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## Xpert<sup>®</sup> MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance (Protocol)

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[Diagnostic Test Accuracy Protocol]

# Xpert® MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance

Mikashmi Kohli<sup>1</sup>, Ian Schiller<sup>2</sup>, Nandini Dendukuri<sup>2</sup>, Hannah Ryan<sup>3</sup>, Keertan Dheda<sup>4</sup>, Claudia M Denkinge<sup>5</sup>, Samuel G Schumacher<sup>5</sup>, Karen R Steingart<sup>6</sup>

<sup>1</sup>All India Institute of Medical Sciences (AIIMS), New Delhi, India. <sup>2</sup>Division of Clinical Epidemiology, McGill University Health Centre - Research Institute, Montreal, Canada. <sup>3</sup>Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK. <sup>4</sup>Lung Infection and Immunity Unit, Department of Medicine, University of Cape Town, Cape Town, South Africa. <sup>5</sup>Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland. <sup>6</sup>Cochrane Infectious Diseases Group, Liverpool School of Tropical Medicine, Liverpool, UK

Contact address: Karen R Steingart, Cochrane Infectious Diseases Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK. [karen.steingart@gmail.com](mailto:karen.steingart@gmail.com).

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## ABSTRACT

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

To determine the diagnostic accuracy of Xpert® MTB/RIF for the detection of extrapulmonary TB by site of disease in people suspected of having extrapulmonary TB. The role of Xpert® MTB/RIF would be a replacement for standard practice.

To determine the diagnostic accuracy of Xpert® MTB/RIF for detection of rifampicin resistance in people suspected of having extrapulmonary TB. The role of Xpert® MTB/RIF would be an initial test for replacement for culture-based DST.

We plan to investigate the impact of covariates on heterogeneity in estimates of test accuracy across the included studies.

### Detection of extrapulmonary TB

The covariates of interest are: HIV prevalence; condition of the specimen (fresh versus frozen); sample input volume (for liquid specimens); homogenization step (for tissue specimens); smear status of specimen; past history of TB; and prevalence of extrapulmonary TB. In addition, we plan to explore whether the WHO standard operating procedures for a given type of specimen were followed and can explain the observed heterogeneity in accuracy estimates. For TB meningitis we plan to investigate whether the use of a concentration step prior to the use of Xpert® MTB/RIF has an impact on accuracy estimates. In addition, for detection of lymph node TB, pleural TB, and TB meningitis, we plan to adjust accuracy estimates by applying a latent class meta-analysis model to account for the imperfect nature of culture as the reference standard.

### Detection of rifampicin resistance detection

The covariate of interest is the prevalence of rifampicin resistance.

## BACKGROUND

Tuberculosis (TB) causes tremendous suffering worldwide and now ranks above HIV/AIDS as the world's leading infectious cause of death. TB is caused by infection with *Mycobacterium tuberculosis* (*M. tuberculosis*) bacteria. The World Health Organization (WHO) estimates that globally in 2015, 10.4 million people became ill with TB and 1.8 million people died, including 0.4 million HIV-positive people (WHO 2016a). In addition, globally in 2015, there were an estimated 480,000 new cases of multidrug-resistant TB (MDR-TB) and 100,000 cases of rifampicin-resistant TB (WHO 2016a). MDR-TB is caused by infection with *M. tuberculosis* bacteria that are resistant to at least rifampicin and isoniazid, two of the most effective anti-TB drugs. When people receive proper treatment, TB is treatable and curable.

TB predominantly affects the lungs (pulmonary TB). Extrapulmonary TB refers to TB in parts of the body other than the lungs, and is known to affect virtually every part, with lymph nodes and pleura being the most common sites (Sharma 2004). While active pulmonary TB is transmissible by droplets spread by coughing in individuals, extrapulmonary TB is thought to result from hematogenous spread from an initial lung infection, and is not infectious. Extrapulmonary TB can occur alone or together with pulmonary TB. Of the 6.1 million new cases of TB notified to WHO in 2015, 15% were extrapulmonary TB cases (WHO 2016a). However, the number of people affected by extrapulmonary TB is likely to be higher, considering that, according to WHO, extrapulmonary TB is notified as pulmonary TB when the two forms exist together (WHO 2014b), and diagnosing extrapulmonary TB is challenging, as described below. Additionally, extrapulmonary TB accounts for an increasing proportion of new TB cases in some countries, in part due to the increased incidence of extrapulmonary TB associated with concurrent HIV infection (Golden 2005; Perkins 2007; Pai 2016). Extrapulmonary TB also affects children in greater proportions than adults (Nelson 2004).

WHO TB treatment guidelines recommend the same drug regimens for extrapulmonary and pulmonary disease with notable mention of other guidelines which recommend longer treatment for TB meningitis and for bone and joint TB (WHO 2010). An updated guideline, published in 2017 provided recommendations on the use of adjuvant steroids in the treatment of extrapulmonary TB disease for TB meningitis (strong recommendation, *moderate certainty evidence*) and TB pericarditis (conditional recommendation, *very low certainty evidence*) (WHO 2017a). TB treatment guidelines published in 2016 include *Index-TB 2016* (India), and those issued by the American Thoracic Society, the Centers for Disease Control and Prevention (CDC), and the Infectious Diseases Society of America (Nahid 2016).

Diagnosis of extrapulmonary TB is challenging for several reasons. Many forms of extrapulmonary TB require invasive diagnostic sampling, and gathering adequate specimens can pose a risk of

harm to the patient and be costly. Most forms of extrapulmonary TB are paucibacillary (TB disease caused by a smaller number of bacteria), making diagnosis by the conventional method of smear microscopy less sensitive. This problem particularly affects resource-limited settings, where the more sensitive methods of mycobacterial culture and histological examination are not widely available. There are also limitations associated with culture and histology: culture takes several weeks, requires a highly-equipped laboratory, and has reduced sensitivity in paucibacillary disease; histology relies on highly trained operators and characteristic morphology is shared with other diseases. As a result of these difficulties, diagnosis of extrapulmonary TB is often made on the grounds of clinical suspicion alone, and many people receive the wrong diagnosis leading to unnecessary TB treatment or poor outcomes from untreated extrapulmonary TB. The demand for faster, reliable diagnostics that are suitable for resource-limited settings is clear, and has been defined by the research community (Denkinger 2015). In 2014, the World Health Assembly unanimously approved the End TB Strategy, a 20-year strategy to end the global TB epidemic. The END TB strategy calls for early diagnosis of TB including universal drug susceptibility testing (DST) (WHO END TB 2014).

Xpert® MTB/RIF is an automated polymerase chain reaction (PCR) test, which accurately detects pulmonary TB and rifampicin resistance when used on sputum specimens (Steingart 2014). The WHO published updated guidance on the use of Xpert® MTB/RIF in 2013 (WHO 2013). The updated policy statement expanded recommendations for the use of Xpert® MTB/RIF for pulmonary TB in adults and added guidance on the use of the test for childhood TB and extrapulmonary TB. Drawing on a systematic review (Denkinger 2014), and using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, the WHO issued the following recommendations related to extrapulmonary TB.

- Xpert® MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for cerebrospinal fluid (CSF) specimens from patients suspected of having TB meningitis (strong recommendation given the urgency for rapid diagnosis, *very low quality evidence*).
- Xpert® MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having extrapulmonary TB (conditional recommendation, *very low quality evidence*).

Subsequently, the use of Xpert® MTB/RIF has been incorporated into the International Standards for TB Care 2014 (TB Care I 2014).

Currently the manufacturer, Cepheid Incorporated (Sunnyvale, CA, USA), has made no specific recommendations for the use of Xpert® MTB/RIF in non-sputum specimens, and accordingly, Xpert® MTB/RIF is approved by the United States Food and Drug Administration (FDA) for use in raw sputum specimens and concentrated sputum sediment only (FDA 2013).

## Target condition being diagnosed

### Extrapulmonary TB

The various forms of extrapulmonary TB cause signs and symptoms related to the structures affected. Table 1 describes the forms of extrapulmonary TB included in this Cochrane Review, as well as the different specimens that may be acquired for diagnosis.

### Rifampicin resistance

Rifampicin inhibits bacterial DNA-dependent RNA polymerase, encoded by the RNA polymerase gene (*rpoB*) (Hartmann 1967). Resistance to this drug has mainly been associated with mutations in a limited region of the *rpoB* gene (Telenti 1993). Rifampicin resistance may occur alone or in association with resistance to isoniazid and other drugs. In settings with a high burden of MDR-TB, the presence of rifampicin resistance alone may serve as a proxy for MDR-TB (WHO 2011).

### Index test(s)

Xpert® MTB/RIF is a diagnostic test for detection of *M. tuberculosis* complex DNA and, when *M. tuberculosis* complex is detected, rifampicin resistance-associated mutations of the *rpoB* gene. The test results are available within two hours after starting the test, and with minimal hands-on technical time. Unlike conventional nucleic acid amplification tests (NAATs), Xpert® MTB/RIF integrates sample processing and PCR amplification and detection into a single self-enclosed test unit, the GeneXpert cartridge (Blakemore 2010). Following sample loading, all steps in the assay are completely automated and self-contained. In addition, the assay's sample reagent, used to liquefy sputum, has potent tuberculocidal (the ability to kill TB bacteria) properties and so largely eliminates biosafety concerns during the test procedure (Banada 2010). Xpert® MTB/RIF detects both live and dead bacteria (Miotto 2012).

