: 352 (37%) HIV positive History of TB: 135 (14%) Clinical setting: 4 hospitals in 3 regions of Cameroon: Mbingo Baptist Hospital and the Nkwen Baptist Health Center (Northwest region), Mutengene Baptist Hospital (Southwest region), and Mboppi Baptist Hospital (Littoral region) Laboratory level: central Country: Cameroon

Ngangue 2022 *(Continued)*

	World Bank income classification: lower middle High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB/HIV burden country: yes
Index tests	Truenat MTB Plus
Target condition and reference standard(s)	Pulmonary TB. Reference standards liquid culture (MGIT960) or solid culture (LJ). All tests were performed in the central laboratory.
Flow and timing	Participants were instructed on how to produce 2 sputum specimens with volume of ≥ 4 mL each. If participant could not expectorate a spot specimen, then a first-morning specimen and a subsequent second specimen were collected on the spot or as a second-morning specimen as possible. Specimens stored at 2–8 °C and transported to central laboratory.
Comparative	
Notes	Truenat MTB Plus was the index test. Adding a second Truenat test for a second sputum specimen increased the sensitivity to detect TB among participants with culture-positive TB to 92% (95% CI 88 to 95) and decreased specificity among those without culture-positive TB to 93% (95% CI 91 to 95), as shown in Table S1 in the supplemental material. We included data of day 1 only.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	

Ngangue 2022 *(Continued)*

Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Yes		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?			
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?			
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Penn-Nicholson 2021

Study characteristics	
Patient Sampling	Prospective, multicentre diagnostic accuracy study of the performance of the Truenat TB assays conducted in 19 clinical sites (with attached microscopy centres) and 7 reference laboratories across Ethiopia, India, Papua New Guinea, and Peru. Study population comprised adults presenting to clinics with symptoms suggestive of pulmonary TB disease. Participants recruited sequentially at each clinic or through neighbouring satellite clinics.
Patient characteristics and setting	Presenting signs and symptoms: not reported Age: > 18 years

Penn-Nicholson 2021 *(Continued)*

	Total recruited for the study: 1917 Number of participants considered for analysis: 1762 Sex: 762 females (43.2%) HIV infection: 48/1762 (2.7%) History of TB: 256/1762 (14.5%) Clinical setting: 19 clinical sites Laboratory level: 19 microscopy centres and 7 reference laboratories Country: India, Peru, Ethiopia, Papua New Guinea World Bank income classification: Ethiopia – low; India and Papua New Guinea – lower middle; Peru – upper middle High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB/HIV burden country: yes
Index tests	Truenat MTB Truenat MTB-RIF Dx
Target condition and reference standard(s)	Pulmonary TB and rifampicin resistance. Solid culture (LJ) and liquid culture (MGIT-960) and MGIT-DST
Flow and timing	Participants enroled at primary healthcare centre clinics asked to provide 3 sputum specimens for reference laboratory testing and an additional specimen for microscopy centre testing. Sputum specimens 1, 2, and 3 were transported to the centralised reference laboratory for culture, Xpert MTB/RIF or Ultra, Truenat, and smear testing. Sputum specimen 4 remained at the attached microscopy centre for Truenat assay testing.
Comparative	Xpert MTB/RIF Ultra (only in Peru)
Notes	A subset of this study containing the same participants was published as another study (Meaza 2021). The author confirmed that they were same sample and hence not evaluated as separate study. All the samples belonging to the parent study were analysed here.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		

Penn-Nicholson 2021 *(Continued)*

Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Yes		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?	Yes		
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		

Penn-Nicholson 2021 *(Continued)*

Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Low risk

Ssengooba 2024

Study characteristics	
Patient Sampling	Adults aged > 18 to 65 years with presumptive pulmonary TB enroled consecutively. The participant samples collected and aliquoted for testing. Samples were subjected to fluorescent microscopy acid-fast bacilli smear, culture (both solid (LJ) and liquid (MGIT 960)), Xpert Ultra, and Truenat MTB and Truenat MTB-RIF.
Patient characteristics and setting	Included: adults aged > 18 years with presumptive pulmonary TB and provided sputum samples Enrolment took place at the outpatients departments of Kampala Capital City Authority (KCCA) Health facilities including: Kisenyi Health Center IV, Kawaala Health Center IV, Kitebi Health Center III, and Kiswa Health Center III, and Namungoona Orthodox Hospital. Study design: cross-sectional study Presenting signs and symptoms: fever, cough for > 2 weeks, unexplained weight loss, night sweats, and chest pain Age: > 18 to 65 years Sex: not reported HIV infection: HIV-positive and HIV-negative individuals History of TB: not reported Clinical setting: outpatient department of Kampala Capital City Authority (KCAA) health facilities Laboratory level: central Country: Uganda World Bank income classification: lower High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB or HIV burden country: yes
Index tests	Truenat MTB
Target condition and reference standard(s)	Pulmonary TB. Reference standard was solid (LJ) or liquid culture (MGIT960). All tests were performed in the central laboratory.
Flow and timing	Participant samples collected, homogenised, and aliquoted for smear, culture, and index tests. If the first sample was insufficient,

Ssengooba 2024 *(Continued)*

	a second sample was requested and then subsequently pooled in the laboratory.
Comparative	Xpert Ultra
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Yes		

Ssengooba 2024 *(Continued)*

Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?	Yes		
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Theron 2024

Study characteristics	
Patient Sampling	Adults aged > 18 years self-presenting with symptoms suggestive of pulmonary TB enroled at 2 outpatient clinics in Cape Town, South Africa, from 2015 to 2021. Samples were biobanked and used retrospectively after procuring Truenat machines and testing kits. Samples were tested with sputum smear, MGIT 960 culture and drug susceptibility testing, Xpert Ultra, Truenat MTB Plus, and Truenat MTB-RIF.
Patient characteristics and setting	Included adults aged > 18 years attending outpatient clinics at Scottsdene and Wallacedene in the northern suburbs of Cape Town, South Africa, who met WHO symptom criteria for pulmonary TB 498 enroled, 384 analysed Study design: cross-sectional study Presenting signs and symptoms: WHO 4-symptom criteria Age: > 18 years Sex: 234 (60.9%) females HIV infection: 54% (269/384) History of TB: included

Theron 2024 *(Continued)*

	Clinical setting: outpatient clinics Laboratory level: central (Biomedical Research Institute laboratory) Country: South Africa World Bank income classification: upper middle High TB burden country: yes High multiple-drug-resistant TB burden country: yes High TB or HIV burden country: yes
Index tests	Truenat MTB Plus
Target condition and reference standard(s)	Pulmonary TB and rifampicin resistance. Reference standard was liquid culture (MGIT960) with drug susceptibility testing. All tests were performed in central laboratory.
Flow and timing	The biobank contained either raw sputum (spot or morning, or both), sputum remnants, or both. When selecting specimens, priority was given to raw sputum, and if not found, sputum remnants that remained after sputum processing for culture were retrieved. When 2 raw sputa were available, the morning sample was chosen over the spot sample.
Comparative	Xpert Ultra
Notes	

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index test (Truenat MTB assays)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	

Theron 2024 *(Continued)*

Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 2: Index test (Xpert MTB/RIF Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference standard			
Were the reference standard results for pulmonary tuberculosis interpreted without knowledge of the results of the index tests?	Yes		
Were the reference standard results for rifampicin resistance interpreted without knowledge of the results of the index tests?	Yes		
Is the reference standards likely to correctly classify the target condition (pulmonary tuberculosis)	Yes		
Is the reference standards likely to correctly classify the target condition (rifampicin resistance)?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

LJ: Lowenstein-Jensen; TB: tuberculosis.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Akhtar 2022	Reference standard not satisfied

Study	Reason for exclusion
Badola 2023	Reference standard not satisfied
Dahiya 2023	Ineligible target condition
Georghiou 2021	Not a diagnostic test accuracy study
Inamdar 2021	Conference abstract
Jose 2021	Ineligible target condition
Kambli 2020	Ineligible article type
Kumara 2021	Ineligible target condition
MacLean 2022	Ineligible target condition
Meaza 2021	Part of an already included study
NCT03712709	Trial protocol of an included study
Nikam 2013	Ineligible population
Nikam 2014	Ineligible population
Sharma 2021	Ineligible target condition
Sharma 2022	Ineligible target condition
Sharma 2023	Ineligible target condition
Sharma 2024a	Ineligible target condition
Sharma 2024b	Ineligible target condition
Shireesha 2020	Conference abstract
Singh 2020	Ineligible article type
Singh 2023	Data not available separately for adolescents and adults
Vajravelu 2022	Ineligible target condition
Valsan 2022	Ineligible population
Vijayalakshmi 2019	Not a diagnostic test accuracy study

Characteristics of ongoing studies *[ordered by study ID]*

NCT02252198

Study name	Evaluation of non-inferiority of two fast follower nucleic acid amplification tests

NCT02252198 *(Continued)*

Target condition and reference standard(s)	Tuberculosis; culture
Index and comparator tests	Epistem Genedrive and MolBio Truenat; GeneXpert MTB/RIF
Starting date	February 2014
Contact information	Dr Susan E Dorman, Johns Hopkins University; dsusan1@jhmi.edu
Notes	Checked on 24 July 2023; last update posted 12 December 2017; ongoing clinical trial with pending results publication.

