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TABLE OF CONTENTS

[Diagnostic Test Accuracy Protocol]

Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults

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ABSTRACT

Objectives

This is a protocol for a Cochrane Review (diagnostic). The objectives are as follows:

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

Secondary objectives

1. To compare the diagnostic accuracy of Truenat assays (MTB, MTB Plus, and MTB RIF Dx) with that of Xpert MTB/RIF Ultra for the detection of pulmonary tuberculosis and rifampicin resistance in adults with presumptive pulmonary tuberculosis.
2. To summarize the frequency of non-determinate Truenat results.
3. To determine the diagnostic accuracy of Truenat after repeat testing in people with non-determinate test results.
4. To investigate potential sources of heterogeneity in the diagnostic accuracy of Truenat for the detection of pulmonary tuberculosis in relation to covariates such as the setting where the index tests were performed, HIV status, previous history of tuberculosis, and sputum smear test status.
5. To investigate heterogeneity in the diagnostic accuracy of Truenat for detecting rifampicin resistance in relation to sputum smear status.

BACKGROUND

Tuberculosis, the second leading infectious killer after Coronavirus disease 2019 (COVID-19), poses a diagnostic and therapeutic enigma. An estimated 10.6 million individuals suffered from tuberculosis in 2021 (WHO Global TB Report 2022). The 30 countries with the most tuberculosis cases accounted for 87% of all estimated incident cases worldwide, with eight low- and middle-income countries (LMICs) accounting for two-thirds of the total cases (WHO Global TB Report 2022). In 2021, 1.6 million people died from tuberculosis, with people living in LMICs accounting for nearly 95% of tuberculosis deaths (WHO Global TB Report 2022). The COVID-19 pandemic has negatively impacted tuberculosis by restricting access to diagnosis and treatment, resulting in reversal of the global progress achieved until 2019 towards eliminating tuberculosis. According to the World Health Organization (WHO), COVID-19 may have resulted in an increase of 200,000 tuberculosis fatalities between 2019 and 2021 and 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 eliminating tuberculosis by 2030 by reducing annual tuberculosis incidence to 80% of the 2015 level (UN 2015). Although cumulative tuberculosis incidence decreased by 10% between 2015 and 2021, this reduction was just halfway to the 2020 goal of the End TB Strategy (WHO Global TB Report 2022). The End TB goals are difficult 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 they 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 multidrug-resistant tuberculosis (MDR-TB). Treatment for RR-TB and MDR-TB is expensive, requires prolonged duration, and has a high probability of adverse events, including death (Jang 2020; Soeroto 2021; WHO Global TB Report 2022). The percentage of people with bacteriologically confirmed tuberculosis who were tested for rifampicin resistance rose from 61% (2.2 million/3.6 million) in 2019 to 71% (2.4 million/3.4 million) in 2021. In 2019, the success rate of RR-TB/MDR-TB treatment was 60% across the globe (WHO Global TB Report 2022). According to mathematical modelling, the percentage of MDR-TB among incident cases of tuberculosis 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 LMICs, is inexpensive, fast, and widely applicable. However, it has limited sensitivity, and a positive result requires between 5000 bacilli/mL and 10000 bacilli/mL (Arora 2020; Steingart 2006). While *Mycobacterium tuberculosis* (*M tuberculosis*) culture has better sensitivity and specificity, it is frequently 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 (DST) is expensive and has a long turnaround time. With increasing drug resistance, detecting resistance to rifampicin is crucial as soon as an individual is diagnosed with tuberculosis.

Innovative rapid molecular-based diagnostic tools have created a revolution in the diagnosis of tuberculosis and rifampicin resistance. The WHO has approved a few molecular-based diagnostic tests, including Xpert MTB/RIF assay (Cepheid Inc, USA; WHO 2011). 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 accuracy of rifampicin-resistance detection (Cepheid 2022b; WHO 2021). However, both Xpert MTB/RIF and Xpert MTB/RIF Ultra require adequate infrastructures, such as continuous power supply and air conditioning (Gomathi 2020). As a result, use of these tests is restricted in low-resource peripheral laboratories. Truenat is another nucleic-acid amplification test-based assay recommended by the WHO. This system may overcome the limitations of Xpert MTB/RIF and Xpert MTB/RIF Ultra, because the test kit is a battery-powered, portable device that can be used effectively at point of care in a limited-resource setting (WHO 2021). Unskilled persons require minimal training to perform the test, which detects *M tuberculosis* in sputum samples within one hour (Lee 2019).

Target condition being diagnosed

Pulmonary tuberculosis

M tuberculosis is the bacteria 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; however, pulmonary tuberculosis is the most prevalent form. 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 (HRZE), followed by four months of daily isoniazid and rifampicin (HR). In some cases, the WHO recommends a four-month regimen (WHO 2022).