Xpert® MTB/RIF uses molecular beacon technology to detect rifampicin resistance. Molecular beacons are nucleic acid probes that recognize and report the presence or absence of the normal, rifampicin-susceptible, 'wild type' sequence of the *rpoB* gene of TB. Five different coloured beacons are used, each covering a separate nucleic acid sequence within the amplified *rpoB* gene.

Although Xpert® MTB/RIF provides testing for both *M. tuberculosis* and rifampicin resistance, it is really only one test. One cannot deselect testing for rifampicin resistance and only run the assay for TB detection. Xpert® MTB/RIF may be used at all levels of the health system. However, with the current device, a stable and uninterrupted electrical supply is required. The WHO has published extensive guidance and practical information on implementing the test (WHO 2014a).

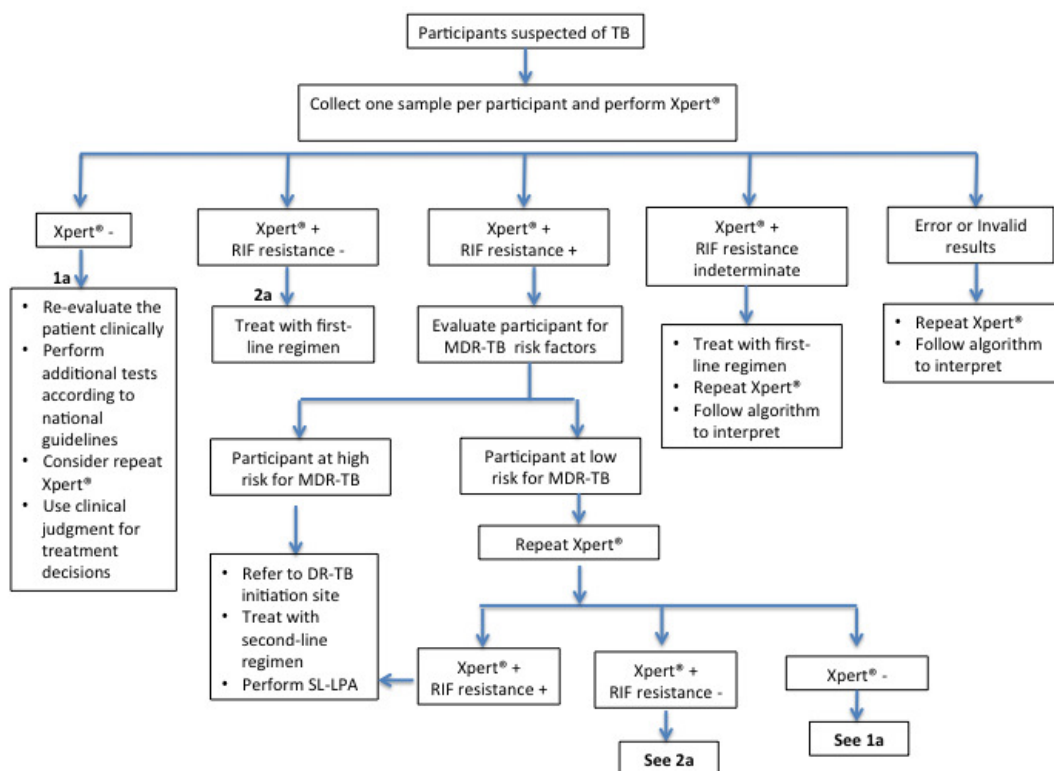
Since Xpert® MTB/RIF was released, there have been four generations (G1, G2, G3, and G4) of the cartridge. G4 is the Xpert® MTB/RIF cartridge in current use and has been in the field for several years (Cepheid 2015). The WHO has recently endorsed the next-generation assay, the Xpert® MTB/RIF Ultra assay, concluding that, compared to G4, the Ultra cartridge showed improved sensitivity for detection of TB, in particular, in populations who are difficult to diagnose, children, people with extrapulmonary TB, and people living with HIV (WHO 2017b). To improve detection of *M. tuberculosis*, Ultra incorporates two different multi-copy amplification targets (IS6110 and IS1081) and to improve detection of rifampicin resistance, Ultra uses melting temperature-based analysis instead of real-time PCR (WHO 2017b). We will include studies that use any of the Xpert® MTB/RIF cartridge generations, as well as the new version, Xpert® MTB/RIF Ultra, in this Cochrane Review.

### Clinical pathway

In this section and Figure 1 we describe the clinical pathway and present the context in which Xpert® MTB/RIF might be used. The target condition is extrapulmonary TB, of which there are several forms (such as pleural TB and TB meningitis). Prior to testing a specimen with Xpert® MTB/RIF, patients suspected of having extrapulmonary TB would receive a history, physical examination, and possibly a chest radiograph. The presentation of extrapulmonary TB varies depending on the body site affected and may imitate other diseases, such as cancer and bacterial and fungal infections. The signs and symptoms of extrapulmonary TB are often non-specific and may include fever, night sweats, fatigue, loss of appetite, and weight loss (as seen in pulmonary TB) or specific complaints related to the involved site (for example, headache for TB meningitis and back pain for TB of the spine). The clinical presentation of extrapulmonary disease may be acute, but is more often subacute (falling between acute and chronic) or chronic, meaning that patients may have symptoms for days to months before they seek care. Signs and symptoms for the forms of extrapulmonary TB included in this review are described in Table 1. The clinician should take a careful history noting history of TB exposure, prior TB disease, and medical conditions that increase the risk for TB disease (such as HIV, diabetes mellitus, and low body weight). In comparison with HIV-negative people, HIV-positive people have higher rates of extrapulmonary TB or mycobacteremia (TB bloodstream infection). HIV-positive patients

with signs or symptoms of extrapulmonary TB should have specimens taken from the suspected site(s) of involvement to increase the likelihood of TB diagnosis. In general, children with extrapulmonary TB present in a similar way to that of adults. However, infants and young children are at the highest risk of developing disseminated TB disease and TB meningitis, the most severe forms of TB. In TB meningitis, diagnosis is often delayed with appalling consequences for patients. For all forms of extrapulmonary TB, patients may be evaluated in a primary or secondary care setting. However, if more complex or invasive tests are needed, patients may be referred to a tertiary medical centre ([Iseman 2000](#); [Sharma 2004](#); [Reuter 2009](#)).

**Figure 1. Abbreviations: DR-TB: drug resistant TB; MDR-TB: multidrug-resistant TB; RIF: rifampicin; SL-LPA: line probe assay for second-line drugs; TB: tuberculosis; Xpert®: Xpert® MTB/RIF.** The Clinical Pathway describes how patients might present and the point in the pathway at which participants would be considered for testing with Xpert® MTB/RIF. Prior to testing a specimen with Xpert® MTB/RIF, participants suspected of having extrapulmonary TB would have received a history, physical examination, and possibly a chest radiograph. The presentation of extrapulmonary TB varies depending on the body site affected and may imitate other diseases such as cancer and bacterial and fungal infections. The signs and symptoms of extrapulmonary TB are often non-specific and may include fever, night sweats, fatigue, loss of appetite, and weight loss (as seen in pulmonary TB) or specific complaints related to the involved site (for example, headache for TB meningitis and back pain for TB of the spine). The clinical presentation of extrapulmonary disease may be acute, but is more often subacute (falling between acute and chronic) or chronic, meaning that patients may have symptoms for days to months before they seek care. Signs and symptoms for the forms of extrapulmonary TB included in this review are described in Table 1. Standard practice includes obtaining specimens for microscopy, culture, and histological examination. We adapted this algorithm for Xpert® MTB/RIF from the Global Laboratory Initiative (GLI 2017).



The purpose of Xpert® MTB/RIF is diagnosis of TB and detection of rifampicin resistance. The role of Xpert® MTB/RIF is a replacement for standard practice, which includes obtaining appropriate specimens from the suspected sites of involvement for microbiological (conventional microscopy and culture) and histological examination. An Xpert® MTB/RIF test is recommended as the preferred initial microbiological test for suspected TB meningitis because of the need for a rapid diagnosis (WHO 2013; TB Care I 2014). In HIV-positive people with a CD4 cell count less than or equal to 100 cells/ $\mu$ L, or HIV-positive people who are seriously ill regardless of CD4 count, the lateral flow urine lipoarabinomannan assay (LF-LAM) (see 'Alternative test(s)' section) may be used to assist in the diagnosis of TB (WHO 2015). The WHO further recommends that: "Individuals suspected of having extrapulmonary TB but who have had a single negative result from Xpert® MTB/RIF should undergo further diagnostic testing, and those for whom there is a high clinical suspicion for TB (especially children) should be treated even if an Xpert® MTB/RIF result is negative or if the test is not available" (WHO 2013). The downstream consequences of Xpert® MTB/RIF testing are as follows.

- True positives (TP): patients would benefit from rapid diagnosis and appropriate treatment.
- True negatives (TN): patients would be spared unnecessary treatment and benefit from reassurance and pursuit of alternative diagnosis.
- False positives (FP): patients would likely experience anxiety and morbidity caused by additional testing, unnecessary treatment, and possible adverse effects; possible stigma associated with a TB or MDR-TB diagnosis; and the chance that a false positive may halt further diagnostic evaluation.
- False negatives (FN): an increased risk of morbidity and mortality, delayed treatment initiation for patients, and the continued risk of TB transmission in the community.