NCT03303963

Study name	DIAgnostics for Multidrug Resistant Tuberculosis in Africa
Target condition and reference standard(s)	Tuberculosis, multiple-drug-resistant; culture, WGS
Index and comparator tests	Deeplex test, Molbio TrueNat for 2nd line, GeneXpert 2nd line, FDA microscopy; whole genome sequencing
Starting date	4 May 2017
Contact information	Dissou AFFOLABI, Laboratoire de Référence des Mycobactéries; affolabi_dissou@yahoo.fr
Notes	Checked 24 July 2023; last update posted 14 March 2023; ongoing clinical trial with pending results publication.

NCT04043390

Study name	A one-stop shop for the same day diagnosis and management of TB and HIV
Target condition and reference standard(s)	Tuberculosis and HIV; culture
Index and comparator tests	CRP, Molbio Truenat MTB, Xpert ULTRA MTB/RIF, Xpert
Starting date	21 January 2019
Contact information	Luis E Cuevas, Professor; Liverpool School of Tropical Medicine; lcuevas@liv.ac.uk
Notes	Checked 24 July 2023; last update posted 2 August 2021; ongoing clinical trial with pending results publication.

NCT04568954

Study name	TB-CAPT CORE Truenat Trial

NCT04568954 *(Continued)*

Target condition and reference standard(s)	Tuberculosis
Index and comparator tests	Truenat TB platform/TB assays
Starting date	28 August 2022
Contact information	Adam Penn-Nicholson, PhD; +41 22 710 05 91; Adam.Penn-Nicholson@finddx.org Morten Ruhwald, MD, PhD; +41 22 710 05 91; Morten.Ruhwald@finddx.org Principal Investigator: Katharina Kranzer; Medical Center of the University of Munich; Katharina.Kranzer@lshtm.ac.uk
Notes	Checked on 24 July 2023; last update posted 2 December 2022; ongoing clinical trial with pending results publication.

NCT05405296

Study name	Evaluation of the Truenat™ MTB Plus/COVID-19 Test for TB (tuberculosis) and COVID-19 (SARS-CoV2)
Target condition and reference standard(s)	Tuberculosis and COVID-19
Index and comparator tests	Truenat MTB Plus/COVID-19
Starting date	June 2022
Contact information	Rita Szekely, PhD; +41 22 749 29 32; Rita.Szekely@finddx.org Adam Penn-Nicholson, PhD; +41 22 749 29 46; Adam.Penn-Nicholson@finddx.org
Notes	Checked 24 July 2023; last update posted 14 June 2022; ongoing clinical trial with pending results publication.

DATA

Presented below are all the data for all of the tests entered into the review.

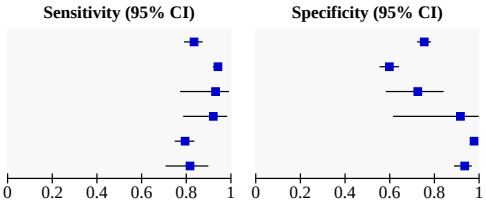
Table Tests. Data tables by test

Test	No. of studies	No. of participants
1 Truenat MTB for pulmonary tuberculosis	6	4081
2 Truenat MTB for pulmonary tuberculosis in peripheral laboratories	0	0
3 HIV-positive, Truenat MTB for pulmonary tuberculosis	1	103
4 HIV-negative, Truenat MTB for pulmonary tuberculosis	1	136

Test	No. of studies	No. of participants
5 Smear-positive, Truenat MTB for pulmonary tuberculosis	3	804
6 Smear negative, Truenat MTB for pulmonary tuberculosis	3	1606
7 History of tuberculosis, Truenat MTB for pulmonary tuberculosis	1	47
8 No history of tuberculosis, Truenat MTB for pulmonary tuberculosis	1	195
9 Truenat MTB for pulmonary tuberculosis in central laboratories	4	1913
10 Xpert MTB/RIF Ultra for pulmonary tuberculosis	3	1011
11 Truenat MTB-RIF Dx for rifampicin resistance	2	966
12 Truenat MTB-RIF Dx for rifampicin resistance in central lab	1	332
13 Truenat MTB-RIF Dx for rifampicin resistance in peripheral laboratories	0	0
14 Truenat MTB Plus for pulmonary tuberculosis – all data	4	3234
15 Truenat MTB Plus for pulmonary tuberculosis	4	3073
16 Truenat MTB Plus for pulmonary tuberculosis in peripheral laboratories	0	0
17 HIV-positive, Truenat MTB Plus for pulmonary tuberculosis	2	555
18 HIV-negative, Truenat MTB Plus for pulmonary tuberculosis	2	772
19 Smear-positive, Truenat MTB plus for pulmonary tuberculosis	3	304
20 Smear-negative, Truenat MTB Plus for pulmonary tuberculosis	3	1388
21 History of tuberculosis, Truenat MTB Plus for pulmonary tuberculosis	1	135
22 No history of tuberculosis, Truenat MTB plus for pulmonary tuberculosis	1	810
23 Truenat MTB Plus for pulmonary tuberculosis in central laboratories	4	3234
24 Truenat MTB for pulmonary tuberculosis (comparative)	3	1004
25 Truenat MTB Plus for tuberculosis (Peru)	1	378
26 Truenat MTB Plus, bronchoalveolar fluid	1	149
27 Smear-positive, Truenat MTB-RIF Dx for rifampicin resistance	1	575
28 Smear-negative, Truenat MTB-RIF Dx for rifampicin resistance	1	59
29 Truenat MTB-RIF Dx for rifampicin resistance (Peru)	1	70
30 Xpert Ultra for rifampicin resistance (Peru)	1	79

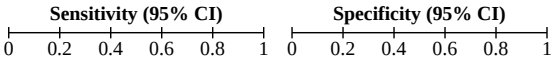
Test 1. Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Gomathi 2020a	273	189	54	581	0.83 [0.79, 0.87]	0.75 [0.72, 0.78]
Gomathi 2020b	535	202	33	301	0.94 [0.92, 0.96]	0.60 [0.55, 0.64]
Mangayarkarasi 2019	27	14	2	37	0.93 [0.77, 0.99]	0.73 [0.58, 0.84]
Meena 2023	35	1	3	11	0.92 [0.79, 0.98]	0.92 [0.62, 1.00]
Penn-Nicholson 2021	275	27	71	1168	0.79 [0.75, 0.84]	0.98 [0.97, 0.99]
Ssengooba 2024	58	11	13	160	0.82 [0.71, 0.90]	0.94 [0.89, 0.97]



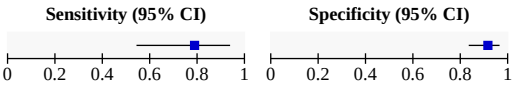
Test 2. Truenat MTB for pulmonary tuberculosis in peripheral laboratories

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)



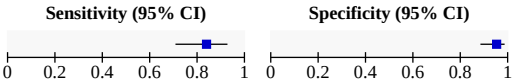
Test 3. HIV-positive, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ssengooba 2024	15	7	4	77	0.79 [0.54, 0.94]	0.92 [0.84, 0.97]



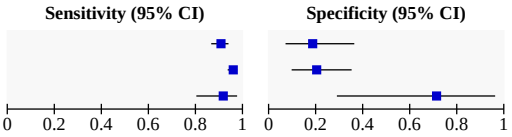
Test 4. HIV-negative, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ssengooba 2024	42	4	8	82	0.84 [0.71, 0.93]	0.95 [0.89, 0.99]



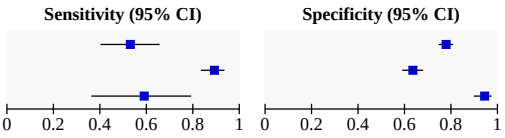
Test 5. Smear-positive, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Gomathi 2020a	239	26	24	6	0.91 [0.87, 0.94]	0.19 [0.07, 0.36]
Gomathi 2020b	393	35	16	9	0.96 [0.94, 0.98]	0.20 [0.10, 0.35]
Ssengooba 2024	45	2	4	5	0.92 [0.80, 0.98]	0.71 [0.29, 0.96]



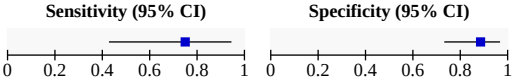
Test 6. Smear negative, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Gomathi 2020a	34	163	30	575	0.53 [0.40, 0.66]	0.78 [0.75, 0.81]
Gomathi 2020b	142	167	17	292	0.89 [0.83, 0.94]	0.64 [0.59, 0.68]
Ssengooba 2024	13	9	9	155	0.59 [0.36, 0.79]	0.95 [0.90, 0.97]



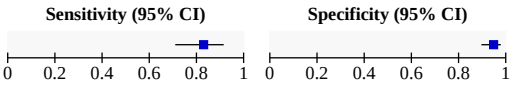
Test 7. History of tuberculosis, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ssengooba 2024	9	4	3	31	0.75 [0.43, 0.95]	0.89 [0.73, 0.97]