Rifampicin resistance

Rifampicin is a potent bactericidal drug that has played a significant role in treating tuberculosis as a first-line drug. Rifampicin acts on the β subunit of the DNA-dependent RNA 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 nine to 12 months for a standardized short oral regimen, and 18 to 20 months for a longer regimen (WHO 2020).

Index test(s)

Our index tests are Truenat and Xpert MTB/RIF Ultra assays. Truenat assays, developed by Molbio diagnostics in Bangalore, India, include Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx**.** Both Truenat and Xpert assays can 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 targets the multicopy genes (*nrdZ and IS6110*) for identifying *M tuberculosis*. 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 similar run time and shelf life. Truenat MTB-RIF Dx targets the *rpoB* gene (RNA polymerase gene's beta subunit) for detecting rifampicin resistance (Nikam 2013; Nikam 2014).

The Trueprep DNA extraction 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 approximately one hour (Beall 2019). Unlike with Xpert assays, users of Truenat can deselect rifampicin resistance testing and use the device for tuberculosis detection only. 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 DNA extraction and *M tuberculosis* detection, rifampicin resistance detection takes approximately one more hour (Gomathi 2020; 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). The same trial showed that Truenat MTB had a lower sensitivity in smear-negative individuals: 36% (95% CI 27% to 47%) for Truenat MTB and 47% (95% CI 37% to 58%) for Truenat MTB Plus (Penn-Nicholson 2021).

The sensitivity of Xpert MTB RIF testing is also lower in smear-negative people (61%; Zifodya 2021). One study compared the performance of Truenat MTB-RIF Dx assay with that of the Xpert MTB RIF assay in a strain panel, and found that both characterized more than 90% of global rifampicin resistance mechanisms (Georghiou 2021).

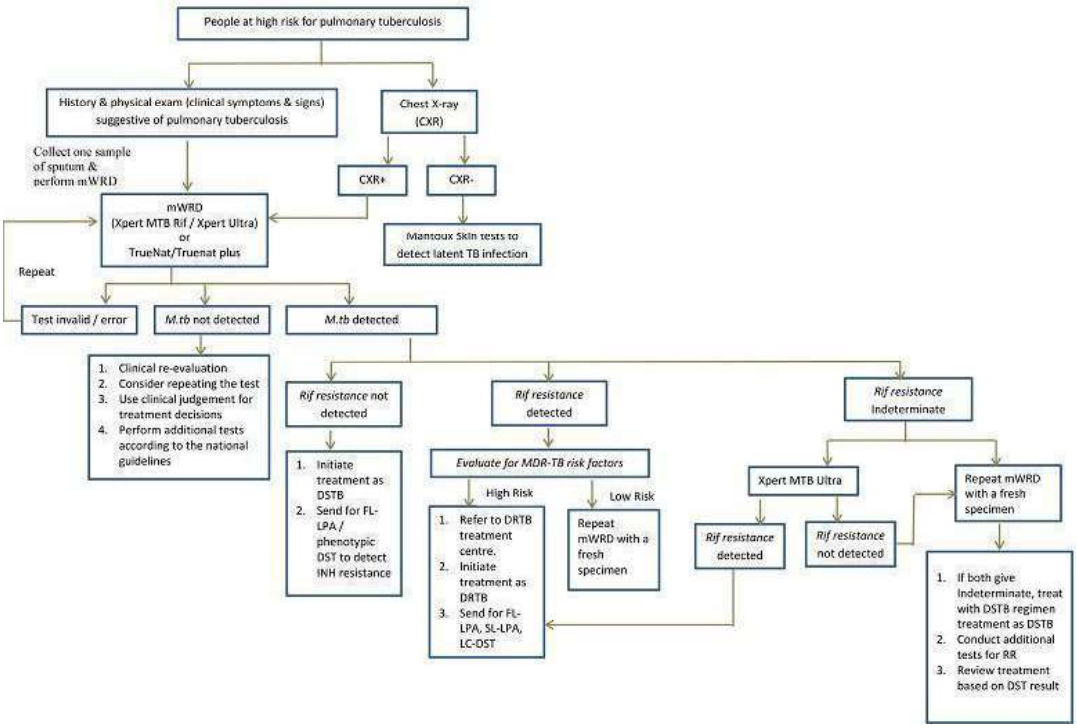
Xpert assays detect the presence of MTB and rifampicin resistance in a single step. Sample processing and the amplification process are combined in a closed Xpert system. Xpert MTB/RIF is based on detecting five overlapping 81-bp regions in the *rpoB* gene (i.e. rifampicin resistance-determining region (RRDR)), and the principle behind the detection is molecular beacon technology (Cepheid 2022a; Rajendran 2022).The Xpert MTB/RIF Ultra test is based on two multicopy targets, IS6110 and IS1081, for MTB detection and rifampicin resistance with improved cartridge design and assay design (Cepheid 2022b; Chakravorty 2017).The test procedure involves mixing the sputum sample with the sample reagent provided by the manufacturer in the ratio of 1:2 in case of a direct specimen and 1:3 in case of processed pellets (Blakemore 2010). After an incubation period of 15 minutes, the mixture is loaded into the cartridge. All the steps after the sample loading are fully automated.