### Alternative test(s)

In this section, we describe alternative tests for detection of extrapulmonary TB and rifampicin resistance. For a comprehensive review of new tests in the diagnostic pipeline, we refer the reader to an excellent resource (Unitaid 2017).

It is recommended that clinicians who evaluate patients considered to have extrapulmonary TB adhere to Standard 4 of the International Standards for TB Care, which states: "For all patients, including children, suspected of having extrapulmonary TB, appropriate specimens from the suspected sites of involvement should be obtained for microbiological and histological examination. An Xpert® MTB/RIF test is recommended as the preferred initial microbiological test for suspected TB meningitis because of the need for a rapid diagnosis" (TB Care I 2014).

Smear microscopy (that is, light microscopy (Ziehl-Neelsen), fluorescence microscopy, or Light-Emitting Diode (LED) fluorescence microscopy) is the examination of smears for acid-fast bacilli (TB bacteria) under a microscope. For extrapulmonary TB, microscopy can be performed on fluid or tissue specimens from sites of disease involvement, for example on CSF in suspected TB meningitis or lymph node tissue biopsy in suspected lymph node TB. The identification of acid-fast bacilli by microscopy in specimens from most extrapulmonary sites is less frequent (because there are usually fewer organisms in extrapulmonary sites) and rapid molecular tests, culture, or both tests are more important (Iseman 2000; TB Care I 2014).

Histological examination involves the examination of tissue specimens under a microscope. Diagnosis of extrapulmonary TB by histological examination is based on finding the presence of granulomatous inflammation with caseous (cheese-like) necrosis and acid-fast bacilli. The contribution of histology to the diagnosis of extrapulmonary TB has been reported to vary for different forms of extrapulmonary TB, as well as for different diagnostic techniques. In patients with TB pleural effusions, granulomatous inflammation has been reported in 50% to 97% of patients (Gopi 2007), whereas in patients with lymph node TB, granulomatous inflammation has been reported in 32% to 88% of patients (Fontanilla 2011). Regarding diagnostic techniques, Diacon 2003 found thoracoscopy to be more sensitive (sensitivity of 100%) than closed needle biopsy (sensitivity of 66%) for establishing a diagnosis of pleural TB. Histological examination carries the additional concern that invasive procedures that are complex and costly may be required to obtain the necessary specimens (Golden 2005).

NAAT is a molecular technique that can detect small quantities of genetic material (DNA or RNA) from microorganisms, such as *M. tuberculosis*. The key advantage of NAATs is that they are rapid diagnostic tests, potentially providing a result in a few hours. This is a particularly important feature of the test in life-threatening forms of extrapulmonary TB, such as TB meningitis. A variety of molecular amplification methods are available, of which PCR is the most common. NAATs are available as commercial kits and in-house tests (based on a protocol developed in a laboratory) and are used routinely in high-income countries for TB detection. In-house PCR is widely used in low-income countries because these tests are less expensive than commercial kits. An older editorial summarizing three systematic reviews (140 studies) of commercial and in-house NAATs (other than Xpert® MTB/RIF) for different forms of extrapulmonary TB found relatively low sensitivity and underscored concerns about the cost and feasibility of this technology in resource-limited areas (Pai 2008). Similarly, a systematic review found NAATs to have relatively low sensitivity for extrapulmonary TB, though high specificity (for example, for TB meningitis and pleural TB), indicating that these tests cannot be



used reliably to rule out TB (Dinnes 2007).

GenoType MTBDR<sub>plus</sub> (Hain Lifescience, Nehren, Germany), is a commercial NAAT that belongs to a category of molecular genetic tests called line probe assays. MTBDR<sub>plus</sub> detects the presence of mutations associated with drug resistance to isoniazid and rifampicin (Nathavitharana 2017). The WHO recommends that MTBDR<sub>plus</sub> be used for cultured isolates of *M. tuberculosis* from both pulmonary and extrapulmonary sites (WHO 2016b).

The LF-LAM (Alere Determine™ TB LAM Ag, Alere Inc, Waltham, USA) is a commercially available point-of-care test for active TB (pulmonary and extrapulmonary TB). The test detects lipoarabinomannan (LAM), a component of the bacterial cell wall, which is present in some people with active TB. The test is performed by placing urine on one end of a test strip, with results appearing as a line (that is, a band) on the strip if TB is present. The test is simple, requires no special equipment, and shows results in 25 minutes (Shah 2016). A Cochrane Review found LF-LAM, whether the test is used for diagnosis or screening, has low sensitivity to detect TB. However, in HIV-positive people with low CD4 counts who are seriously ill, LF-LAM may help with the diagnosis of TB (Shah 2016).

## Rationale

Existing diagnostic tests for extrapulmonary TB are not sensitive enough or are invasive and costly. This Cochrane Review will estimate sensitivity and specificity of Xpert® MTB/RIF for detection of extrapulmonary TB and rifampicin resistance. We are aware of six systematic reviews previously published on this topic: Chang 2012 (seven studies), Denkinger 2014 (18 studies), Maynard-Smith 2014 (27 studies), Penz 2015 (37 studies), Sehgal 2016 (24 studies; pleural fluid only), and Li 2017 (26 studies) (Table 2). Chang 2012 performed literature searching up to 1 October 2011, Denkinger 2014 up to 15 October 2013, Maynard-Smith 2014 up to 6 November 2013, Penz 2015 up to 15 August 2014, Sehgal 2016 up to 31 August 2015, and Li 2017 up to 20 June 2015. The reviews found different pooled accuracy estimates for different forms of extrapulmonary TB and noted several limitations, including the following: small number of samples for a given specimen type, incomplete information on HIV status, concerns about accuracy of the reference standards used, limited data for assessing the accuracy of Xpert® MTB/RIF for detection of rifampicin resistance, and considerable differences in the preparation of specimens for testing. Concerning the latter, the WHO has prepared standard operating procedures for preparation of non-respiratory specimens for use with Xpert® MTB/RIF (WHO 2014a). This Cochrane Review will enable us to update the literature and provide an opportunity to address some of the noted limitations.

## OBJECTIVES

To determine the diagnostic accuracy of Xpert® MTB/RIF for the detection of extrapulmonary TB by site of disease in people suspected of having extrapulmonary TB. The role of Xpert® MTB/RIF would be a replacement for standard practice.

To determine the diagnostic accuracy of Xpert® MTB/RIF for detection of rifampicin resistance in people suspected of having extrapulmonary TB. The role of Xpert® MTB/RIF would be an initial test for replacement for culture-based DST.

## Secondary objectives

We plan to investigate the impact of covariates on heterogeneity in estimates of test accuracy across the included studies.

## Detection of extrapulmonary TB

The covariates of interest are: HIV prevalence; condition of the specimen (fresh versus frozen); sample input volume (for liquid specimens); homogenization step (for tissue specimens); smear status of specimen; past history of TB; and prevalence of extrapulmonary TB. In addition, we plan to explore whether the WHO standard operating procedures for a given type of specimen were followed and can explain the observed heterogeneity in accuracy estimates. For TB meningitis we plan to investigate whether the use of a concentration step prior to the use of Xpert® MTB/RIF has an impact on accuracy estimates. In addition, for detection of lymph node TB, pleural TB, and TB meningitis, we plan to adjust accuracy estimates by applying a latent class meta-analysis model to account for the imperfect nature of culture as the reference standard.

## Detection of rifampicin resistance detection

The covariate of interest is the prevalence of rifampicin resistance.

## METHODS

### Criteria for considering studies for this review

#### Types of studies

We will include primary studies that compare the results of the index test with the reference standard. We will include randomized controlled trials, cross-sectional studies, and observational cohort studies. We will include studies that report data from which we can extract TP, FP, FN, and TN. We will exclude case-control studies. In addition, we will exclude data reported only in abstracts from conference proceedings and case reports.

## Participants

For both review questions, we will include participants of all ages thought to have extrapulmonary TB from all settings and countries. We will include all patients providing non-respiratory specimens (may include blood, urine, pericardial fluid, ascitic fluid, or bone biopsy tissue, for example), except as noted. We will exclude sputum and other respiratory specimens, such as fluid obtained from bronchial alveolar lavage and tracheal aspiration. Studies will need to provide data for at least five specimens. We have added this criterion because we are aware that some studies may include data for only a few non-respiratory specimens, which we feel would contribute relatively little in relation to the additional resources required. We will exclude studies that evaluate Xpert® MTB/RIF by aspiration of gastric fluid, as this specimen is used mostly for investigating pulmonary TB in children. We will also exclude stool specimens because TB bacteria may be swallowed and passed into stool as a marker of pulmonary TB. We will exclude studies evaluating the use of Xpert® MTB/RIF to diagnose relapse of previously treated extrapulmonary TB so as to avoid the selection bias that may arise by limiting to a group that is already at elevated risk of extrapulmonary TB. Our intent is to identify studies that include patients with diagnostic uncertainty who are not taking anti-TB drugs or who have taken anti-TB drugs for less than seven days. However, if we find that some studies do include some patients on TB drugs, we will address this concern in a sensitivity analysis.

