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Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

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TABLE OF CONTENTS

ABSTRACT	1
PLAIN LANGUAGE SUMMARY	3
SUMMARY OF FINDINGS	5
BACKGROUND	9
Figure 1.	11
OBJECTIVES	13
METHODS	13
RESULTS	17
Figure 2.	18
Figure 3.	19
Figure 4.	20
Figure 5.	22
Figure 6.	24
Figure 7.	27
Figure 8.	28
Figure 9.	29
DISCUSSION	29
AUTHORS' CONCLUSIONS	32
ACKNOWLEDGEMENTS	33
REFERENCES	34
CHARACTERISTICS OF STUDIES	43
ADDITIONAL TABLES	70
WHAT'S NEW	77
HISTORY	77
CONTRIBUTIONS OF AUTHORS	77
DECLARATIONS OF INTEREST	77
SOURCES OF SUPPORT	78
DIFFERENCES BETWEEN PROTOCOL AND REVIEW	78
INDEX TERMS	79

[Diagnostic Test Accuracy Review]

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children

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ABSTRACT

Background

Every year, an estimated one million children and young adolescents become ill with tuberculosis, and around 226,000 of those children die. Xpert MTB/RIF Ultra (Xpert Ultra) is a molecular World Health Organization (WHO)-recommended rapid diagnostic test that simultaneously detects *Mycobacterium tuberculosis* complex and rifampicin resistance. We previously published a Cochrane Review 'Xpert MTB/RIF and Xpert MTB/RIF Ultra assays for tuberculosis disease and rifampicin resistance in children'. The current review updates evidence on the diagnostic accuracy of Xpert Ultra in children presumed to have tuberculosis disease. Parts of this review update informed the 2022 WHO updated guidance on management of tuberculosis in children and adolescents.

Objectives

To assess the diagnostic accuracy of Xpert Ultra for detecting: pulmonary tuberculosis, tuberculous meningitis, lymph node tuberculosis, and rifampicin resistance, in children with presumed tuberculosis.

Secondary objectives

To investigate potential sources of heterogeneity in accuracy estimates. For detection of tuberculosis, we considered age, comorbidity (HIV, severe pneumonia, and severe malnutrition), and specimen type as potential sources.

To summarize the frequency of Xpert Ultra trace results.

Search methods

We searched the Cochrane Infectious Diseases Group Specialized Register, MEDLINE, Embase, three other databases, and three trial registers without language restrictions to 9 March 2021.

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

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Selection criteria

Cross-sectional and cohort studies and randomized trials that evaluated Xpert Ultra in HIV-positive and HIV-negative children under 15 years of age. We included ongoing studies that helped us address the review objectives. We included studies evaluating sputum, gastric, stool, or nasopharyngeal specimens (pulmonary tuberculosis), cerebrospinal fluid (tuberculous meningitis), and fine needle aspirate or surgical biopsy tissue (lymph node tuberculosis). For detecting tuberculosis, reference standards were microbiological (culture) or composite reference standard; for stool, we also included Xpert Ultra performed on a routine respiratory specimen. For detecting rifampicin resistance, reference standards were drug susceptibility testing or MTBDR_{plus}.

Data collection and analysis

Two review authors independently extracted data and, using QUADAS-2, assessed methodological quality judging risk of bias separately for each target condition and reference standard. For each target condition, we used the bivariate model to estimate summary sensitivity and specificity with 95% confidence intervals (CIs). We stratified all analyses by type of reference standard. We summarized the frequency of Xpert Ultra trace results; trace represents detection of a very low quantity of *Mycobacterium tuberculosis* DNA. We assessed certainty of evidence using GRADE.

Main results

We identified 14 studies (11 new studies since the previous review). For detection of pulmonary tuberculosis, 335 data sets (25,937 participants) were available for analysis. We did not identify any studies that evaluated Xpert Ultra accuracy for tuberculous meningitis or lymph node tuberculosis. Three studies evaluated Xpert Ultra for detection of rifampicin resistance. Ten studies (71%) took place in countries with a high tuberculosis burden based on WHO classification. Overall, risk of bias was low.