The total run time for Xpert MTB/RIF assay is two hours, and for the Xpert MTB/RIF Ultra assay, one to 1.5 hours (Chakravorty 2017; Theron 2014). According to one systematic review, Xpert MTB/RIF Ultra compared with Xpert MTB/RIF had a higher sensitivity (90.9%, 95% credible interval 86.2 to 94.7 for Xpert MTB/RIF Ultra versus 84.7%, 95% credible interval 78.6 to 89.9 for Xpert MTB/RIF) and lower specificity (95.6%, 95% credible interval 93.0 to 97.4 for Xpert MTB/RIF Ultra versus 98.4%, 95% credible interval 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 2021).

Clinical pathway

In LMICs, 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; DS-TB: drug-sensitive tuberculosis; DR-TB: drug-resistant tuberculosis; FL-LPA: first-line line probe assay; INH: isoniazid; LC-DST: liquid culture drug susceptibility testing; MDRTB: multidrug-resistant tuberculosis; MTB: *Mycobacterium tuberculosis*; mWRD: molecular WHO-recommended rapid diagnostics; RIF: rifampicin; SL-LPA: second-line line probe assay. Adapted from WHO 2021.



Clinical suspicion of tuberculosis is based on symptoms of weight loss, fever, night sweats, cough, and haemoptysis, determined through history-taking and physical examination (Heemskerk 2015; Lewinsohn 2017). People with these symptoms 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 clinical manifestations with or without chest X-ray abnormalities are considered to have presumptive tuberculosis. In these cases, a sputum sample should be collected and tested with an mWRD for rapid bacteriological confirmation of *M tuberculosis,* with or without rifampicin resistance detection (WHO 2022). These rapid molecular tests detect tuberculosis and rifampicin resistance within a few hours, irrespective of the setting.

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 cases 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 chest imaging.

If the mWRD does not detect rifampicin resistance, the individual is considered to have drug-sensitive tuberculosis and should be started on the drug-sensitive tuberculosis regimen. Positive rifampicin resistance detection leads to a diagnosis of drug-resistant tuberculosis and administration of RR-TB or MDR-TB 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. On the other hand, false-negative results could increase morbidity and mortality related to tuberculosis and community transmission due to delayed diagnosis and treatment (WHO 2021).

Settings of interest

The settings of interest for this review are peripheral and intermediate reference laboratories. 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.

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 lesser turnaround time and cost-effectiveness, smear microscopy has the major drawback of reduced sensitivity (50% to 60%). Detection under a microscope requires a concentration of 5,000 CFU/mL to 10,000 CFU/mL of bacilli (Arora 2020; Steingart 2006), and smear microscopy 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 people being evaluated for tuberculosis (WHO 2021).

Culture

Sputum culture is the most sensitive method and 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 pulmonary tuberculosis cases than smear microscopy and can also be used for drug sensitivity testing (Acharya 2020). However, it takes four to 12 weeks for solid culture 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 sensitivity 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 specialized laboratories.

Genotypic tests

The genotypic tests for the diagnosis of *M tuberculosis* include probes and gene amplification techniques; various molecular methods have been developed from these techniques since the early 2010s. The gene-specific PCRs targeting *sdaA*, *devR*, *IS6110*, *MPB4*, and *rpoB* genes are reliable diagnostic tests (Nimesh 2013). In 2016, the WHO approved loop-mediated isothermal amplification (LAMP) technology (Eiken Chemical, Japan) as a diagnostic test for peripheral labs (Notomi 2000; WHO 2016). The amplification process utilizes at least four different sets of primers and is carried out in a single step, comprising of a strand displacement reaction at 65 °C for 15 to 60 minutes. LAMP 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 setting varies from 76% to 80%, and specificity from 97% to 98% (WHO 2016).