## Index tests

The index test is Xpert® MTB/RIF. The index test results are automatically generated and the user is provided with a printable test result as follows.

- MTB (*M. tuberculosis*) DETECTED; Rif (rifampicin) resistance DETECTED.
- MTB DETECTED; Rif resistance NOT DETECTED.
- MTB detected; Rif resistance INDETERMINATE.
- MTB NOT DETECTED.
- INVALID (The presence or absence of MTB cannot be determined).
- ERROR (The presence or absence of MTB cannot be determined).
- NO RESULT (The presence or absence of MTB cannot be determined).

Non-interpretable results for detection of MTB are classified as 'invalid', 'error', or 'no result'. Non-interpretable results for detection of rifampicin resistance are classified as rifampicin resistance 'indeterminate'.

## Target conditions

The target condition is extrapulmonary TB, stratified by site of disease. We will consider the subcategories of the target condition

as separate diagnostic classifications. We will include the most common forms of extrapulmonary TB (Sharma 2004; Sandgren 2013; CDC 2014). Table 1 lists forms of extrapulmonary TB and the specimens suggested for diagnostic testing.

- Lymph node TB.
- Pleural TB.
- TB meningitis.
- Bone and joint TB.
- Genitourinary TB.
- Peritoneal TB.
- Pericardial TB.
- Disseminated TB.

We anticipate that we will find very few studies that evaluate Xpert® MTB/RIF for bone and joint TB, genitourinary TB, peritoneal TB, pericardial TB, and disseminated TB. If so, we will describe these studies in an appendix and will focus the review on three subcategories of the target condition: lymph node TB, pleural TB, and TB meningitis.

## Reference standards

### Detection of all forms of extrapulmonary TB

The primary reference standard will be solid or liquid mycobacterial culture.

- 'TB' is defined as a positive *M. tuberculosis* culture.
- 'Not TB' is defined as a negative *M. tuberculosis* culture.

For detection of pleural TB by biopsy of pleural tissue, we will also include a composite reference standard defined as 'culture or granulomatous inflammation on histopathological examination, either test positive'. There is evidence to support including histopathological examination in the composite reference standard for pleural TB. Around 60% of patients undergoing pleural biopsy will show granulomatous inflammation (American Thoracic Society 2000). In a prospective cohort study of patients with clinical and radiological findings consistent with pleural TB, Conde 2003 found histological examination of tissue obtained from pleural biopsy to have a higher diagnostic yield, 78% (66/84), than that of culture (62% 52/84). For other forms of TB, we decided against the use of a composite reference standard due to the differing definitions of the composite reference standards, the difficulty in interpreting them, and the concern for bias (Schiller 2016).

Culture is considered the best reference standard for TB. However, culture may misclassify cases of extrapulmonary TB as not TB due to the paucibacillary nature of the disease. This means that index test TPs may be misclassified as FPs by culture. Therefore, when evaluating Xpert® MTB/RIF against culture, the number of FPs (classified as positive by the index test and negative by the reference test) may be increased and Xpert® MTB/RIF specificity may be underestimated. Another drawback of using culture as a

reference standard is that it amounts to ignoring the dependence that arises between culture and Xpert® MTB/RIF among true extrapulmonary TB cases due to their common dependence on the bacterial load. Both culture and Xpert® MTB/RIF are likely to pick up cases with a higher bacterial load, and both are likely to miss cases with a lower bacterial load. Ignoring this dependence could lead to an overestimation of the sensitivity of Xpert® MTB/RIF. To improve the estimation of diagnostic accuracy, we plan to apply latent class analysis to the three most commonly studied forms of extrapulmonary TB on which we believe it is possible to gather the data necessary to make the adjustment. We discuss this

approach further in the [Statistical analysis and data synthesis](#) section.

### Detection of rifampicin resistance

The reference standard will be culture-based DST using solid or liquid media or MTBDR<sub>plus</sub> as recommended by the WHO ([WHO 2012](#); [WHO 2016b](#)).

### Search methods for identification of studies

We will attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, and ongoing).

#### Electronic searches

Vittoria Lutje (VL), the Information Specialist for the Cochrane Infectious Diseases Group (CIDG), will perform literature searches without language restrictions. To identify all relevant studies, VL will search the following databases using the search terms and strategy described in [Appendix 1](#): CIDG Specialized Register; MEDLINE (OVID); Embase (OVID); Science Citation Index Expanded (SCI-EXPANDED), Conference Proceedings Citation Index-Science, (CPCI-S), and BIOSIS Previews; all three from Web of Science; LILACS (BIREME; <http://lilacs.bvsalud.org/en/>); and Scopus® (Elsevier). VL will also search the ISRCTN registry (<http://isrctn.com>) and the search portal of the WHO International Clinical Trials Registry Platform (ICTRP; <http://apps.who.int/trialsearch/>) to identify ongoing trials, and ProQuest Dissertations & Theses A&I to identify relevant dissertations.

#### Searching other resources

We will review reference lists of included articles and any relevant review articles identified through the above methods. We will contact the test manufacturer (Cepheid Inc.) to identify unpublished studies. We will also handsearch WHO reports on Xpert® MTB/RIF. We will contact researchers at FIND, members of the Stop

TB Partnership's New Diagnostics Working Group, and other experts in the field of TB diagnostics for information on ongoing or unpublished studies.

### Data collection and analysis

#### Selection of studies

Two review authors will independently scrutinize titles and abstracts identified by electronic literature searching to identify potentially eligible studies. We will select any citation identified by either review author as potentially eligible for full-text review. Two review authors will then independently assess the full-text papers for study eligibility using the predefined inclusion and exclusion criteria. The review authors will resolve any discrepancies by discussion, or if they are unable to resolve, by decision of a third review author. We will list all studies excluded after full-text assessment and their reasons for exclusion in a 'Characteristics of excluded studies' table. We will illustrate the study selection process in a PRISMA diagram.

#### Data extraction and management

Two review authors will extract a set of data from five studies using a piloted data extraction form ([Appendix 3](#)). Based on the pilot, we will finalize the extraction form. Next, two review authors working independently will extract data on the following characteristics.

- Author; publication year; country; setting (outpatient, inpatient, or both outpatient and inpatient); study design; manner of participant selection; number of participants enrolled; number of participants for whom results are available.
- Characteristics of participants: % female; age; % HIV-positive; % with history of TB.
- Index test version.
- Target condition and subcategories.
- Reference standard.
- QUADAS-2 items.
- Details of specimen: type (such as CSF, pleural fluid, lymph node aspirate or tissue); condition (fresh or frozen); smear positive or negative.
- Specimen preparation; homogenization step (for tissue specimens); concentration step; sample input volume; adherence to WHO standard operating procedures.
- Number of TP, FP, FN, and TN; the number of non-interpretable results; the number of indeterminate rifampicin-resistant results.
- Number of missing or unavailable test results.

When possible, we will extract data for subgroups by HIV status. We will classify country income status as either low- and middle-income or high-income, according to the World Bank List of Economies ([World Bank 2017](#)).

We will extract TP, FP, FN, and TN values for non-respiratory specimens: pleural, CSF, joint aspirate, urine, peritoneal, pericardial, blood, and biopsy specimens such as lymph node and pleural tissue, corresponding to the forms of extrapulmonary TB in the review. In situations where a participant contributes more than one specimen, but of a different type, we will extract data for all specimens. When a study includes data for both raw specimens and concentrated sediment involving the same participants, we will preferentially extract data for raw specimens, except in the case of CSF where we will extract data for concentrated sediment as recommended by the WHO (WHO 2014a). We will only extract accuracy data for the participants who received the defined reference standard (see Reference standards). In situations where a subset of participants in a study received the reference standard but others did not, we will only extract information on the subset together with information on the percentage of patients who received the reference standard. This will allow us to make corrections for verification bias in the statistical analysis (Begg 1983). We will contact authors of primary studies for missing data or clarifications. We will enter all data into a database manager, Microsoft Excel 2014.

### Assessment of methodological quality

We will use the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool to assess the quality of the included studies (Whiting 2011). QUADAS-2 consists of four domains: patient selection, index test, reference standard, and flow and timing. We will assess all domains for the potential for risk of bias and the first three domains for concerns regarding applicability. As recommended, we will first develop guidance on how to appraise each question and interpret this information tailored to this review. Then, one review author will pilot the tool with two of the included studies. Based on experience gained from the pilot, we will finalize the tool. Two review authors will independently complete QUADAS-2. We will resolve disagreements through discussion or by consulting a third review author. We will present the results of the quality assessment in the text, table, and graphs.

We will follow Cochrane policy which states that "authors of primary studies will not extract data from their own study or studies. Instead, another author will extract these data, and check the interpretation against the study report and any available study registration details or protocol".

### Statistical analysis and data synthesis

We will perform descriptive analyses of the characteristics of the included studies using Stata 12 (Stata 2011), and will present key study characteristics in the 'Characteristics of included studies' table. We will use data reported in the TP, FP, FN, and TN format to calculate sensitivity and specificity estimates and 95% confidence intervals (CI) for individual studies. We will present individual

study results graphically by plotting the estimates of sensitivity and specificity (and their 95% CIs) in forest plots and receiver operating characteristic (ROC) space using Review Manager 5 (RevMan 5) (RevMan 2014).