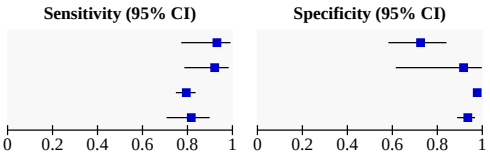
Test 8. No history of tuberculosis, Truenat MTB for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ssengooba 2024	49	7	10	129	0.83 [0.71, 0.92]	0.95 [0.90, 0.98]



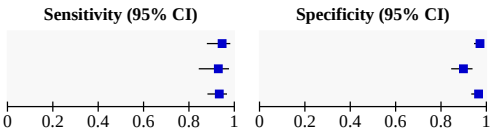
Test 9. Truenat MTB for pulmonary tuberculosis in central laboratories

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Mangayarkarasi 2019	27	14	2	37	0.93 [0.77, 0.99]	0.73 [0.58, 0.84]
Meena 2023	35	1	3	11	0.92 [0.79, 0.98]	0.92 [0.62, 1.00]
Penn-Nicholson 2021	275	27	71	1168	0.79 [0.75, 0.84]	0.98 [0.97, 0.99]
Ssengooba 2024	58	11	13	160	0.82 [0.71, 0.90]	0.94 [0.89, 0.97]



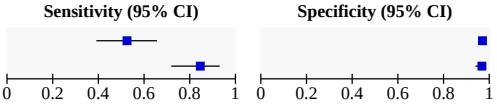
Test 10. Xpert MTB/RIF Ultra for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	88	8	5	277	0.95 [0.88, 0.98]	0.97 [0.95, 0.99]
Ssengooba 2024	66	18	5	160	0.93 [0.84, 0.98]	0.90 [0.84, 0.94]
Theron 2024	141	8	10	225	0.93 [0.88, 0.97]	0.97 [0.93, 0.99]



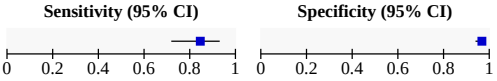
Test 11. Truenat MTB-RIF Dx for rifampicin resistance

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Gomathi 2020c	31	17	28	558	0.53 [0.39, 0.66]	0.97 [0.95, 0.98]
Penn-Nicholson 2021	44	9	8	271	0.85 [0.72, 0.93]	0.97 [0.94, 0.99]



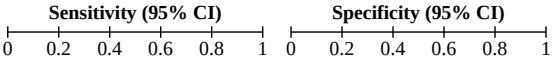
Test 12. Truenat MTB-RIF Dx for rifampicin resistance in central lab

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	44	9	8	271	0.85 [0.72, 0.93]	0.97 [0.94, 0.99]



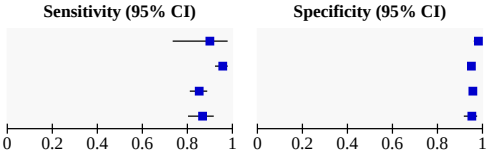
Test 13. Truenat MTB-RIF Dx for rifampicin resistance in peripheral laboratories

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)



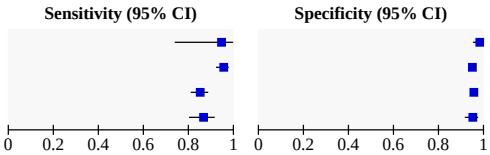
Test 14. Truenat MTB Plus for pulmonary tuberculosis – all data

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Jose 2024	27	6	3	328	0.90 [0.73, 0.98]	0.98 [0.96, 0.99]
Ngangue 2022	224	35	10	676	0.96 [0.92, 0.98]	0.95 [0.93, 0.97]
Penn-Nicholson 2021	295	51	51	1144	0.85 [0.81, 0.89]	0.96 [0.94, 0.97]
Theron 2024	131	11	20	222	0.87 [0.80, 0.92]	0.95 [0.92, 0.98]



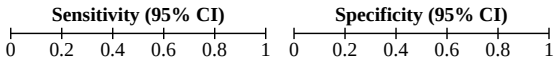
Test 15. Truenat MTB Plus for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Jose 2024	18	3	1	181	0.95 [0.74, 1.00]	0.98 [0.95, 1.00]
Ngangue 2022	224	35	10	676	0.96 [0.92, 0.98]	0.95 [0.93, 0.97]
Penn-Nicholson 2021	295	51	51	1144	0.85 [0.81, 0.89]	0.96 [0.94, 0.97]
Theron 2024	131	11	20	222	0.87 [0.80, 0.92]	0.95 [0.92, 0.98]



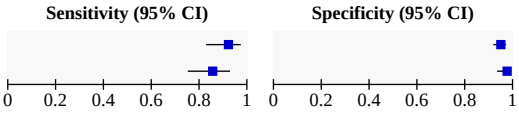
Test 16. Truenat MTB Plus for pulmonary tuberculosis in peripheral laboratories

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)



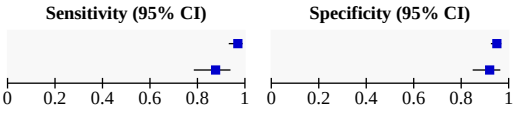
Test 17. HIV-positive, Truenat MTB Plus for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ngangue 2022	60	14	5	273	0.92 [0.83, 0.97]	0.95 [0.92, 0.97]
Theron 2024	60	3	10	130	0.86 [0.75, 0.93]	0.98 [0.94, 1.00]



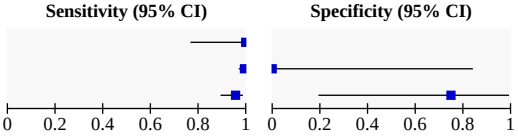
Test 18. HIV-negative, Truenat MTB Plus for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ngangue 2022	163	21	5	402	0.97 [0.93, 0.99]	0.95 [0.93, 0.97]
Theron 2024	71	8	10	92	0.88 [0.78, 0.94]	0.92 [0.85, 0.96]



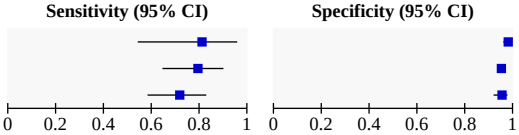
Test 19. Smear-positive, Truenat MTB plus for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Jose 2024	14	0	0	0	1.00 [0.77, 1.00]	Not estimable
Ngangue 2022	189	2	1	0	0.99 [0.97, 1.00]	0.00 [0.00, 0.84]
Theron 2024	90	1	4	3	0.96 [0.89, 0.99]	0.75 [0.19, 0.99]



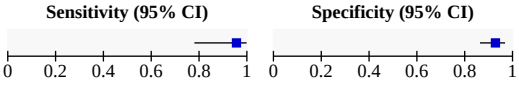
Test 20. Smear-negative, Truenat MTB Plus for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Jose 2024	13	6	3	328	0.81 [0.54, 0.96]	0.98 [0.96, 0.99]
Ngangue 2022	35	33	9	676	0.80 [0.65, 0.90]	0.95 [0.94, 0.97]
Theron 2024	41	10	16	218	0.72 [0.58, 0.83]	0.96 [0.92, 0.98]



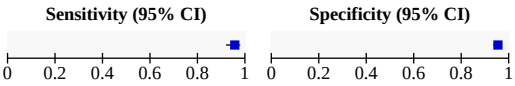
Test 21. History of tuberculosis, Truenat MTB Plus for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ngangue 2022	22	8	1	104	0.96 [0.78, 1.00]	0.93 [0.86, 0.97]



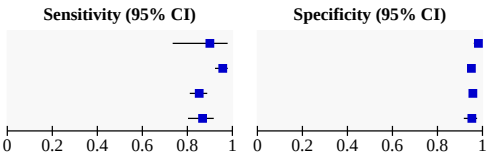
Test 22. No history of tuberculosis, Truenat MTB plus for pulmonary tuberculosis

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ngangue 2022	202	27	9	572	0.96 [0.92, 0.98]	0.95 [0.94, 0.97]



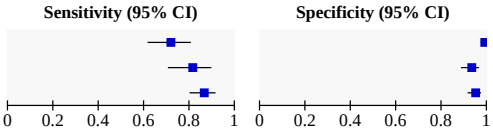
Test 23. Truenat MTB Plus for pulmonary tuberculosis in central laboratories

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Jose 2024	27	6	3	328	0.90 [0.73, 0.98]	0.98 [0.96, 0.99]
Ngangue 2022	224	35	10	676	0.96 [0.92, 0.98]	0.95 [0.93, 0.97]
Penn-Nicholson 2021	295	51	51	1144	0.85 [0.81, 0.89]	0.96 [0.94, 0.97]
Theron 2024	131	11	20	222	0.87 [0.80, 0.92]	0.95 [0.92, 0.98]