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 hybridization on a strip with a particular oligonucleotide probe (Nathavitharana 2017). GenoType MTBDRplus 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 MTBDRplus VER 2.0 detects both rifampicin and isoniazid from respiratory samples (Crudu 2012; Hain 2022). One systematic review evaluating the diagnostic accuracy of all three line probe assay techniques found that they had 96.7% sensitivity and 98.8% specificity for rifampicin resistance, and 90.2% sensitivity and 99.2% specificity for Isoniazid resistance among smear-positive cases (Nathavitharana 2017). Commercial line probe assays 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 smear-negative people (indirect testing), as per WHO recommendations (WHO 2008). GenoType MTBDRplus 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 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 is conditional and based on moderate-certainty evidence from a multicentric prospective clinical evaluation of 1336 people. The guidelines express serious concerns about imprecision and inconsistency of evidence related to sensitivity, and conclude that the certainty of evidence is low for sensitivity and high for specificity for the detection of pulmonary tuberculosis in adults (WHO 2021). 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. The WHO guideline development group expresses concerns of indirectness and inconsistency on sensitivity estimates for the detection of rifampicin resistance due to the small number of participants contributing to the analysis, and concludes that the evidence on rifampicin resistance might not be generalizable to all settings (WHO 2021). The guideline contains a conditional recommendation based on low-certainty evidence for the use Truenat for detecting rifampicin resistance (WHO 2021).There is also uncertainty about the use of this assay in people living with HIV (WHO 2021). Therefore, we intend to carry out a robust systematic review and meta-analysis to synthesize evidence on Truenat that may aid the WHO and other agencies to formulate future tuberculosis guidelines and policies. In addition, given the established role of Xpert assays in the clinical pathway, we will compare the accuracy of Truenat and Xpert head-to-head (i.e. direct comparison). Since Xpert MTB/RIF Ultra has superseded Xpert MTB/RIF, and the manufacturer will discontinue Xpert MTB/RIF in most countries in 2023, we will consider only Xpert MTB/RIF Ultra (Kay 2022).

OBJECTIVES

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

Secondary objectives

1. To compare the diagnostic accuracy of Truenat assays (MTB, MTB Plus, and MTB RIF Dx) with that of Xpert MTB/RIF Ultra for the detection of pulmonary tuberculosis and rifampicin resistance in adults with presumptive pulmonary tuberculosis.
2. To summarize the frequency of non-determinate Truenat results.
3. To determine the diagnostic accuracy of Truenat after repeat testing in people with non-determinate test results.
4. To investigate potential sources of heterogeneity in the diagnostic accuracy of Truenat for the detection of pulmonary tuberculosis in relation to covariates such as the setting where the index tests were performed, HIV status, previous history of tuberculosis, and sputum smear test status.
5. To investigate heterogeneity in the diagnostic accuracy of Truenat for detecting rifampicin resistance in relation to sputum smear status.

METHODS

Criteria for considering studies for this review

Types of studies

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

Participants

We will include adults (aged 15 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 cavity, previously diagnosed tuberculosis, and a previous history of anti-tuberculosis therapy are at significant risk for MDR-TB (Xi 2022). We will include studies that have recruited participants with HIV, diabetes mellitus, and a previous history of tuberculosis. We will exclude participants who are receiving treatment for tuberculosis or who have received treatment within the past seven days, as this could interfere with index tests and reference standard results. We will include studies from all healthcare settings and peripheral, intermediate, and central laboratories. We will also include studies from community and healthcare facilities, irrespective of the burden of tuberculosis in those settings. We will place no restrictions on sex of participants or geographical location.

Index tests

Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx are the primary index tests. Truenat MTB-RIF Dx can detect rifampicin resistance in *Mtuberculosis* in Truenat MTB- and Truenat MTB Plus-positive specimens. We will compare the Truenat assays (MTB, MTB Plus, and MTB RIF Dx) with Xpert MTB/RIF Ultra for the secondary objective related to comparative diagnostic accuracy. For brevity, we will refer to the Truenat assays as Truenat and Xpert MTB/RIF Ultra as Xpert, 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 either automated liquid culture, solid culture, or a combination of both solid and liquid culture methods. The reference standard for rifampicin resistance is culture-based DST or LPAs. 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 will consider any commercially available culture method as a reference standard. A culture reporting positive *M tuberculosis* indicates the presence of pulmonary tuberculosis, and a culture reporting negative *M tuberculosis* indicates the absence of pulmonary tuberculosis. A positive culture-based result of drug resistance indicates the presence of rifampicin resistance, and a negative result indicates the absence of rifampicin resistance.