When possible, we will perform meta-analyses to estimate the pooled sensitivity and specificity and corresponding 95% credible (CrI, defined below) and prediction intervals using an adaptation of the bivariate random-effects approach of Reitsma (Reitsma 2005; Chu 2009). The bivariate random-effects approach will allow us to calculate the pooled estimates of sensitivity and specificity while dealing with potential sources of variation caused by: (1) imprecision of sensitivity and specificity estimates within individual studies; (2) correlation between sensitivity and specificity across studies; and (3) variation in sensitivity and specificity between studies.

We will use the following approach. We will perform separate analyses grouped by type of non-respiratory specimen (for example pleural, CSF, or peritoneal), rather than determine summary accuracy estimates for all forms of extrapulmonary TB combined, because we think the former approach is most clinically meaningful.

For the analysis of Xpert® MTB/RIF accuracy for rifampicin resistance detection, we will include patients who are (1) culture-positive; (2) have a valid phenotypic DST (or MTBDR<sub>plus</sub>) result; are (3) Xpert® MTB/RIF TB-positive; and have (4) a valid Xpert® RIF-result.

Sensitivity = Xpert® MTB/RIF RIF-resistant/DST RIF-resistant  
Specificity = Xpert® MTB/RIF RIF-susceptible/DST RIF-susceptible

Culture negative-Xpert® positive-rifampicin resistant positive results have rarely been described in the literature (Boyles 2014; Kelly 2014). When reported in the included studies, we will extract these data and describe them narratively in the 'Findings' and 'Strengths and weaknesses' sections of the review.

For our primary analysis and examination of heterogeneity, we will estimate all models using a Bayesian approach with low-information prior distributions and implement the models using WinBUGS (Version 1.4.3) (Lunn 2000). Under the Bayesian approach, all unknown parameters must be provided a prior distribution that defines the range of possible values of the parameter and the weight of each of those values, based on information external to the data. In order to let the observed data dominate the final results, we will choose to use low-information prior distributions. We will define prior distributions on the log-odds scale over the pooled sensitivity and specificity parameters, their corresponding between-study standard deviations and the correlation between the sensitivities and specificities across studies (see Steingart 2014 and Shah 2016). We will summarize the meta-analysis models used and corresponding WinBUGS programs in an appendix. It is known that meta-analysis models can be sensitive to the choice of prior distributions over the between-study standard deviation parameters. Therefore, we will carry out sensitivity analyses con-

sidering alternative prior distributions that are less informative, allowing a wider range of possible values.

We will combine information from the prior distribution with the likelihood of the observed data, in accordance with Bayes' theorem using the WinBUGS program, which will provide a sample from the posterior distribution of each unknown parameter. We are particularly interested in the pooled sensitivity and specificity of Xpert® and the between-study variance in the sensitivity and specificity of Xpert® on the log-odds scale. Using a sample from the posterior distribution, we will calculate various descriptive statistics of interest. We will estimate the median pooled sensitivity and specificity and their 95% CrI. The median or the 50% quantile is the value below which 50% of the posterior sample lies. We will report the median because the posterior distributions of some parameters may be skewed and the median would be considered a better point estimate of the unknown parameter than the mean in such cases. The 95% CrI is the Bayesian equivalent of the classical (frequentist) 95% CI (we will indicate 95% CI for individual study estimates and 95% CrI for pooled study estimates as appropriate). The 95% CrI may be interpreted as an interval that has a 95% probability of capturing the true value of the unknown parameter given the observed data and the prior information.

We will also determine the predicted sensitivity and specificity of Xpert® and their 95% CrIs. The predicted values are our best guess for the sensitivity and specificity in a future study. They will be close to the pooled estimates. However, their CrIs may be different. If there is no heterogeneity at all between studies, the CrI around the predicted estimate will be the same as the CrI around the pooled estimate. On the other hand, if there is considerable heterogeneity between studies, the CrI around the predicted estimate will be much wider than the CI around the pooled estimate. This will be reflected in a much wider prediction region compared to the credible region on a bivariate meta-analysis plot.

In addition, in a secondary analysis for three forms of extrapulmonary TB (pleural TB, lymph node TB, and TB meningitis), we plan to adjust accuracy estimates by applying a latent class meta-analysis model to account for the imperfect nature of culture as the reference standard (Dendukuri 2012).

Latent class analysis is a statistical modelling technique that allows unbiased estimation of test accuracy in the absence of an adequate reference standard to define the presence or absence of disease (Van Smeden 2014). This model will expand the traditional model in two ways: (1) we will add parameters for the sensitivity and specificity of culture; and (2) we will add a conditional dependence term to adjust for the dependence between Xpert® MTB/RIF and culture among disease positive participants. Each study will contribute a 2 x 2 table to this model or three degrees of freedom. However, there are six unknown parameters at the study level: sensitivity and specificity of Xpert® MTB/RIF and culture, prevalence of extrapulmonary TB and the covariance between the two tests among extrapulmonary TB positive patients. The excess

of three unknown parameters over the number of degrees of freedom will render the model non-identifiable. Therefore, we will have to use an informative prior distribution over at least three parameters. The covariance term is bounded by the values of the sensitivities of the two tests. In addition, we plan to use informative prior distributions over the sensitivity and specificity of culture, obtained from latent class analyses of large cohort studies where data are available at the patient level and on more than two tests. For the analyses where we have an informative prior distribution for the sensitivity and specificity of culture, we will prepare plots of the prior and posterior density functions of the different parameters of interest in order to visually display the impact of the prior information on the posterior distribution.

Based on recent work evaluating Xpert® MTB/RIF for childhood TB (Schumacher 2016), we anticipate that these adjustments will lead to a lowering of the estimated pooled sensitivity of Xpert® MTB/RIF and an increase in the estimated pooled specificity of Xpert® MTB/RIF compared to the primary analyses.

#### Approach to non-interpretable index test results

For detection of extrapulmonary TB, we will define non-interpretable (invalid, error, no result) index results as a third category. If we find very few non-interpretable results reported, we will exclude non-interpretable results from the quantitative analysis and analyse them descriptively. Similarly, for detection of rifampicin resistance, we will define indeterminate index results as a third category. If we find very few non-interpretable results reported, we will exclude indeterminate results from the quantitative analysis

and analyse them descriptively. In the 'Discussion section' of the review, we will discuss the consequences of an indeterminate index test result considered to be a false negative result (may lead to missed or delayed diagnosis, with potential for increased morbidity, mortality, and TB transmission), or considered to be false positive result (may lead to unnecessary treatment with adverse effects and increased anxiety).

#### Investigations of heterogeneity

Initially, we will investigate heterogeneity through visual examination of forest plots of sensitivities and specificities and through visual examination of the ROC space of the raw data. If there are sufficient studies, we will assess heterogeneity through subgroup analyses or regression modelling. We include the prevalence of extrapulmonary TB as a covariate because changes in disease prevalence have often found to be associated with other, important changes such as changes in the disease spectrum, which may affect diagnostic accuracy estimates (Leeftang 2009). All covariates added to the model will be study level and categorical.

- Smear status (positive or negative).
- Percent HIV-positive individuals (> 10% or ≤ 10%).
- Past history of TB (> 30% or ≤ 30%).

- Condition of specimen (fresh or frozen).
- Sample input volume for body fluid specimens (> 2 mL or ≤ 2 mL).
- Homogenization step for tissue specimens (yes or no).
- WHO standard procedures for preparing specimen followed (yes or no).
- Prevalence of extrapulmonary TB (> 20% or ≤ 20%).
- Prevalence of rifampicin resistance (> 5% or ≤ 5%).
- For TB meningitis, concentration step used for preparing specimen (yes or no).

### Sensitivity analyses

If there are sufficient data, we will perform sensitivity analyses to explore the contribution of risk of bias and patient characteristics on Xpert® MTB/RIF accuracy by limiting inclusion in the meta-analysis to the following.

- Studies that used consecutive or random selection of participants.
- Studies where the reference standard results were interpreted without knowledge of the index test results.
- Studies that included only untreated patients.

### Assessment of reporting bias

We will not perform a formal assessment of publication bias using methods such as funnel plots or regression tests because such techniques have not been helpful for diagnostic test accuracy studies (Macaskill 2010).

### Assessment of the certainty of the evidence

Two review authors (MK and KRS) will assess the certainty of the evidence (also called quality of the evidence) using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (Schünemann 2008; Balshem 2011; GRADE 2013), and GRADEpro Guideline Development Tool (GDT) software (GRADEpro GDT 2015). In the context of a systematic review, the ratings of the certainty of the evidence reflect the extent of our confidence that the estimates of the effect (including test accuracy and associations) are correct. As recommended, we will rate the certainty of the evidence as either high (not downgraded), moderate (downgraded by one level), low (downgraded by two levels), or very low (downgraded by more than two levels) for five domains: risk of bias, indirectness, inconsistency, imprecision, and publication bias. For each outcome, we will consider the certainty of the evidence to begin as high when there are high quality observational studies (cross-sectional or cohort studies) that enrolled participants with diagnostic uncertainty. If we find a reason for downgrading, we will use our judgment to classify the reason as either serious (downgrade by one level) or very serious (downgrade by two levels). We will summarize this information in the 'Summary of findings' tables (Schünemann 2011).