Detection of pulmonary tuberculosis

Sputum, 5 studies

Xpert Ultra summary sensitivity verified by culture was 75.3% (95% CI 64.3 to 83.8; 127 participants; high-certainty evidence), and specificity was 97.1% (95% CI 94.7 to 98.5; 1054 participants; high-certainty evidence).

Gastric aspirate, 7 studies

Xpert Ultra summary sensitivity verified by culture was 70.4% (95% CI 53.9 to 82.9; 120 participants; moderate-certainty evidence), and specificity was 94.1% (95% CI 84.8 to 97.8; 870 participants; moderate-certainty evidence).

Stool, 6 studies

Xpert Ultra summary sensitivity verified by culture was 56.1% (95% CI 39.1 to 71.7; 200 participants; moderate-certainty evidence), and specificity was 98.0% (95% CI 93.3 to 99.4; 1232 participants; high certainty-evidence).

Nasopharyngeal aspirate, 4 studies

Xpert Ultra summary sensitivity verified by culture was 43.7% (95% CI 26.7 to 62.2; 46 participants; very low-certainty evidence), and specificity was 97.5% (95% CI 93.6 to 99.0; 489 participants; high-certainty evidence).

Xpert Ultra sensitivity was lower against a composite than a culture reference standard for all specimen types other than nasopharyngeal aspirate, while specificity was similar against both reference standards.

Interpretation of results

In theory, for a population of 1000 children:

- where 100 have pulmonary tuberculosis in sputum (by culture):
 - 101 would be Xpert Ultra-positive, and of these, 26 (26%) would not have pulmonary tuberculosis (false positive); and
 - 899 would be Xpert Ultra-negative, and of these, 25 (3%) would have tuberculosis (false negative).
- where 100 have pulmonary tuberculosis in gastric aspirate (by culture):
 - 123 would be Xpert Ultra-positive, and of these, 53 (43%) would not have pulmonary tuberculosis (false positive); and
 - 877 would be Xpert Ultra-negative, and of these, 30 (3%) would have tuberculosis (false negative).
- where 100 have pulmonary tuberculosis in stool (by culture):
 - 74 would be Xpert Ultra-positive, and of these, 18 (24%) would not have pulmonary tuberculosis (false positive); and
 - 926 would be Xpert Ultra-negative, and of these, 44 (5%) would have tuberculosis (false negative).

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

- where 100 have pulmonary tuberculosis in nasopharyngeal aspirate (by culture):
- 66 would be Xpert Ultra-positive, and of these, 22 (33%) would not have pulmonary tuberculosis (false positive); and
- 934 would be Xpert Ultra-negative, and of these, 56 (6%) would have tuberculosis (false negative).

Detection of rifampicin resistance

Xpert Ultra sensitivity was 100% (3 studies, 3 participants; very low-certainty evidence), and specificity range was 97% to 100% (3 studies, 128 participants; low-certainty evidence).

Trace results

Xpert Ultra trace results, regarded as positive in children by WHO standards, were common. Xpert Ultra specificity remained high in children, despite the frequency of trace results.

Authors' conclusions

We found Xpert Ultra sensitivity to vary by specimen type, with sputum having the highest sensitivity, followed by gastric aspirate and stool. Nasopharyngeal aspirate had the lowest sensitivity. Xpert Ultra specificity was high against both microbiological and composite reference standards. However, the evidence base is still limited, and findings may be imprecise and vary by study setting. Although we found Xpert Ultra accurate for detection of rifampicin resistance, results were based on a very small number of studies that included only three children with rifampicin resistance. Therefore, findings should be interpreted with caution. Our findings provide support for the use of Xpert Ultra as an initial rapid molecular diagnostic in children being evaluated for tuberculosis.