Test 24. Truenat MTB for pulmonary tuberculosis (comparative)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	67	2	26	283	0.72 [0.62, 0.81]	0.99 [0.97, 1.00]
Ssengooba 2024	58	11	13	160	0.82 [0.71, 0.90]	0.94 [0.89, 0.97]
Theron 2024	131	11	20	222	0.87 [0.80, 0.92]	0.95 [0.92, 0.98]

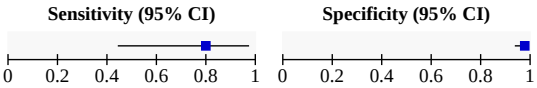


Test 25. Truenat MTB Plus for tuberculosis (Peru)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	73	7	20	278	0.78 [0.69, 0.86]	0.98 [0.95, 0.99]

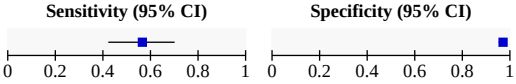
Test 26. Truenat MTB Plus, bronchoalveolar fluid

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Jose 2024	8	3	2	136	0.80 [0.44, 0.97]	0.98 [0.94, 1.00]



Test 27. Smear-positive, Truenat MTB-RIF Dx for rifampicin resistance

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Gomathi 2020c	30	15	23	507	0.57 [0.42, 0.70]	0.97 [0.95, 0.98]

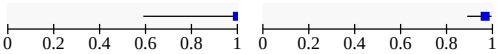


Test 28. Smear-negative, Truenat MTB-RIF Dx for rifampicin resistance

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Gomathi 2020c	1	2	5	51	0.17 [0.00, 0.64]	0.96 [0.87, 1.00]

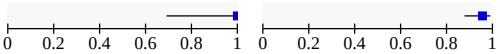
Test 29. Truenat MTB-RIF Dx for rifampicin resistance (Peru)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	7	2	0	61	1.00 [0.59, 1.00]	0.97 [0.89, 1.00]		



Test 30. Xpert Ultra for rifampicin resistance (Peru)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	10	3	0	66	1.00 [0.69, 1.00]	0.96 [0.88, 0.99]		



ADDITIONAL TABLES

Table 1. Characteristics of the included studies

Study	Index test	Country	Study design	Reference standard	Clinical setting	Proportion of people with HIV	Type of specimens	Truenat non-determinate[a] % (number/total)
Gomathi 2020a	Truenat MTB	India	Cross-sectional	LJ and MGIT	Outpatients from tertiary care hospitals and National Tuberculosis Elimination Program (NTEP) clinics	Not reported	Unpooled fresh sputum	1.5% (17/1097)
Gomathi 2020b	Truenat MTB	India	Cross-sectional	LJ and MGIT	Outpatients from tertiary care hospitals and National Tuberculosis Elimination Program clinics	Not reported	Pooled fresh sputum	19.7% (211/1071)
Gomathi 2020c	Truenat MTB, Truenat MTB RIF Dx	India	Cross-sectional	MGIT	Outpatients from all settings	Not reported	Pooled frozen sputum	6.4% (142/2188)
Jose 2024	Truenat MTB Plus	India	Cross-sectional	LJ and MGIT	Outpatients and inpatients (majority) from tertiary care hospital	Not reported	Unpooled fresh sputum	Not reported
Mangayarkarasi 2019	Truenat MTB	India	Cross-sectional	LJ	Tertiary care hospital	Not reported	Pooled fresh sputum	Not reported
Meena 2023	Truenat MTB	India	Cross-sectional	MGIT	Outpatient and inpatient setting of a tertiary care hospital	Not reported	Pooled fresh sputum	Not reported
Ngangue 2022	Truenat MTB Plus	Cameroon	Prospective cohort	LJ and MGIT	Outpatients from tertiary and secondary care	37% (352/945)	Unpooled fresh sputum	10% (136/1353) – before repeat testing 12.2% (166/1353) – after repeat testing
Penn-Nicholson 2021	Truenat MTB, Truenat MTB RIF Dx, Truenat MTB Plus	India, Peru, Ethiopia, Papua New Guinea	Prospective cohort	LJ and MGIT	Outpatients from peripheral clinics and tertiary hospitals	2.7% (48/1762)	Pooled fresh sputum	**Truenat MTB** 6.2% (293/4720) –before repeat testing

Table 1. Characteristics of the included studies *(Continued)*

								21.2% (62/293) – after repeat testing **Truenat MTB Plus** 9.2% (434/4720) – before repeat testing 36.8% (159/432) –after repeat testing **Truenat MTB RIF Dx** 22.5% (232/1042) – before repeat testing 762.7% (157/216) – after repeat testing
Ssengooba 2024	Truenat MTB	Uganda	Cross-sectional	LJ and MGIT	Outpatients	43.6% (109/250)	Unpooled fresh sputum	1.2% (3/250) invalid tests
Theron 2024	Truenat MTB Plus	South Africa	Cross-sectional	MGIT	Outpatients	54% (269/384)	Unpooled frozen sputum	86/501 (17.1%) – before repeat testing; 34/501 (6.7%) – after repeat testing

LJ: Löwenstein-Jensen medium (solid culture); MGIT: Mycobacteria Growth Indicator Tube (liquid culture).

Table 2. Accuracy of Truenat assays for pulmonary tuberculosis and rifampicin resistance detection in adults and adolescents

Analysis	Number			Summary sensitivity (95% CI)	Summary specificity (95% CI)
	Studies	People with tuberculosis	Total		
Truenat MTB					
Main analysis	6	1379	4081	87.6 (81.6 to 91.8)	86.1 (70.1 to 94.3)
HIV positive	1	19	103	—	—
HIV negative	1	50	136	—	—
Smear positive	3	721	804	93.7 (89.7 to 96.2)	29.1 (12.1 to 54.9)
Smear negative	3	245	1606	71.3 (46.5 to 87.6)	82.1 (61.2 to 93.0)
History of TB	1	12	47	—	—
No history of TB	1	59	195	—	—
Central laboratory	4	484	1913	84.1 (73.9 to 90.8)	92.7 (80.5 to 97.5)
Truenat MTB Plus					
Main analysis	4	3073	750	90.6 (83.7 to 94.8)	95.7 (94.7 to 96.5)
HIV positive	2	135	555	—	—
HIV negative	2	249	772	—	—
Smear positive[a]	3	298	304	—	—
Smear negative	3	117	1388	76.1 (67.5 to 82.9)	96.4 (94.4 to 97.7)
History of TB	1	23	135	—	—
No history of TB	1	211	810	—	—
Rifampicin resistance					
Truenat MTB-RIF Dx	2	111	966	—	—
Comparison of Truenat MTB and Xpert Ultra					
Truenat MTB	3	315	1004	81.0 (72.8 to 87.2)	97.0 (91.9 to 98.9)
Xpert Ultra	3	315	1011	93.7 (90.4 to 95.9)	95.3 (90.9 to 97.7)
Absolute difference (95% CI)	—	—	—	−12.7 (−20.3 to −5.00); P = 0.001	1.64 (−2.79 to 6.06); P = 0.47

TB: tuberculosis.

[a]specificity was not estimable for one study, 0% for one study, and 75% for the third study. Meta-analysis not performed.

APPENDICES

Appendix 1. Detailed search strategies

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions <1946 to October 16, 2023>

1 Extensively Drug-Resistant Tuberculosis/ or Tuberculosis, Multidrug-Resistant/ or Tuberculosis, Pulmonary/ or Mycobacterium Tuberculosis/

2 ((tuberculosis or TB) adj3 (lung* or pulmonic or bronchial or pulmonary)) or ((tuberculosis or TB) adj3 (respiratory or respirational)).mp.

3 (tuberculosis adj3 (drug resistan* or multidrug resistan* or mdr or xdr)).mp.

4 (((isoniazid adj3 resistance) or isoniazid) adj3 resistant).mp.

5 ((Ethionamide adj3 resistance) or (ethionamide adj3 resistant)).mp

6 ((Amikacin adj3 resistance) or (amikacin adj3 resistant)).mp.

7 ((Fluoroquinolone adj3 resistance) or (Fluoroquinolone adj3 resistant)).mp.

8 (Second-line injectable drug adj3 resistance).mp.

9 (MDR-TB or XDR-TB).mp.

10 1-9/or

11 (Truenat* or Molbio).mp

12 (Genexpert* or Xpert MTB*RIF or Xpert ultra).mp

13 exp Point-of-Care Systems/

14 (drug susceptibility test* or drug resistance test* or (rapid adj3 (detect* or test* or diagnos*)) or (poc or poct or "point of care")).mp.

15 11 or 12 or 13 or 14

16 10 and 15

Embase 1947-Present, updated daily

1 drug resistant tuberculosis/ or extensively drug resistant tuberculosis/ or lung tuberculosis/ or Mycobacterium Tuberculosis/

2 (((tuberculosis or TB) adj3 (lung* or pulmonic or bronchial or pulmonary)) or ((tuberculosis or TB) adj3 (respiratory or respirational))).mp.

3 (tuberculosis adj3 (drug resistan* or multidrug resistan* or mdr or xdr)).mp.

4 (((isoniazid adj3 resistance) or isoniazid) adj3 resistant).mp.

5 ((Ethionamide adj3 resistance) or (ethionamide adj3 resistant)).mp.