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\* Indicates the major publication for the study

**ADDITIONAL TABLES****Table 1. Forms of extrapulmonary TB**

Form of extrapulmonary TB	Characteristics	Diagnostic specimens and means of collection
Lymph node TB, also called TB lymphadenitis	MTB infection of lymph nodes. May affect one node or a group of nodes, or multiple groups within a chain. Lymph node TB is relatively more common among children than adults. The most common presentation is of a single, firm, non-tender enlarged node in the neck, although any lymph node group can be affected. This may be accompanied by fever, weight loss, and night sweats, particularly in people with HIV. Patients with TB in deep lymph nodes, such as the mediastinal or mesenteric lymph nodes, may present with fever, night sweats, and weight loss, or more rarely with symptoms related to compression of adjacent structures. Over time lymph nodes become fluctuant and may discharge via a sinus to the skin or an adjacent viscus. It should be noted that lymphadenopathy may also be seen in other forms of TB as part of the immune response, but this is not usually caused by direct infection of the lymph nodes	Fine needle aspirate from affected lymph node, with or without radiological guidance; excisional biopsy of superficial lymph nodes; endoscopic biopsy of deep lymph nodes with ultrasound guidance

**Table 1. Forms of extrapulmonary TB** (Continued)

Pleural TB, also called TB pleurisy	MTB infection of the pleura presents with the gradual onset of pleuritic chest pain, shortness of breath, fever, night sweats, and weight loss. Chest X-ray may demonstrate unilateral or occasionally bilateral pleural effusion. The severity of symptoms is highly variable, with many patients experiencing spontaneous resolution of symptoms, while others may develop severe pleural effusions requiring drainage. Pleuropulmonary TB, where there is parenchymal lung involvement visible on a chest X-ray, is associated with higher mortality than isolated pleural infection, which appears to be rarely fatal (Shu 2011).	Pleural aspirate; pleural biopsy, which may be performed via thoracoscopy or percutaneously with an Abram's needle, with or without ultrasound guidance
TB meningitis, also called tuberculous meningitis	MTB infection of the meninges affects people of all ages, but is most common in children and people with untreated HIV infection. In adults, TB meningitis presents with the gradual onset of headache, neck stiffness, malaise, fever, and, if untreated, can progress to altered sensorium, focal neurological deficits, coma, and death. Young children may present with poor weight gain, low-grade fever, and listlessness. Infants, may present with fever, cough (related to the primary pulmonary infection which occurs before TB meningitis develops), change of consciousness at presentation, bulging anterior fontanel, and seizures (Thwaites 2013). TB meningitis is sometimes associated with a concurrent cerebral tuberculoma, or more rarely a tuberculous abscess	CSF, acquired by lumbar puncture with or without radiological guidance; biopsy of tuberculoma, acquired surgically
Bone and joint TB	MTB infection of the bones or joints or both causes chronic pain, deformity and disability, and TB of the cervical spine can be life-threatening. The usual presenting symptom is pain. Fever and weight loss, with or without signs of spinal cord compression, may be present. Patients with advanced disease may have severe pain, spinal deformity, paraspinal muscle wasting, and neurological deficit. Children may have failure to thrive and difficulty walking	Aspiration of joint fluid or periarticular abscesses; percutaneous computed tomography guided biopsy of lesions is preferred, but some patients may require open biopsy

**Table 1. Forms of extrapulmonary TB** (Continued)

Genitourinary TB	MTB infection of the genitourinary tract. Renal TB presents with flank pain, haematuria, and dysuria. Female genital TB presents with infertility (and may be otherwise asymptomatic), pelvic pain, and vaginal bleeding. Testicular TB presents with a scrotal mass and infertility	Urine; biopsy of affected organs, acquired under radiological guidance or surgically
Pericardial TB, also called TB pericarditis	MTB infection of the pericardium presents with fever, malaise, night sweats, and weight loss. Chest pain and shortness of breath are also commonly experienced symptoms. Pericardial TB may be associated with pericardial effusion, which can be severe and lead to tamponade, which is life-threatening. Some patients go on to develop pericardial constriction, which can lead to heart failure and death, and may require surgical intervention even after mycobacterial cure	Pericardial fluid acquired by pericardiocentesis; pericardial biopsy, acquired under radiological guidance or surgically
Peritoneal TB	MTB infection of the peritoneum. Peritoneal TB usually presents with pain and abdominal swelling, which may be accompanied by fever, weight loss, and anorexia	Ascitic fluid acquired by paracentesis; peritoneal biopsy ( <a href="#">Chow 2002</a> )
Disseminated TB, also called miliary TB. It has been proposed that the designation miliary TB be restricted to disseminated TB with miliary shadows on chest radiograph ( <a href="#">Reuter 2009</a> )	Disseminated TB refers to TB that involves two or more distinctly separate sites. Manifestations may be quite varied, ranging from acute fulminant disease to non-specific symptoms of fever, weight loss, and weakness. HIV-positive people are more likely to have disseminated TB than HIV-negative people. In a systematic review of the prevalence of TB in post-mortem evaluations of HIV-positive people, in adults disseminated TB was found in 87.9% (82.2% to 93.7%) of TB cases and considered the cause of death in 91.4% (95% CI 85.8% to 97.0%) of TB cases ( <a href="#">Gupta 2015</a> ).	Blood; specimens acquired from affected extrapulmonary sites

Abbreviations: CSF: cerebrospinal fluid; HIV: human immunodeficiency virus; MTB: *Mycobacterium tuberculosis*; TB: tuberculosis. We adapted the table from [Index-TB 2016](#).

**Table 2. Systematic reviews of Xpert® MTB/RIF for extrapulmonary TB**

Systematic review	Search period	Number of studies (total number of extrapulmonary specimens)	Forms of extrapulmonary TB or specimens	Accuracy against culture reference standard		
				Lymph node TB	Pleural TB (pleural fluid)	TB meningitis
<a href="#">Chang 2012</a> <sup>1</sup>	Up to 1 October 2011	7 (1058)	Multiple forms combined	Not reported	Not reported	Not reported
<a href="#">Denkinger 2014</a>	Up to 15 October 2013	18 (4461)	Lymph node, pleural fluid, CSF	Sensitivity 83%; specificity 94%	Sensitivity 46%; specificity 99%	Sensitivity 81%; specificity 98%
<a href="#">Maynard-Smith 2014</a>	Up to 6 November 2013	27 (6026)	Lymph node, pleural fluid, CSF, other forms	Sensitivity 96%; specificity 93%	Sensitivity 34%; specificity 98%	Median sensitivity 85% (IQR 75% to 100%); median specificity 100% (IQR 98% to 100%)
<a href="#">Penz 2015</a>	Up to 15 August 2014	36 (9523)	Lymph node, pleural fluid, CSF, other forms	Sensitivity 87%; specificity 92%	Sensitivity 37%; specificity 98%	Sensitivity 69%; specificity 97%
<a href="#">Sehgal 2016</a>	Up to 31 August 2015	24 (2486)	Pleural fluid	Not applicable	Sensitivity 51%; specificity 99%	Not applicable
<a href="#">Li 2017</a> <sup>2</sup>	Up to 20 June 2015	26 (Not reported)	Multiple forms combined	Not reported	Not reported	Not reported

Abbreviations: CI: confidence interval; CSF: cerebrospinal fluid; IQR: interquartile range; TB: tuberculosis.

<sup>1</sup>For all forms of extrapulmonary TB combined, [Chang 2012](#) reported pooled sensitivity and specificity of 80.4% (95% confidence interval (CI) 75.0% to 85.1%) and 86.1% (95% CI 83.5% to 88.4%), respectively.

<sup>2</sup>For both pulmonary and extrapulmonary TB, the authors included 106 studies involving 52,410 samples. For all forms of extrapulmonary TB combined, [Li 2017](#) reported pooled sensitivity and specificity of 80% (95% CI 69% to 88%) and 97% (95% CI 94% to 98%), respectively.

## APPENDICES

### Appendix 1. Search strategy

- 1 Mycobacterium tuberculosis/
- 2 Tuberculosis/ or “Tuberculosis, Multidrug-Resistant”/ or Extensively Drug-Resistant Tuberculosis/
- 3 (Tuberculosis or MDR-TB or XDR-TB or “Multidrug Resistant Tuberculosis” or “Extensively Drug Resistant Tuberculosis” or tuberculous).ti. ab .
- 4 (extrapulmonary or extra-pulmonary or EPTB).ti. ab .
- 5 (lymphadenitis or disseminated or miliary or pleur\* or skeletal or spine or mening\* or intracranial or intra-ocular or ocular or abdominal or splenic or genitourinary or pericardial).ti. ab .
- 6 “Tuberculosis, Central Nervous System”/ or “Tuberculosis, Urogenital”/ or “Tuberculosis, Splenic”/ or “Tuberculosis, Spinal”/ or “Tuberculosis, Renal”/ or “Tuberculosis, Pleural”/ or “Tuberculosis, Osteoarticular”/ or “Tuberculosis, Oral”/ or “Tuberculosis, Ocular”/ or “Tuberculosis, Meningeal”/ or “Tuberculosis, Lymph Node”/ or “Tuberculosis, Laryngeal”/ or “Tuberculosis, Hepatic”/ or “Tuberculosis, Gastrointestinal”/ or “Tuberculosis, Female Genital”/ or “Tuberculosis, Endocrine”/ or “Tuberculosis, Cutaneous”/ or “Tuberculosis, Cardiovascular”/ or Tuberculosis, Miliary/ or Tuberculosis, Male Genital/
- 7 1 or 2 or 3
- 8 4 or 5
- 9 7 and 8
- 10 9 or 6
- 11 Xpert\*.ti. ab .
- 12 (GeneXpert or cepheid).ti.ab .
- 13 (near\* patient or near-patient).ti.ab
- 14 11 or 12 or 13
- 15 10 and 14

This is the preliminary search strategy for MEDLINE (OVID). It will be adapted for other electronic databases. We will report all search strategies in full in the final version of the review.