Search methods for identification of studies

Electronic searches

The Information Specialist from the Cochrane Infectious Diseases Group (CIDG) will perform the search using terms and strategies described in Appendix 1 without language restriction. We will search the following databases: CIDG Specialized Register, MEDLINE (Ovid), Embase (Ovid), Science Citation Index and Biosis previews (ISI Web of Knowledge), Global Index Medicus, and SCOPUS (Elsevier). We will also search the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/clinical-trials-registry-platform) and ClinicalTrials.gov (clinicaltrials.gov) to identify any ongoing trials. We will also search for systematic reviews.

Searching other resources

We will perform bibliography mining and screen the bibliographies of included articles. We will identify abstracts of conferences on tuberculosis and search in ProQuest Dissertations & Theses A&I for dissertations, using terms for tuberculosis and Truenat. We will also look for reviews, guidelines, and reference lists related to tuberculosis as sources of studies. We will contact researchers at the New Diagnostic Working Group of the Stop TB Partnership, FIND (the global alliance for diagnostics), and other experts who are working on tuberculosis diagnostics for any ongoing and unpublished studies. We will contact the test manufacturers (Molbio diagnostics, India) for unpublished studies.

Data collection and analysis

Selection of studies

We will upload all articles retrieved though the electronic literature search to EndNote software to remove duplicates, then upload the filtered list of articles to the systematic review software Rayyan. Two review authors (VA and MKS) will independently screen the preliminary list of articles based on the titles and abstracts and mark those that appear eligible (Step 1). Two review authors (VA and MKS) will independently assess the full texts of potentially eligible articles against our eligibility criteria (Step 2). We will check the reference lists of shortlisted articles for any potentially relevant articles not retrieved in the computerized searches. If any discrepancies arise between the review authors, a third review author (JD) will aid in resolving the disagreement. We will list the reasons for exclusion in the 'Characteristics of excluded studies' table.

Data extraction and management

Two review authors (VA and MKS) will independently extract data using a predesigned piloted data collection form (Appendix 2). We will collect the following information from the studies.

1. 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, enrolled and excluded; study funding.
2. Study participants: history of pulmonary tuberculosis, comorbidity status (diabetes, HIV, acid-fast bacilli smear).
3. Target conditions: pulmonary tuberculosis, rifampicin resistance, or both.
4. Reference standards: solid culture (LJ) or automated liquid culture (MGIT), DST or LPA, manufacturer, cross-contamination of the culture media.
5. Index tests: Truenat MTB, Truenat MTB Plus, and Truenat MTB RIF Dx. In addition, for comparative studies, details of Xpert MTB/RIF Ultra.
6. Sputum collection: type (such as expectorated sputum, induced sputum, bronchoalveolar lavage), condition (fresh or frozen), and smear status (positive or negative).
7. Outcomes: number of true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), and the number of missing or unavailable test results. We will record the time of treatment initiation since sputum collection date and the time to diagnose pulmonary tuberculosis after running the Truenat assay.
8. 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 define non-determinate results as a combination of operator and equipment errors or 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 indeterminate in Truenat MTB RIF Dx rifampicin resistance testing can be due to low bacilli load or a run error. There are different types of errors depending on the parts of the device that are malfunctioning. We will extract the proportion of non-determinate (pulmonary tuberculosis) results and indeterminate (rifampicin resistance) results. We will consider a trace result in Xpert/RIF Ultra as a positive result for *M tuberculosis* (WHO 2017).

We will extract the number of cultures contaminated with non-tuberculous bacteria or fungi, or cross-contaminated, and express this as a proportion of the total samples cultured. If any publication (including supplementary material) does not provide the relevant data, we will contact the primary study author for further details. We will enter data through Epi-info 7.

Assessment of methodological quality

Two review authors (VA and MKS) will assess 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, we will use the Quality Assessment of Diagnostic Accuracy Studies-Comparative (QUADAS-C) tool to assess risk of bias (Yang 2021). We have 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. The final modified version is available in Appendix 3. We will summarize 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 will categorize the results of *M tuberculosis* detection and rifampicin resistance as follows.

1. *M tuberculosis* detected, rifampicin resistance not detected.
2. *M tuberculosis* detected, rifampicin resistance detected.
3. *M tuberculosis* not detected, rifampicin resistance not detected.
4. *M tuberculosis* detected; rifampicin resistance indeterminate.

The unit of analysis will be the participant rather than the specimen. We will perform descriptive analyses of the included studies and summarize key characteristics in a table. We will graphically present individual study estimates of sensitivity and specificity in forest plots and receiver operating characteristic (ROC) space using Review Manager 5 (Review Manager 2020).

We will perform meta-analysis separately for each target condition and objective. We will fit bivariate models to estimate the summary sensitivity and specificity of each Truenat assay (Chu 2006; Reitsma 2005). If we cannot fit a bivariate model due to sparse data, or if we observe little or no variability in sensitivity or specificity between studies included in an analysis, we will meta-analyse sensitivity and specificity separately using univariate random-effects logistic regression modes (Kay 2020; Takwoingi 2017). As rifampicin resistance testing is not performed for MTB negative samples, the numbers and groups of participants included in the meta-analysis for MTB detection is expected to differ from those for resistance testing.