6 ((Amikacin adj3 resistance) or (amikacin adj3 resistant)).mp.

7 ((Fluoroquinolone adj3 resistance) or (Fluoroquinolone adj3 resistant)).mp.

8 (Second-line injectable drug adj3 resistance).mp.

9 (MDR-TB or XDR-TB).mp.

10 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9

11 (Truenat* or Molbio).mp.

12 (Genexpert* or Xpert MTB*RIF or Xpert ultra).mp.

13 exp Point-of-Care Systems/

14 (drug susceptibility test* or drug resistance test* or (rapid adj3 (detect* or test* or diagnos*)) or (poc or poct or "point of care")).mp.

15 11 or 12 or 13 or 14

16 10 and 15

Scopus
(TITLE-ABS-KEY ((truenat* OR molbio) AND tuberculosis)) OR ((TITLE-ABS-KEY (truenat* OR molbio)) AND (TITLE-ABS-KEY (drug AND resistant AND tuberculosis OR extensively AND drug AND resistant AND tuberculosis OR lung AND tuberculosis OR mycobacterium AND tuberculosis OR mdr-tb OR xdr-tb)))

WHO Global index medicus

(tw:(tuberculosis OR tb OR MDR-TB or XDR-TB)) AND (tw:(truenat or molbio))

Clinicaltrials.gov, WHO ICTRP: Tuberculosis and Truenat, MDR-TB and Truenat, XDR-TB and Truenat.

SCI-EXPANDED (Web of Science), BIOSIS Previews (Web of Science)

#1 drug resistant tuberculosis or extensively drug resistant tuberculosis (Topic) OR (MDR-TB or XDR-TB) (Topic)
#2 truenat (Topic) OR molbio (Topic)
#3 #1 AND #2

Appendix 2. Data extraction form

Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults

Study name:		
- Screening number:		
- Publication month & year:		
- First author:		
- Author contact email:		
- Was the author contacted? Yes/No**. If yes, when? _____**		
- Language of the article: English or Other _____		
- Funding: Industry sponsors/Institutional funds/Research grants/Unknown		
-Country of study origin		
-World Bank Classification: Low/Middle/High (circle If more than one)		
Study details	**Study design**	1. Cohort selection cross-sectional study / 2. Randomized comparative study – paired design / 3. Randomized comparative study – randomized design / 4. Not mentioned / 5. Other _____
	Participant selection	Consecutive / Convenient / Random / Not reported / Other _____
	Index tests	Truenat only / Xpert and Truenat

(Continued)

	Direction of study	Prospective / Retrospective / Ambi-directional / Not reported / Other _____
	Primary objective	Detect pulmonary TB (PTB) / Detect rifampicin (RIF) resistance / Both
	Number of people recruited	_____
	Number of people included in the analysis	Total: _____, Males: ___ (___ %), Females: ___ (___ %)
	Unit of analysis	Participant / Sputum / Not reported / Other _____
	Comments	
Sputum	**How was the sputum collected?**	Usual expectoration / Induced Sputum / Bronchoalveolar lavage / Tracheal aspirates / Multiple mixed methods / Not reported / Other ____
	How was the sputum processed?	Not processed / N-acetyl-l-cysteine–sodium hydroxide (NALC-NaOH) / Sodium hydroxide (Petroff's method) / Other _____
	Was the same sample used for Truenat and culture?	Yes / No
	Was the same sample used for Xpert and culture?	Yes / No / Not applicable
	Was the same sample used for Xpert and Truenat?	Yes / No / Not applicable
	Was the same sample used for Truenat, Xpert, and culture?	Yes / No / Not applicable
	Was the same sample used for Line Probe assay and Truenat/Xpert?	Yes / No / Not applicable
	How was the acid-fast bacillus (AFB) smear performed?	Not performed / Ziehl-Neelsen / Fluorescent microscopy / Both
	Number of smears	None / 1 / 2 / 3 / Other _____
	Smear type	Direct / Concentrated / Not reported
	Sample status	Fresh / Frozen / Not reported / Other
	Comments	
Reference standard for tuberculosis detection	**Solid culture**	Lowenstein-Jensen (LJ) / 7H10 / 7H11 / Other
	Liquid culture	Mycobacteria Growth Indicator Tube (MGIT) 960 / BACTEC 460 / Other
	Both solid and liquid/Either solid or liquid	
	Sample status	Fresh/Frozen/Not reported/Other

(Continued)

	Comments	
Reference standard for rifampicin resistance	**Solid culture**	Lowenstein-Jensen (LJ)/ Middlebrook 7H10/Middlebrook 7H11/Other
	Liquid culture	Mycobacteria Growth Indicator Tube (MGIT) 960 / BACTEC 460 / Other
	Both solid and liquid/Either solid or liquid	
	Polymerase chain reaction (PCR) test	Line Probe Assay
	Both culture and PCR/Either culture or PCR	
	Sample status	Fresh/Frozen/Not reported/Other
	Comments	
Contamination status	**Total number of cultures:**	_____
	Total number of contaminated cultures:	_____
Recruitment	Inpatient/Outpatient/Community/Laboratory/Not specified/Other _____	
Truenat	**Where was Truenat performed?**	Point of care / Peripheral Lab / Intermediate Lab / Central Lab
	Acceptable time from sputum collection to testing?	Yes / No
	Truenat assay type	MTB / MTB Plus / MTB-RIF Dx / All
	Truenat versions	
Xpert	**Where was Xpert performed (ignore if not performed)?**	Point of care / Peripheral Lab / Intermediate Lab / Central Lab
	Acceptable time from sputum collection to testing?	Yes / No
	Xpert assay type	Only Xpert Ultra / Both Xpert & Xpert Ultra
	Xpert Ultra version	
Smear	**Number of smear-positive participants**	Number _____ (_____%)
	Number of smear-negative participants	Number _____ (_____%)
History	**Number of participants with previous history of tuberculosis**	Number_____ (_____%)
	Number of participants with HIV positive status	Number_____ (_____%)
	Number of participants with diabetes	Number_____ (_____%)
Time to outcome	**Time to initiation of treatment**	_____

(Continued)

	Time to diagnosis	_____

Data for Truenat MTB

1.

Overall Truenat	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			
Truenat MTB Plus only	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			

RS: reference standard.

2.

Overall Truenat non-determinate	**RS positive**	**RS negative**	**Total**
Invalid			
Error			
No result			
Indeterminate			

RS: reference standard.

3.

Overall Truenat after repeat testing	**RS positive**	**RS negative**	**Total**

(Continued)

Truenat positive			
Truenat negative			
Total			
Non-determinate			

RS: reference standard.

4.

Smear positive	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			
Smear negative	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			

RS: reference standard.

5.

HIV positive	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			
HIV negative	**RS positive**	**RS negative**	**Total**
Truenat positive			

(Continued)

Truenat negative			
Total			
Non-determinate			

RS: reference standard.

6.

Past history of tuberculosis	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			
No past history of tuberculosis	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			

RS: reference standard.

7.

High tuberculosis prevalence setting	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			
Low tuberculosis prevalence setting	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			

(Continued)

Total			
Non-determinate			

RS: reference standard.

8.

Community setting	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			
Hospital setting	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			

RS: reference standard.

Data for Xpert Ultra

9.

XPERT Ultra	**RS positive**	**RS negative**	**Total**
Xpert positive			
Xpert negative			
Total			
Non-determinate			

RS: reference standard.

Data for rifampicin resistance

10.

Truenat RIF resistance	RS resistance positive	RS resistance negative	Total
Truenat RIF positive			
Truenat RIF negative			
Total			
Indeterminate			

RIF: rifampicin; RS: reference standard.

11.

Smear positive RIF resistance	RS resistance positive	RS resistance negative	Total
Truenat RIF positive			
Truenat RIF negative			
Total			
Indeterminate			
Smear negative RIF resistance	**RS resistance positive**	**RS resistance negative**	**Total**
Truenat RIF positive			
Truenat RIF negative			
Total			
Indeterminate			

RIF: rifampicin; RS: reference standard.

12.

Xpert Ultra RIF resistance	RS resistance positive	RS resistance negative	Total
Xpert RIF positive			
Xpert RIF negative			

(Continued)

Total			
Indeterminate			

RIF: rifampicin; RS: reference standard.

13.

Liquid culture – RIF resistance	RS Resistance positive	RS Resistance negative	Total
Truenat RIF positive			
Truenat RIF negative			
Total			
Indeterminate			

RIF: rifampicin; RS: reference standard.

14.

Solid culture – RIF resistance	RS resistance positive	RS resistance negative	Total
Truenat RIF positive			
Truenat RIF negative			
Total			
Indeterminate			

RIF: rifampicin; RS: reference standard.

15.

One of liquid/solid culture – RIF resistance	RS Resistance positive	RS Resistance negative	Total
Truenat RIF positive			
Truenat RIF negative			
Total			

(Continued)

Indeterminate

RIF: rifampicin; RS: reference standard.

16.