### Appendix 2. Rules for QUADAS-2

#### Domain 1: Patient selection

##### Risk of bias: Could the selection of patients have introduced bias?

##### Signalling question 1: Was a consecutive or random sample of patients enrolled?

We will score “yes” if the study enrolled a consecutive or random sample of eligible patients, “no” if the study selected patients by convenience, and “unclear” if the study did not report the manner of patient selection or we cannot tell.

##### Signalling question 2: Was a case-control design avoided?

We will not include studies using a case-control design in the review because this study design, especially when used to compare results in severely ill patients with those in relatively healthy individuals, may lead to overestimation of accuracy in diagnostic studies. We will score “yes” for all studies.

**Signalling question 3: Did the study avoid inappropriate exclusions?**

We will score “yes” if the study included specimens regardless of prior testing and appearance. We will judge “no” if the study excluded specimens based on the results of prior testing, appearance of the specimen (for example, purulence), other biochemical analysis (for example, ADA, cell analysis) or smear status. For example, if specimens were excluded based on microscopic examination or histological appearance, we will score “no”. We will score “unclear” if we cannot tell.

**Applicability: Are there concerns that the included patients and setting do not match the review question?**

We are interested in how Xpert® MTB/RIF performs in patients presumed to have extrapulmonary TB whose specimens were evaluated as they would be in routine practice. We will score “low concern” if Xpert® MTB/RIF was evaluated in district hospitals or primary health clinics and “high concern” if Xpert® MTB/RIF was evaluated in central (reference) laboratories or tertiary care centres. We will judge applicability to be of “unclear concern” if we cannot tell the level of the health system that ran the Xpert® MTB/RIF assay.

**Domain 2: Index test****Risk of bias: Could the conduct or interpretation of the index test have introduced bias?****Signalling question 1: Were the index test results interpreted without knowledge of the results of the reference standard?**

We will answer this question “yes” for all studies because Xpert® MTB/RIF test results are automatically generated and the user is provided with printable test results. Thus, there is no room for subjective interpretation of test results.

**Signalling question 2: If a threshold was used, was it prespecified?**

As, the threshold is prespecified in all versions of Xpert® MTB/RIF, we will answer this question “yes” for all studies.

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

We note that variations in the execution of the test might affect accuracy estimates. We will judge “low concern” if specimens were unprocessed and the index test was performed as recommended by the manufacturer for sputum. In addition, we will also judge “low concern” if the test was performed according to WHO standard operating procedures (WHO 2014a). We will score ‘high concern’ if the test was performed in a way that deviates from these recommendations, for example, by adding a mechanical homogenization step, because it is unclear what duration of homogenization would be sufficient and, as well, we would not be able to tell what the final specimen input volume would be. We will score “unclear concern” if we cannot tell.

**Domain 3: Reference standard****Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?**

We will consider this domain separately for the reference standard for detection of extrapulmonary TB and the reference standard for detection of rifampicin resistance.

**Signalling question 1: Is the reference standard likely to correctly classify the target condition?*****For detection of extrapulmonary TB***

Culture is considered the best reference standard for TB. For the diagnosis of all forms of extrapulmonary TB (except as noted for pleural TB below) culture is a criterion for inclusion in the review. Therefore we will score “yes” for all studies. However, there are limitations

associated with culture; bacillary load is usually low in extrapulmonary TB leading to a reduction in the sensitivity of culture. We will discuss this further in the 'Accuracy of reference standards used' section.

#### ***For detection of pleural TB***

The use of culture or a composite reference standard are criteria for inclusion in the review. We will answer this question "yes" for all studies of pleural TB.

#### ***For detection of rifampicin resistance***

Culture-based drug susceptibility testing (DST, also called conventional phenotypic method) is considered to be the best reference standard. MTBDR<sub>plus</sub> is also a WHO-endorsed test for rifampicin resistance. We will answer this question "yes" for all studies using culture-based DST or MTBDR<sub>plus</sub>. We will judge studies that do not use culture-based DST or MTBDR<sub>plus</sub> as "no" and judge "unclear" if we cannot tell.

#### **Signalling question 2: (TB) Were the reference standard results interpreted without knowledge of the results of the index test?**

We will score "yes" if the reference test provides an automated result (for example, MGIT 960), blinding was explicitly stated, or it was clear that the reference standard was performed at a separate laboratory and/or performed by different people. We will score "no" if the study stated that the reference standard result was interpreted with knowledge of the Xpert® MTB/RIF test result. We will score "unclear" if we cannot tell.

#### **Signalling question 3: (Rifampicin resistance) We will add a signalling question for rifampicin resistance because judgments might differ for TB detection and for rifampicin resistance detection, the two target conditions.**

#### ***Were the reference standard results interpreted without knowledge of the results of the index test?***

We will score "yes" if the reference test provided an automated result (for example, MGIT 960), blinding was explicitly stated, or it was clear that the reference standard was performed at a separate laboratory or performed by different people, or both. We will score "no" if the study stated that the reference standard result was interpreted with knowledge of the Xpert® MTB/RIF test result. We will score "unclear" if we cannot tell.

#### ***Applicability: Are there concerns that the target condition as defined by the reference standard does not match the question?***

We will judge "high concern" if included studies did not speciate mycobacteria isolated in culture, "low concern" if speciation was performed, and "unclear concern" if we could not tell.

### **Domain 4: Flow and timing**

#### **Risk of bias: Could the patient flow have introduced bias?**

#### **Signalling question 1: Was there an appropriate interval between the index test and reference standard?**

In most included studies, we expect that specimens for Xpert® MTB/RIF and culture will be obtained at the same time when patients were evaluated for presumed TB. However, even if there were a delay of several days or weeks between index test and reference standard, TB is a chronic disease and we consider misclassification of disease status to be unlikely, as long as treatment was not initiated in the



interim. We will judge “yes” if the index test and reference standard were performed at the same time or if the time interval is less than or equal to 30 days, “no” if the time interval is greater than 30 days, and “unclear” if we cannot tell.

**Signalling question 2: Did all patients receive the same reference standard?**

For the diagnosis of any form of extrapulmonary TB, except pleural TB, we will answer this question “yes” if all participants in the study or a subset of participants in the study received the acceptable reference standard (solid culture, liquid culture, or both), which we specified as a criterion for inclusion in the review. However, we acknowledge that it is possible that some specimens could undergo solid culture and others liquid culture. This could potentially result in variations in accuracy, but we think the variation will be minimal. For the diagnosis of pleural TB as measured against a composite reference standard, we will answer this question “yes” if all participants received the same reference standard, “no” if not all participants received the same reference standard, and “unclear” if we cannot tell. For rifampicin resistance detection, we will answer “yes” if all participants received the same reference standard (either culture-based DST or MTBDR*plus*), “no” if not all participants received the same reference standard, and “unclear” if we cannot tell.

**Signalling question 3: Were all patients included in the analysis?**

We will determine the answer to this question by comparing the number of patients enrolled with the number of patients included in the 2 x 2 tables. We will answer “yes” if the numbers matched and “no” if there were patients enrolled in the study that were not included in the analysis. We will answer “unclear” if we cannot tell.

**Judgements for overall Risk of bias’ assessments**

- If we answer all signalling questions for a domain “yes” then we will judge risk of bias as “low”.
- If we answer all or most signalling questions for a domain “no”, then we will judge risk of bias as “high”.
- If we answer only one signalling question for a domain “no”, we will discuss with a third review author the “Risk of bias” judgement.
- If we answer all or most signalling questions for a domain “unclear”, then we will judge risk of bias as “unclear”.
- If we answer only one signalling question for a domain “unclear”, we will discuss with a third review author the “Risk of bias” judgement for the domain.

**Appendix 3. Data extraction form**

Data extractor	MK KRS
First author	
Corresponding author and email	
Title of paper	
Journal	
Language if other than English	
Year	

**I. Study details**

Type of study: Randomized controlled trial Cross-sectional cohort (with follow-up) Case-control (exclude) Unclear/not reported

Study data collection: Prospective Retrospective Unclear/not reported  
Participant selection: Convenience Consecutive Random Other Unclear/not reported  
Country:  
Country income status: Low Middle High

## II. Presenting signs and symptoms, setting

Presenting signs and symptoms?