To compare the accuracy of Truenat and Xpert, we will include only comparative accuracy studies (Takwoingi 2013). For the pairwise comparison of each Truenat assay (Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx) with Xpert MTB/RIF Ultra, we will estimate and compare summary sensitivities and specificities by adding a covariate for test type to a bivariate model. We will add separate covariate terms for logit sensitivity and logit specificity. If possible,

given the number of included studies, we will allow the variance parameters of the bivariate model to differ between tests (Macaskill 2022; Takwoingi 2022). After estimating the model, will calculate absolute differences in sensitivity and specificity using the bivariate model parameters. We will obtain the 95% CIs for the differences using the delta method. We will use Wald tests to assess the statistical significance of differences in sensitivity and specificity. For the meta-analysis, we will use the meqrlogit command in Stata 17.0.

Approach to non-determinate and indeterminate index test results

To estimate the accuracy of Truenat assays and the comparative accuracy of Truenat and Xpert assays, we will exclude non-determinate and indeterminate index test results from the analyses. We will report the proportion of such results for individual studies and, if appropriate, combine the proportions using univariate random-effects logistic regression.

Investigations of heterogeneity

We will visually explore heterogeneity in the sensitivity and specificity of Truenat and Xpert assays using forest plots. To explore whether updating of software and cartridge has changed the accuracy of Truenat assays for detection of MTB and rifampicin resistance, we will sort the studies by the year of publication on a forest plot and visually assess the trend over time. Where possible, we will evaluate the following potential sources of heterogeneity using subgroup analysis and meta-regression.

1. Setting (location of the laboratories, community versus healthcare setting) and the year the index tests were performed.
2. HIV status.
3. History of tuberculosis.
4. Sputum smear status.
5. Low and high tuberculosis prevalence settings.
6. Automated liquid culture only as reference standard and a composite reference with automated liquid culture and solid culture.
7. Blinded versus unblinded reference standard.

Tuberculosis laboratory networks are categorized as peripheral, intermediate, and central laboratories. Peripheral laboratories are located at community and sub-district levels and are involved in screening, case-finding, referral, and treatment. An intermediate reference laboratory caters to a district or a region. Central reference laboratories, also known as national reference laboratories, perform surveillance, develop and distribute reference methods and standards, and supervise laboratories in the network (WHO 2015).

We will perform meta-regression by adding one covariate at a time to the bivariate model to explore the effect of the covariate on sensitivity, specificity, or both. We will use likelihood ratio tests to compare models with and without covariate terms to assess statistical significance.

Sensitivity analyses

Depending on the number of included studies, we will assess the robustness of the results by excluding studies that we judge at high or unclear risk of bias with respect to the following signalling questions.

1. Did the study enrol a consecutive or random sample of people?
2. Did the study use a fully paired or randomized design?
3. Did all participants receive a reference standard?
4. Did the study only use fresh specimens?
5. Did the study include all participants in the analysis?
6. Did the study exclude people with previous history of tuberculosis or untreated tuberculosis?

Assessment of reporting bias

We will not formally investigate reporting bias because the methodology for this is not well developed for test accuracy reviews (Takwoingi 2022). We will attempt to contact the study authors for relevant missing information. Our search strategy includes contacting experts and relevant organizations for unpublished and ongoing studies to minimize publication bias risk.

Assessment of the certainty of evidence

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

If there are high-quality cross-sectional or cohort studies that enrolled participants with diagnostic uncertainty, we will assess the certainty of the evidence as high for both sensitivity and specificity. If there is a reason for downgrading, we will use our best 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). At least two review authors (LR and JD) will discuss the judgements of certainty of the evidence and apply GRADE in the following format (GRADEpro GDT; Schünemann 2020a; Schünemann 2020b).

Risk of bias

We will use QUADAS-2 and QUADAS-C tools to assess the risk of bias.

Indirectness

We will assess indirectness in relation to the target population (including disease spectrum), setting, index tests, reference standards, and accuracy outcomes. We will check whether the study population matched that of our review. We will also use the prevalence of tuberculosis as a guide to check whether there was indirectness in the population.

Inconsistency

Inconsistency can be caused by clinical or methodological heterogeneity, or sometimes it cannot be explained. We will carry out per-protocol analyses to investigate potential sources of heterogeneity. GRADE recommends downgrading for unexplained inconsistency in sensitivity and specificity estimates.