Line probe assay – RIF resistance	RS resistance positive	RS resistance negative	Total
Truenat RIF positive			
Truenat RIF negative			
Total			
Indeterminate			

RIF: rifampicin; RS: reference standard.

Form completed by:

Date:

Appendix 3. Methodological quality assessment form

METHODOLOGICAL QUALITY ASSESSMENT USING QUADAS-2 AND QUADAS-C TOOLS

Study name:

Screening number:

Publication month & year:

Objectives of the review to be assessed:

1)

2)

3)

4)

5)

6)

Participants:	1. Presumptive tuberculosis
	2. Confirmed tuberculosis not on treatment
	3. Confirmed tuberculosis on treatment
	4. Stored laboratory sample

(Continued)

	5. Other _____
Index test A:	1. Truenat MTB 2. Truenat MTB Plus 3. Truenat RIF Dx
Index test B:	1. Xpert MTB/RIF Ultra
Reference standard and target condition:	

Study design

Which of the following study designs did the primary study most strongly resemble?	1. Fully paired 2. Randomized 3. Partially paired with random subset 4. Partially paired with non-random subset 5. Unpaired non-randomized 6. Other _____

Flow diagram

Domain 1: Patient selection

Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults and adolescents

Relevant details:

Single test accuracy (QUADAS-2)		**Answers for Truenat**	**Answers for Xpert**
Signalling questions	1.1 Was a consecutive or random sample of patients enrolled?	Yes/No/Unclear	Yes/No/Unclear
	1.2 Was a case-control design avoided?	Yes/No/Unclear	Yes/No/Unclear
	1.3 Did the study avoid inappropriate exclusions?	Yes/No/Unclear	Yes/No/Unclear
Risk of bias	1.4 Could the selection of participants have introduced bias?	Low/High/Unclear	Low/High/Unclear
Concerns regarding applicability	1.5 Are there concerns that the included patients and setting do not match the review question?	Low/High/Unclear	Low/High/Unclear
Comparative accuracy (QUADAS-C)		**Answers for the test comparison**	

(Continued)

Signalling questions	C1.1 Was the risk of bias for each index test judged 'low' for this domain?	Yes/No
	C1.2 Was a fully paired or randomized design or a partially paired design with random subset used?	Yes/No/Unclear
	C1.3 Was the allocation sequence random?[a]	Yes/No/Unclear/NA
	C1.4 Was the allocation sequence concealed until patients were enrolled and assigned to index tests?[a]	Yes/No/Unclear/NA
Risk of bias	C1.5 Could the selection of patients have introduced bias in the comparison?	Low/High/Unclear

Footnotes:
[a]Only applicable to randomized designs.
NA: not applicable.

Signalling question 1.1: Was a consecutive or random sample of patients enrolled?

We answered 'yes' if enrolment was either consecutive or random, 'no' if selection was based on convenience, and 'unclear' if not described in the study.

Signalling question 1.2: Was a case-control design avoided?

We answered 'yes' for all studies by default as we decided to avoid case-control designs in our review.

Signalling question 1.3: Did the study avoid inappropriate exclusions?

We expected the studies to include a representative presumptive tuberculosis population that may include both people who were treatment-naive and who have previously received treatment for tuberculosis, irrespective of sputum smear status or the result of other related investigations such as Xpert. We answered 'yes' if the study included a representative population; 'no' if selection was based on a particular treatment, or sputum smear positive status, or positive status of other investigations; and unclear if the report did not provide this information.

Risk of bias (1.4): Could the selection of participants have introduced bias?

We judged risk of bias as 'low' if we answered 'yes' to signalling questions 1.1 to 1.3, 'high' if we answered 'no' to at least one question, and 'unclear' if the answer to at least one question was 'unclear' and any remaining answers are 'yes'.

Applicability (1.5): Are there concerns that the included people and setting do not match the review question?

We were interested in knowing if the Truenat MTB/MTB Plus/RIF performs well as a point-of-care testing method in the community or peripheral medical centres. We answered 'low concern' if participants were tested in the community or in peripheral medical centres; 'high concern' if participants were tested in tertiary care hospitals or medical colleges, or if the specimens were from stored samples in a central laboratory; and 'unclear concern' if the report did not clearly describe the clinical setting.

Signalling question C1.1 Was the risk of bias for each index test judged 'low' for this domain?

If the answer to 1.4 was 'low' for each index test, we answered 'yes'; otherwise, we answered 'no'.

Signalling Question C1.2 Was a fully paired or randomized design used?

A partially paired, random subset design guards against confounding, just like a completely paired or a randomized study design, and may imply a 'low' risk of bias assessment for this domain. We responded 'yes' if the study used any of the three designs (partially paired with random subsets, completely paired, and randomized designs), 'no' if it used none of them, and 'unclear' if the report did not describe the design in sufficient detail.

Signalling question C1.3 Was the allocation sequence random?

We answered 'yes' if the study used computer-generated random numbers, random number tables, or drawing lots for randomization; 'no' if the study used non-random allocation sequences such as alternation, procedures based on dates, or investigators' subjective judgements; 'unclear' if the report did not adequately describe the allocation sequence; and 'NA' if the study has a non-randomized design.

Signalling question C1.4 Was the allocation sequence concealed until patients were enrolled and assigned to index tests?

We answered 'yes' if the study used central randomization methods or sealed envelopes, 'no' if the allocation sequence was not hidden, 'unclear' if the explanation is inadequate, and 'NA' if the study had a non-randomized design.

Signalling question C1.5 Could the selection of patients have introduced bias in the comparison?

If we answered 'yes' to questions C1.1 to C1.4, we judged risk of bias to be 'low' (questions C1.3 and C1.4 only apply to randomized designs). If we answered 'no' to at least one question, or if the bias connected with the design element was sufficiently troublesome that the domain as a whole is deemed problematic, we considered a 'high' risk of bias judgement. We considered a 'high' risk of bias if C1.2 was answered 'no'; however, if a partially paired with random subset design is used, we still considered it as a 'low' risk of bias. If we answered 'unclear' to at least one question and 'yes' to any remaining questions, we considered risk of bias to be 'unclear'.

Domain 2: Index Test

Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults and adolescents

Relevant details:

Single test accuracy (QUADAS-2)		**Answers for Truenat**	**Answers for Xpert**
Signalling questions	2.1 Were the index test results interpreted without knowledge of the results of the reference standard?	Yes/No/Unclear	Yes/No/Unclear
	2.2 If a threshold was used, was it prespecified?	Yes/No/Unclear	Yes/No/Unclear
Risk of bias	2.3 Could the conduct or interpretation of the index test have introduced bias?	Low/High/Unclear	Low/High/Unclear
Concerns regarding applicability	2.4 Are there concerns that the index test, its conduct, or its interpretation differ from the review question?	Low/High/Unclear	Low/High/Unclear
Comparative accuracy (QUADAS-C)		**Answers for the test comparison**	
Signalling questions	C2.1 Was the risk of bias for each index test judged 'low' for this domain?	Yes/No	
	C2.2 Were the index test results interpreted without knowledge of the results of the other index test(s)?[a]	Yes/No/Unclear/NA	
	C2.3 Is undergoing one index test unlikely to affect the performance of the other index test(s)?[a]	Yes/No/Unclear/NA	
	C2.4 Were the index tests conducted and interpreted without advantaging one of the tests?	Yes/No/Unclear	
Risk of bias	C2.5 Could the conduct or interpretation of the index tests have introduced bias in the comparison?	Low/High/Unclear	

Footnotes:

[a]Only applicable if patients received multiple index tests (fully or partially paired designs).
NA: not applicable

Signalling question 2.1: Were the index test results interpreted without knowledge of the results of the reference standard?

We answered 'yes' for all studies because both Truenat and Xpert test results are machine-generated and objective in nature.

Signalling question 2.2: If a threshold was used, was it prespecified?

We answered 'yes' for all studies since the threshold is predefined in Truenat and Xpert.

Risk of bias (2.3): Could the conduct or interpretation of the index test have introduced bias?

As the answer to signalling questions 2.1 and 2,2 were always 'yes', we considered the risk of bias to be 'low'. Both index tests have well-defined thresholds. The machine gives a positive or a negative test result.

Applicability (2.4): Are there concerns that the index test, its conduct, or its interpretation differ from the review question?

We answered 'low concern' if standard methods were followed, as recommended by the test manufacturer. It is important to mix the specimen with reagents in an appropriate ratio and load the sample into the machine as per the manufacturer's instructions. We answered 'high concern' if the persons administering and interpreting the test clearly did not follow the manufacturer's instructions, and 'unclear concern' if the article did not describe these processes in sufficient detail.

Signalling question C2.1: Was the risk of bias for each index test judged 'low' for this domain?

For our research question, the answer to both signalling questions of QUADAS-2 domain 2 was yes'; therefore, the answer to C2.1 was also 'yes'.

Signalling question C2.2: Were the index test results interpreted without knowledge of the results of the results of the other index test(s)?

Blinding was not necessary, as none of the index tests involves subjective interpretation. Therefore, the response was always 'yes'.

Signalling question C2.3 Is the first index test unlikely to have affected the performance of the other index test(s)?