Clinical setting: Inpatient Outpatient Both Unclear/Not reported

Level of laboratory running Xpert? Peripheral Intermediate Central (reference)

Comments, describe exclusions

*(Tests at laboratory levels)*

*Peripheral: AFB (Ziehl-Neelsen, Auramine-rhodamine, Auramine-O staining) and Xpert MTB/RIF*

*Intermediate: Peripheral laboratory tests and culture on solid media and line probe assay (LPA) from smear positive sputum*

*Central: Intermediate laboratory tests and culture on liquid media and DST (1<sup>st</sup> and 2<sup>nd</sup> line anti-TB drugs) on solid or in liquid media and LPA on positive cultures and rapid speciation tests*

## III. Other demographics

HIV patients included? Yes No Unclear/not reported; if yes ## and percentage? *(Denominator is number tested, when possible)*

Age? Median age in years (IQR); mean (SD); range Unclear/not reported

Children (< 15 years old) included: Yes No Unclear/not reported; if yes, percentage?

Percentage female included? Unclear/not reported

Past history of TB? Yes No Unclear/not reported; if yes, percentage?

Only patients who received TB treatment for  $\leq 7$  days were included? Yes No Unclear/not reported; if no, percentage on treatment included?

## IV. Reference standard

### A. Reference standard for TB detection

Solid culture (specify): LJ 7H10 7H11 Other

Liquid culture (specify): MGIT Bactec 460 Other

Solid and liquid culture (indicate which kind above)

Were reference standard results interpreted without knowledge of index test results? Yes No Unclear/not reported

### B. Composite reference standard for pleural TB

Solid culture (specify): LJ 7H10 7H11 Other

Liquid culture (specify): MGIT Bactec 460 Other

Solid and liquid culture (indicate which kind above)

Histopathology (specify): Granulomas Caseating granulomas

Were reference standard results interpreted without knowledge of index test results? Yes No Unclear/not reported

Did all patients receive the same reference standard? Yes No Unclear/not reported, if no, describe

### C. Reference standard for rifampicin resistance

LJ DST MGIT DST MTBDR<sub>plus</sub>

Were reference standard results interpreted without knowledge of index test results? Yes No Unclear/not reported

## V. Sites with > five specimens (check all that apply)

- A. Lymph node TB fluid tissue both fluid and tissue
- B. Pleural TB fluid tissue both fluid and tissue
- C. TB meningitis CSF
- D. Bone and joint TB fluid tissue both fluid and tissue
- E. Genitourinary TB urine other, specify
- F. Peritoneal TB fluid tissue both fluid and tissue
- G. Pericardial TB fluid tissue both fluid and tissue
- H. Disseminated TB blood
- I. Other, specify

## VI. Specimen processing

Condition of specimens: fresh frozen

*If frozen for > 7 days, indicate WHO not followed*

For a given site, how many specimens were collected per patient? one multiple Unclear/not reported

### A. Lymph node tissue, other tissue

Was the WHO standard operating procedure (SOP) followed for each specimen type?

- 1a. Lymph node tissue WHO followed: Yes No Unclear
  - 1b. Lymph node tissue homogenisation step for tissue specimens: Yes No Unclear/not reported
  - 2a. Other tissue, specify WHO followed: Yes No Unclear
  - 2b. Other tissue homogenisation step for tissue specimens: Yes No Unclear/not reported
- (For tissue, if the WHO SOP not followed, briefly describe specimen processing in comments)*

### WHO SOPs for specimen processing, lymph node and other tissue, sterile specimen

1. *Cut the tissue specimen into small pieces in a sterile mortar.*
2. *Add approximately 2 mL of sterile phosphate buffered saline (PBS).*
3. *Grind solution of tissue and PBS until homogeneous suspension has been obtained.*
4. *Place approximately 0.7 mL of the homogenized tissue in a sterile, conical screw-capped tube.*
5. *Double volume of specimen with Xpert® Sample Reagent (1.4 mL Sample Reagent to 0.7 mL of homogenized tissue).*
6. *Shake tube vigorously 10 to 20 times or vortex for at least 10 seconds.*
7. *Incubate specimen for 10 minutes at room temperature, and again shake specimen 10-20 times or vortex for at least 10 seconds.*
8. *Incubate specimen at room temp. for an additional 5 minutes.*
9. *Transfer 2mL to Xpert® MTB/RIF cartridge*
10. *Load into GeneXpert and per manufacturer's instructions*

(Note: For specimens **not collected in a sterile manner**, the WHO SOP suggests a NaOH decontamination/concentration protocol similar to that used for sputum)

### B. CSF

- 3a. CSF WHO followed: Yes No Unclear
  - 3b. CSF concentration step: Yes No Unclear/not reported
  - 3c. CSF sample input volume specify, Unclear/not reported
- (For CSF, if WHO SOP not followed, briefly describe specimen processing in comments)*

### WHO SOPs for CSF

*If there is more than 5 mL of CSF available for testing*

1. Transfer all of the CSF specimen to a conical centrifuge tube and concentrate the specimen at 3000 x g for 15 minutes.
2. Resuspend the pellet to a final volume of 2 mL by adding Xpert® MTB/RIF Sample Reagent.
3. Transfer 2 mL of the resuspended CSF sample to the Xpert® MTB/RIF cartridge.
4. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

***If there is 1 mL to 5 mL of CSF available***

1. Add an equal volume of Sample Reagent to the CSF.
2. Mix the specimen and the Sample Reagent by vortexing as described above. After 7 to 8 minutes at room temperature, vortex the sample as above a second time.
3. Incubate for an additional 7 to 8 minutes (15 minutes total incubation) at room temp
4. Add 2 mL of the sample mixture directly to the Xpert® MTB/RIF cartridge.
5. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

**C. Body fluids, other than CSF**

4a. Body fluid specify, processed as per manufacturer for sputum

Yes No Unclear

4b. Body fluid specify, Sample input volume specify, Unclear/not reported

5a. Body fluid specify, processed as per manufacturer for sputum (WHO followed)

Yes No Unclear

5b. Body fluid specify, sample input volume, specify, Unclear/not reported

*(Add additional specimens as needed)*

*(For body fluids other than CSF, if manufacturer's instructions not followed, briefly describe specimen processing in comments)*

**Manufacturer's instructions for sputum**

***Raw specimen***

1. Pour or pipette (pipette not provided) approximately 2 times the volume of the Sample Reagent into the specimen (2:1 dilution, Sample Reagent: specimen).
2. Shake vigorously 10 to 20 times or vortex for at least 10 seconds.
3. Incubate sample for a total of 15 minutes at 20°C to 30°C.
4. Between 5 and 10 minutes into the incubation period, shake vigorously 10 to 20 times or vortex for at least 10 seconds.

***Specimen sediment***

*Assay requires at least 0.5 mL of resuspended specimen sediment after digestion, decontamination, and concentration.*

1. Use the method of Kent and Kubica and resuspend the sediment in a 67 mM phosphate/H<sub>2</sub>O buffer.
2. After resuspension, keep at least 0.5 mL of the resuspended sediment for the Xpert® MTB/RIF assay.
3. Add 1.5 mL of Sample Reagent to 0.5 mL of resuspended sediment (3:1 dilution, Sample Reagent: specimen)
4. Follow steps 2 to 4 above.

Comments on specimen processing:

**VII. Results**

TB detection: number error or invalid or both Xpert® MTB/RIF results over total number of cultures performed. The denominator includes contaminated cultures and cultures that were uninterpretable.

Unclear/not reported

RIF resistance: number indeterminate Xpert results (over total number of cultures performed)

Unclear/not reported

Nontuberculous mycobacteria (NTM): number of cultures with NTM (over total number of cultures performed)

Unclear/not reported

### VIII. Tables

*(Nontuberculous mycobacteria (NTM) should be included as not TB)*

**TB detection (example of table; add additional tables by site of extrapulmonary disease)**

TB detection, studies on lymph node fluid with CULTURE standard		Definite TB		
		Yes	No	Total
Xpert® MTB/RIF result	Positive			
	Negative			
	Total			
	Error/invalid			

**By smear status (extrapulmonary specimens)**

TB detection, microscopy smear positive		Definite TB		
		Yes	No	Total
Xpert® MTB/RIF result	Positive			
	Negative			
	Total			
	Error/invalid			

TB detection, microscopy smear negative		Definite TB		
		Yes	No	Total
Xpert® MTB/RIF result	Positive			
	Negative			

(Continued)

	<b>Total</b>			
	<b>Error/invalid</b>			

**Rifampicin resistance detection (for all culture positive, extrapulmonary specimens)**

RIF resistance detection		RIF resistance		
		Yes	No	Total
Xpert® MTB/RIF result	Positive			
	Negative			
	Total			
	Indeterminate			

## CONTRIBUTIONS OF AUTHORS

MK, HR, and KRS wrote early drafts of the protocol. MK designed the data extraction form. KD contributed clinical expertise. CMD and SGS tailored QUADAS-2 to the review. ND wrote the statistical analysis and data synthesis section with contributions from IS and KRS. All review authors contributed to the final manuscript.

## DECLARATIONS OF INTEREST

We have no financial involvement with any organization or entity with a financial interest in, or financial conflict with, the subject matter or materials discussed in the review apart from those disclosed.

CMD and SGS are employed by FIND, Geneva. FIND is a non-for-profit foundation, whose mission is to find diagnostic solutions to overcome diseases of poverty in low- and middle-income countries. FIND has received funding from Cepheid to evaluate Xpert® MTB/RIF. FIND affirms no undue influences in its work or the publication of its findings.

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