Imprecision

We believe a precise estimate to be one that would enable a clinically meaningful decision. We will consider the width of 95% CIs. We will determine projected ranges for true positives (TP), false negatives (FN), true negatives (TN), and false positives (FP) for a given prevalence of tuberculosis, and make judgements on imprecision from these calculations.

Publication bias

We will take into account the thoroughness of the literature search and outreach to tuberculosis researchers, the presence of only studies that produce precise estimates with high accuracy despite a small sample size, and knowledge about studies that were conducted but not published, while assessing publication bias.

We will use the GRADEpro GDT online tool to create summary of findings tables for each target condition.

The summary of findings tables will include the following details.

1. The review question and its components, population, setting, index test(s), and reference standard(s).
2. Summary estimates of sensitivity and specificity with 95% CIs.
3. The number of included studies and participants contributing to the estimates of sensitivity and specificity.
4. Different estimates of prevalence of the target condition with an explanation of why the prevalence has been chosen.
5. An assessment of the certainty of the evidence (GRADE).
6. Explanations for downgrading, as needed.

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Editorial and peer-reviewer contributions

The following people conducted the editorial process for this article:

- Sign-off Editor (final editorial decision): Dr Karen Steingart (CIDG) and Dr Mariska Leeflang (DTA).
- Managing Editor (selected peer reviewers, collated peer-reviewer comments, provided editorial guidance to authors, edited the article): Dr Deirdre Walshe, CIDG Managing Editor.
- Copy Editor (copy editing and production): Julia Turner
- Peer-reviewers (provided comments and recommended an editorial decision):
  - Clinical/content review: Mikashmi Kohli, FIND; Professor Gerry Davies, CIDG Editor*; Dr James Millard, University of Liverpool.
  - Methods review: Mohammad Yaghoobi.
  - Search review: April Coombe

*Professor Gerry Davies is a CIDG Editor, and provided peer-review comments on this article, but was not otherwise involved in the editorial process or decision making for this article.

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APPENDICES

Appendix 1. MEDLINE search strategy

MEDLINE (Ovid)

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

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
	Direction of study	Prospective / Retrospective / Ambi-directional / Not reported / Other _____

(Continued)

	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
	Comments	

(Continued)

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**	_____
	Time to diagnosis	_____

Data for Truenat MTB

1.

Overall Truenat	**RS positive**	**RS negative**	**Total**
Truenat positive			
Truenat negative			
Total			
Non-determinate			
Truenet 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**
Truenat positive			
Truenat negative			

(Continued)

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			
Truenat negative			
Total			

(Continued)

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			
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

(Continued)

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			
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			
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 5. Other _____
Index test A:	1. Truenat MTB 2. Truenat MTB Plus 3. Truenat RIF Dx

(Continued)

Index test B:	1. Xpert MTB/RIF Ultra
Reference standard and target condition:	

Study design

Which of the following study designs does 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

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**
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

(Continued)

	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 will answer '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 will answer '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 expect the studies to include a representative presumptive tuberculosis population that may include both people who are 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 will answer '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 does not provide this information.

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

We will judge 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 is '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 are 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 will answer '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 does 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 is 'low' for each index test, we will answer 'yes'; otherwise, we will answer '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 will respond '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 does not describe the design in sufficient detail.

Signalling question C1.3 Was the allocation sequence random?

We will answer '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 judgments; 'unclear' if the report does 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 will answer '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 has 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 will judge 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 is sufficiently troublesome that the domain as a whole is deemed problematic, we will consider a 'high' risk of bias judgement. We will consider a 'high' risk of bias if C1.2 was answered 'no'; however, if a partially paired with random subset design is used, we will still consider it as a 'low' risk of bias. If we answered 'unclear' to at least one question and 'yes' to any remaining questions, we will consider risk of bias to be 'unclear'.

Domain 2: Index Test **Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults**			
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 will answer '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 will answer '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 will be 'yes', we will consider 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 will answer '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 will answer 'high concern' if the persons administering and interpreting the test clearly did not follow the manufacturer's instructions, and 'unclear concern' if the article does 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 will be 'yes'; therefore, the answer to C2.1 will also be '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 is not necessary, as none of the index tests involves subjective interpretation. Therefore, the response will always be '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 will always be '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 will answer '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 does 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 will consider risk of bias to be 'low', since responses to C2.1 to C2.3 will always be 'yes' (C2.2 and C2.3 are only relevant to fully or partially paired designs). If we answered 'no' to C2.4, we will consider a 'high' risk of bias judgment. If the answer to C2.4 is 'unclear', we will consider 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

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
	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 does not match the review question?	Low/High/Unclear	Low/High/Unclear
Comparative accuracy (QUADAS-C)		**Answers for the**	

(Continued)

		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	AC3.3Could 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 will answer '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 does 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 will answer '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 does not provide this information.