Since both index tests are performed on sputum samples and produce findings that are objectively calculated by machines, the answer was always 'yes', as one index test cannot affect or interfere with the outcome of an index test that is conducted later.

Signalling question C2.4: Were the index tests conducted and interpreted without advantaging one of the tests?

We answered 'yes' if both index tests were performed on the same sputum sample or in different samples processed in the same way, or if unprocessed sputum was used for both samples; 'no' if the sputum samples used for the two index tests were different in nature; and 'unclear' if the report did not provide this information.

Risk of bias (C2.5): Could the conduct or interpretation of the index tests have introduced bias in the comparison?

If the answer to C2.4 is 'yes', we considered risk of bias to be 'low', since responses to C2.1 to C2.3 was always 'yes' (C2.2 and C2.3 were only relevant to fully or partially paired designs). If we answered 'no' to C2.4, we considered a 'high' risk of bias judgement. If the answer to C2.4 was 'unclear', we considered the whole domain to be at 'unclear' risk of bias.

Domain 3 A: Reference Standard

Truenat MTB assays for detection of pulmonary tuberculosis in adults **and adolescents**

Relevant details:

Single test accuracy (QUADAS-2)		**Answers for Truenat**	**Answers for Xpert**
Signalling questions	A3.1 Is the reference standard likely to correctly classify the target condition (pulmonary tuberculosis)?	Yes/No/Unclear	Yes/No/Unclear

(Continued)

	A3.2 Were the reference standard results interpreted without knowledge of the results of the index test?	Yes/No/Unclear	Yes/No/Unclear
Risk of bias	A3.3 Could the reference standard, its conduct, or its interpretation have introduced bias?	Low/High/Unclear	Low/High/Unclear
Concerns regarding applicability	A3.4 Are there concerns that the target condition as defined by the reference standard did not match the review question?	Low/High/Unclear	Low/High/Unclear
Comparative accuracy (QUADAS-C)		**Answers for the test comparison**	
Signalling questions	AC3.1 Was the risk of bias for each index test judged 'low' for this domain?	Yes/No	
	AC3.2 Did the reference standard avoid incorporating any of the index tests?	Yes/No/Unclear	
Risk of bias	AC3.3 Could the reference standard, its conduct, or its interpretation have introduced bias in the comparison?	Low/High/Unclear	

Signalling question A3.1: Is the reference standard likely to correctly classify the target condition (pulmonary tuberculosis)?

We answered 'yes' if a study used any of the solid or automated liquid culture methods, or a combination of these methods; 'no' if the study used no culture methods; and 'unclear' if the report did not mention the reference standard.

Signalling question A3.2: Were the reference standard results interpreted without knowledge of the results of the index test?

We answered 'yes' if the reference standard was automated (e.g. Mycobacteria Growth Indicator Tube culture), or if the assessor was blinded, or if the culture process and the Truenat/Xpert test took place in different locations; 'no' if the person interpreting the reference standard result knew index test result; and 'unclear' if the report did not provide this information.

Risk of bias (A3.3): Could the reference standard, its conduct, or its interpretation have introduced bias?

We judged risk of bias as 'low' if we have answered 'yes' to signalling questions A3.1 and A3.2, 'high' if we have answered 'no' to at least one question, and 'unclear' if the answer to at least one question was 'unclear' and any remaining answers were 'yes'.

Applicability (A3.4): Are there concerns that the target condition as defined by the reference standard d not match the question?

Diagnosis of tuberculosis is not complete if *M tuberculosis* is not isolated from the culture specimen. We judged 'high concern' if the culture methods used in the study did not result in speciation with specific mention of *M tuberculosis* (present or not). A different *Mycobacterium* species or a contaminant may be present. We judged 'low concern' if speciation was performed appropriately; and 'unclear concern' if the report did not provide this information.

Signalling question AC3.1 Was the risk of bias for each index test judged'low'for this domain?

If the answer to A3.3 was 'low' for each index test, we answered 'yes'; otherwise, we answered 'no'.

Signalling question AC3.2 Did the reference standard avoid incorporating any of the index tests?

We answered 'yes' if both Truenat MTB/MTB Plus and Xpert/RIF were NOT part of the reference standard; 'no' if they were part of the reference standard; and unclear if the report did not provide this information.

Risk of bias (C3.3): Could the reference standard, its conduct, or its interpretation have introduced bias in the comparison?

We considered risk of bias to be 'low' if we answered 'yes' to signalling questions AC3.1 and AC3.2. We considered a 'high' risk of bias judgement if we answered 'no' to at least one question or if the bias associated with the design element raised enough red flags to make the domain as a whole problematic. If the answer to at least one question was 'unclear' and any remaining answers were 'yes', we considered risk of bias to be 'unclear'.

Domain 3 B: Reference Standard			
Truenat MTB assays for rifampicin resistance in adults**and adolescents**			
Relevant details:			
Single test accuracy (QUADAS-2)		**Answers for Truenat**	**Answers for Xpert**
Signalling questions	B3.1 Is the reference standard likely to correctly classify the target condition (rifampicin resistance)?	Yes/No/Unclear	Yes/No/Unclear
	B3.2 Were the reference standard results interpreted without knowledge of the results of the index test?	Yes/No/Unclear	Yes/No/Unclear
Risk of bias	B3.3 Could the reference standard, its conduct, or its interpretation have introduced bias?	Low/High/Unclear	Low/High/Unclear
Concerns regarding applicability	B3.4 Are there concerns that the target condition as defined by the reference standard did not match the review question?	Low/High/Unclear	Low/High/Unclear
Comparative accuracy (QUADAS-C)		**Answers for the test comparison**	
Signalling questions	BC3.1 Was the risk of bias for each index test judged 'low' for this domain?	Yes/No	
	BC3.2 Did the reference standard avoid incorporating any of the index tests?	Yes/No/Unclear	
Risk of bias	BC3.3 Could the reference standard, its conduct, or its interpretation have introduced bias in the comparison?	Low/High/Unclear	

Signalling question B3.1: Is the reference standard likely to correctly classify the target condition (rifampicin resistance)?

We answered 'yes' if a study used any of the solid or liquid culture methods or phenotypic drug susceptibility testing, either alone or in combination; 'no' if the study used no culture method, phenotypic drug-susceptibility testing, or any other valid method for rifampicin resistance detection, and 'unclear' if the report did not provide this information.

Signalling question B3.2: Were the reference standard results interpreted without knowledge of the results of the index test?

We answered 'yes' if the reference standard was culture drug susceptibility testing, the interpreter was blinded, or if culture was performed in a different laboratory to where the Truenat or Xpert tests were performed; 'no' if the reference standard result was interpreted knowing the result of the index test; and 'unclear' if the report did not provide this information.

Risk of bias (B3.3): Could the reference standard, its conduct, or its interpretation have introduced bias?

We judged risk of bias as 'low' if we have answered 'yes' to signalling questions B3.1 and B3.2, 'high' if we have answered 'no' to at least one question, and 'unclear' if the answer to at least one question was 'unclear' and any remaining answers were 'yes'.

Applicability (B3.4): Are there concerns that the target condition as defined by the reference standard did not match the question?

We judged 'high concern' if the culture methods used in the study did not result in speciation with specific mention of *M tuberculosis* (present or not). A different Mycobacterium species or a contaminant may be present. In addition further sensitivity of the culture isolate (if positive for *M tuberculosis*) to isoniazid and rifampicin have been performed and reported. We answered 'low concern' if the study performed speciation and sensitivity testing appropriately; and 'unclear' if the report did not provide this information.

BC3.1 Was the risk of bias for each index test judged 'low' for this domain?

If the answer to B3.3 was 'low' for each index test, we answered 'yes'; otherwise, we answered 'no'.

BC3.2 Did the reference standard avoid incorporating any of the index tests?

We answered 'yes' if both Truenat MTB-RIF Dx and Xpert/RIF did NOT form part of the reference standard, 'no' if they did form part of the reference standard, and unclear if the report did not provide this information.

Risk of bias (BC3.3): Could the reference standard, its conduct, or its interpretation have introduced bias in the comparison?

We considered risk of bias to be 'low' if we answered 'yes' to signalling questions BC3.1 and BC3.2. We considered a 'high' risk of bias judgement if we answered 'no' to at least one question or if the bias associated with the design element raised enough red flags to make the domain as a whole problematic. If the answer to at least one question was 'unclear' and any remaining answers were yes, we considered risk of bias to be 'unclear'.

Domain 4: Flow and timing

Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults and adolescents

Relevant details:

Single test accuracy (QUADAS-2)		**Answers for Truenat**	**Answers for Xpert**
Signalling questions	4.1 Was there an appropriate interval between index tests and reference standard?	Yes/No/Unclear	Yes/No/Unclear
	4.2 Did all patients receive a reference standard?	Yes/No/Unclear	Yes/No/Unclear
	4.3 Did all patients receive the same reference standard?	Yes/No/Unclear	Yes/No/Unclear
	4.4 Were all patients included in the analysis?	Yes/No/Unclear	Yes/No/Unclear
Risk of bias	4.5 Could the patient flow have introduced bias?	Low/High/Unclear	Low/High/Unclear
Comparative accuracy (QUADAS-C)		**Answers for the test comparison**	
Signalling questions	C4.1 Was the risk of bias for each index test judged 'low' for this domain?	Yes/No	
	C4.2 Was there an appropriate interval between the index tests?	Yes/No/Unclear	
	C4.3 Did the study use the same reference standard for all index tests?	Yes/No/Unclear	
	C4.4 Are the proportions and reasons for missing data similar across index tests?	Yes/No/Unclear	
Risk of bias	C4.5 Could the patient flow have introduced bias in the comparison?	Low/High/Unclear	

Signalling question 4.1: Was there an appropriate interval between the index test and reference standard?

We answered 'yes' if the index test and reference standard were performed at the same time, or if the time interval was less than or equal to seven days. We answered 'no' if the time interval was greater than seven days and we answered 'unclear' If we were unable to make a judgement of yes or no based on the available information.

Signalling question 4.2: Did all patients receive a reference standard?

We answered 'yes' if all sputum samples were subjected to solid or liquid culture; 'no' if no culture method was used; or 'unclear' if not described

Signalling question 4.3: Did all patients receive the same reference standard?

We answered 'yes' if either a liquid or solid culture medium was used as a standalone or in combination; 'no' if neither culture method were used; or 'unclear' if not described

Signalling question 4.4: Were all patient included in the analysis?

We answered 'yes' if the number of people enrolled and the number of people included in the 2 × 2 tables match, 'no' if the numbers did not match, and unclear if the report did not provide this information.

Risk of bias (4.5): Could the patient flow have introduced bias?

We judged risk of bias as 'low' if we answered 'yes' to signalling questions 4.1 to 4.4, 'high' if we answered 'no' to at least one question, and 'unclear' if we answered 'unclear' to at least one question and 'yes' to any remaining questions.

C4.1 Was the risk of bias for each index test judged 'low' for this domain?

If the answer to 4.5 was 'low' for each index test, we answered 'yes'; otherwise, we answered 'no'.

C4.2 Was there an appropriate interval between the index tests?

We answered 'yes' if both index tests were performed within 3 days if the sputum sample was unrefrigerated, 'no' if more than 3 days, or 'unclear' if not described. If the studies used a preservative to extend the viability of the sputum, the appropriate interval of sputum collection and testing for each preservative was obtained from the existing literature.

C4.3 Was the same reference standard used for all index tests?

We answered 'yes' if a solid or liquid culture was used for all index tests, alone or in combination; 'no' if no culture method was used as the reference standard (even for a few tests); and 'unclear' if the report did not provide this information.

C4.4 Are the proportions and reasons for missing data similar across index tests?

We answered 'yes' if the proportion of missing data across both index tests was 5% or less, no if it was more than 5%, and 'unclear' if the report did not provide this information.

Risk of bias (C4.5): Could the patient flow have introduced bias in the comparison?

We considered risk of bias to be 'low' if we answered 'yes' to signalling questions C4.1 to C4.4. We considered a 'high' risk of bias judgement if at least one question was answered 'no'. If the answer to at least one question was 'unclear' and any remaining answers were 'yes', we considered risk of bias to be 'unclear'.

HISTORY

Protocol first published: Issue 1, 2023

CONTRIBUTIONS OF AUTHORS

LRI conceived the idea, trained the team, supervised the article inclusion and data extraction, contributed to the writing of the protocol and review, co-ordinated the tasks, and edited and reviewed the final manuscript.

JD wrote the protocol sections, trained the team, developed the data extraction form and led modifications of the QUADAS-2 and QUADAS-C tools to the review question, supervised data extraction, supervised risk of bias and applicability assessment, and reviewed the manuscript.

MKSN was involved in assessing the articles, data extraction, and assessment of the risk of bias and applicability.

VAS was involved in assessing the articles, data extraction, and assessment of the risk of bias and applicability.

AB assisted in data extraction and data entry.

KS assisted YT in data analysis and reviewed the manuscript.

PR provided technical input and wrote sections of the review.

RK provided input on the methodology and critically reviewed the manuscript.

HDS provided input on the methodology and critically reviewed the manuscript.

MM provided input on the methodology and critically reviewed the manuscript.

CP critically reviewed the manuscript and supervised the work.

YT wrote the sections of the protocol and review, provided methodological and statistical supervision, performed statistical analysis, critically reviewed the manuscript, and mentored the team.

All review authors reviewed and approved the final version of the review.

DECLARATIONS OF INTEREST

Author team

LRI: none. Is employed at ICMR*.

JD: none.

MKSN: none. Is employed at ICMR*.

VAS: none. Is employed at ICMR*.

AB: none. Is employed at ICMR*.

KS: none.

PR: none. Is employed at ICMR*.

RK: none. He is on the editorial board of the Cochrane Statistical Methods group.

HDS: none. Is employed at ICMR*.

MM: none. Is employed at ICMR*.

CP: none. Is employed at ICMR*.

YT: none. She is a co-convenor of the Cochrane Screening and Diagnostic Tests Methods Group and an editor of the Cochrane Infectious Diseases Group. She was not involved in the editorial process or decision-making for this review.

*ICMR – this organization has published opinions in medical journals relevant to the interventions in the work and has declared its opinion on this topic.

Editors involved in editorial processing

CIDG Editor: Dr Karen Steingart reviewed data on Truenat and prepared GRADE tables for a WHO Guideline Development Meeting in December 2019 at the request of the WHO Global Tuberculosis Programme and received payment for this work. She has authored several Cochrane reviews on a similar technology: Cepheid's Xpert MTB/RIF and Xpert MTB/RIF Ultra.

DTA Editor: Dr Mariska Leeflang has no known conflicts of interest.

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Internal sources

- Liverpool School of Tropical Medicine (LSTM), UK

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External sources

- World Health Organization (WHO), Switzerland

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- Foreign, Commonwealth, and Development Office (FCDO), UK

Project number: 300342-104

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

This review contributed to the 2024 update of the World Health Organization (WHO) consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection. The Guideline Development Group meeting was held from 6 to 10 May 2024 in Geneva, Switzerland. WHO introduced a class-based recommendation approach in December 2020 instead of an approach based on individual technologies. We changed a few sections of the review to be consistent with the other five systematic reviews in our generic protocol for the 2024 WHO policy update. The generic protocol is available at https://osf.io/26wg7/.

We made the following changes from the published protocol (Inbaraj 2023).

Title

We changed the title to "Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults and adolescents."

Age

We changed the age category to 10 years or older instead of 15 years and older to conform with WHO age categories.

Reference standard

We removed line probe assay as one of the reference standards. We have also considered composite reference standards in addition to culture. However, none of the included studies evaluated the index tests against a composite reference standard.

Settings of interest

We modified the settings of interest to "We were interested in how our index tests were performed in adults and adolescents with presumptive tuberculosis presenting to local hospitals or primary care centres."

Searching for other sources

We included a statement: "A WHO public call for data was made between 30 November 2023 and 15 February 2024 for ongoing and unpublished studies from manufacturers and researchers."

Methodological quality assessment

For the judgement regarding the risk of bias for all the domains, if only one signalling question was answered 'no' or 'unclear,' we discussed further before making the risk of bias judgement for the domain. We judged 'low' if all signalling questions were answered 'yes.' We judged 'high' if all or most signalling questions were answered 'no.' We judged 'unclear' if all or most signalling questions were answered unclear.

Applicability (1.5): are there concerns that the included people and setting do not match the review question?

We were interested in how the index test was performed in adults and adolescents who were evaluated for pulmonary tuberculosis as they would be in routine practice. We answered 'low concern' if participants were evaluated in local hospitals, community, or primary care centres, or if the sample was collected at a peripheral centre but processed in a tertiary laboratory. We answered 'high concern' if participants were evaluated exclusively as inpatients in tertiary care centres or medical colleges, or if the specimens were from stored samples in a central laboratory, or if the setting did not match the review question (e.g. using the index for decisions about the need for airborne isolation). We answered 'unclear concern' if the clinical setting was not reported or the information available was insufficient to make a judgement. We also answered 'unclear concern' if the index test was performed at a central-level laboratory, and the clinical setting was not reported for the following reason: it is difficult to determine if a given reference laboratory provided services mainly to very sick people (inpatients in tertiary care) or to all people, including very sick people and those with less-severe disease (primary, secondary, and tertiary care).

Signalling question 4.1: was there an appropriate interval between the index test and reference standard?

We answered 'yes' if the index test and reference standard were performed at the same time or if the time interval was seven days or less. We answered 'no' if the time interval was greater than seven days, and we answered 'unclear' if we were unable to make a judgement of yes or no based on the available information.

Statistical analysis and data synthesis

Our investigations of heterogeneity were limited due to limited data. We did not perform sensitivity analyses using the QUADAS-2 signalling questions specified in the protocol due to limited data. We also did not assess the diagnostic accuracy of Truenat after repeat testing in people with non-determinate test results as written in the protocol, as most of the studies did not report this information.