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

We will judge 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 is 'unclear' and any remaining answers are 'yes'.

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

Diagnosis of tuberculosis will not be complete if *M tuberculosis* is not isolated from the culture specimen. We will judge '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 will judge 'low concern' if speciation was performed appropriately; and 'unclear concern' if the report does 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 is 'low' for each index test, we will answer 'yes'; otherwise, we will answer 'no'.

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

We will answer 'yes' if both Truenat MTB/MTB Plus and Xpert/RIF are NOT part of the reference standard; 'no' if they are part of the reference standard; and unclear if the report does 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 will consider risk of bias to be 'low' if we answered 'yes' to signalling questions AC3.1 and AC3.2. We will consider a 'high' risk of bias judgement if we answered 'no' to at least one question or if the bias associated with the design element raises enough red flags to make the domain as a whole problematic. If the answer to at least one question is 'unclear' and any remaining answers are 'yes', we will consider risk of bias to be 'unclear'.

Domain 3 B: Reference Standard		
Truenat MTB assays for rifampicin resistance in adults		
Relevant details:		
Single test accuracy (QUADAS-2)	**Answers for Truenat**	**Answers for Xpert**

(Continued)

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 does 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 will answer '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 does not provide this information.

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

We will answer 'yes' if the reference standard is 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 does not provide this information.

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

We will judge 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 is 'unclear' and any remaining answers are 'yes'.

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

We will judge '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 *Mtuberculosis*) to isoniazid and rifampicin should have been performed and reported. We will answer 'low concern' if the study performed speciation and sensitivity testing appropriately; and 'unclear' if the report does 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 is 'low' for each index test, we will answer 'yes'; otherwise, we will answer 'no'.

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

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

Domain 4: Flow and Timing **Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults**			
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?

Specimens should be sent to the lab as soon as feasible after collection. The specimens should be refrigerated if a delay is unavoidable to prevent the growth of undesirable microorganisms. A suitable preservative must be added if refrigeration is not possible and a delay of more than two days is predicted (CTD India 2009). Therefore, we will answer 'yes' if the index tests and reference standard were performed at the same time, or if the time interval was not more than three days if kept at room temperature; 'no' if the time interval was greater than three days at room temperature; or 'unclear' if these details were not available (Paramasivan 1983).

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

We will answer 'yes' if all sputum samples are subject 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 will answer 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 will answer 'yes' if the number of people enrolled and the number of people included in the 2 × 2 tables match, 'no' if the numbers do not match, and unclear if the report does not provide this information.

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

We will judge 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 is 'low' for each index test, we will answer 'yes'; otherwise, we will answer 'no'.

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

We will answer '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 will be obtained from the existing literature.

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

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

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

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

CONTRIBUTIONS OF AUTHORS

LRI conceived the idea, contributed to the writing of the protocol, supervised the training, co-ordinated the tasks, and edited and reviewed the final manuscript. JD wrote the protocol sections, trained the team, developed a data extraction form and risk of bias tool, and reviewed the manuscript. HDS and RK provided input on the methodology and critically reviewed the manuscript. PR, AB, VAS, MKSN, KS, and MM wrote the protocol sections and reviewed the manuscript. YT wrote the protocol sections, gave methodological and statistical inputs, critically reviewed the protocol, and mentored the team. CP critically reviewed the protocol and supervised the work. All protocol authors reviewed and approved the final version of the protocol.

DECLARATIONS OF INTEREST

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LRI is employed at ICMR. This organization has published opinions in medical journals relevant to the interventions in the work, and has a declared opinion on this topic. LRI has no known conflicts of interest.
JD has no known conflicts of interest.
PR was involved in a primary diagnostic study that may be included in this review. She is employed at ICMR. This organization has published opinions in medical journals relevant to the interventions in the work, and has a declared opinion on this topic. PR has no known conflicts of interest.
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KS has no known conflicts of interest.
MM is employed at ICMR. This organization has published opinions in medical journals relevant to the interventions in the work, and has a declared opinion on this topic. MM has no known conflicts of interest.
YT is a co-convenor of the Cochrane Screening and Diagnostic Tests Methods Group, an editor of the Cochrane Infectious Diseases Group, and a statistical editor of the Cochrane Bone, Muscle, Joint and Trauma Group. She was not involved in the editorial process or decision making for this protocol, and has no known conflicts of interest.
CP is employed at ICMR. This organization has published opinions in medical journals relevant to the interventions in the work, and has a declared opinion on this topic. CP has no known conflicts of interest.

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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