PLAIN LANGUAGE SUMMARY

Xpert Ultra for diagnosing tuberculosis and rifampicin resistance in children

Why is improving the diagnosis of tuberculosis important?

Every year, an estimated one million children and young adolescents become ill with tuberculosis, and around 226,000 die from the disease. Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis* and mostly affects the lungs (pulmonary tuberculosis), though it can affect other sites in the body (extrapulmonary tuberculosis). Signs and symptoms of pulmonary tuberculosis include cough, fever, night sweats, and weight loss. Signs and symptoms of extrapulmonary tuberculosis depend on the site of disease. When detected early and treated effectively, tuberculosis is largely curable.

Not recognizing tuberculosis (false negative) early may result in delayed diagnosis and treatment, severe illness, and death. An incorrect tuberculosis diagnosis (false positive) may result in anxiety, unnecessary treatment (which can involve medication side effects), and the possibility of missing alternative diagnoses which warrant treatment.

What was the aim of this review?

To determine the accuracy of Xpert Ultra in children with symptoms of tuberculosis for diagnosing pulmonary tuberculosis, tuberculous meningitis (affecting membranes that surround the brain and spinal cord), lymph node tuberculosis (a painful swelling of one or more lymph nodes, which are bean-shaped structures that help fight infection), and rifampicin resistance.

What did this review study?

Xpert Ultra, a World Health Organization-recommended rapid test that simultaneously detects tuberculosis and rifampicin resistance in adults and children with tuberculosis symptoms. Rifampicin is an important medicine used to treat tuberculosis. For tuberculosis diagnosis, we assessed results against two different benchmarks: tuberculosis culture (a method used to grow bacteria on nutrient-rich media) and a composite definition based on symptoms, chest X-ray, sputum microscopy (examination under a microscope of mucus and other matter coughed up from the lungs), and culture. For rifampicin resistance detection, we assessed results against drug susceptibility testing or line probe assay (a rapid laboratory-based test for detecting tuberculosis bacteria).

What were the main results in this review?

We included 14 studies. For pulmonary tuberculosis, we analysed 335 data sets (around 26,000 participants). No studies evaluated Xpert Ultra accuracy for tuberculous meningitis or lymph node tuberculosis. Three studies evaluated Xpert Ultra accuracy for detection of rifampicin resistance.

For a population of 1000 children:

- where 100 have pulmonary tuberculosis in sputum according to culture results:
- 101 would be Xpert Ultra-positive, and of these, 26 (26%) would not have pulmonary tuberculosis (false positive); and

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

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- 899 would be Xpert Ultra-negative, and of these, 25 (3%) would have tuberculosis (false negative).
- where 100 have pulmonary tuberculosis in gastric aspirate (collection of lung and oral secretions from the stomach) according to culture results:
 - 97 would be Xpert Ultra-positive, and of these, 27 (28%) would not have pulmonary tuberculosis (false positive); and
 - 903 would be Xpert Ultra-negative, and of these, 30 (3%) would have tuberculosis (false negative).
- where 100 have pulmonary tuberculosis in stool according to culture results:
 - 74 would be Xpert Ultra-positive and of these, 18 (24%) would not have pulmonary tuberculosis (false positive); and
 - 926 would be Xpert Ultra-negative, and of these, 44 (5%) would have tuberculosis (false negative).
- where 100 have pulmonary tuberculosis in nasopharyngeal aspirate (secretions from the uppermost part of the throat, behind the nose) according to culture results:
 - 66 would be Xpert Ultra-positive, and of these, 22 (33%) would not have pulmonary tuberculosis (false positive); and
 - 934 would be Xpert Ultra-negative, and of these, 56 (6%) would have tuberculosis (false negative).

Xpert Ultra accurately detected rifampicin resistance, but there were few studies and only three children with rifampicin resistance included.

How confident are we in the results of this review?

For pulmonary tuberculosis, we are fairly confident because we included studies from different countries and used two different benchmarks, though neither is perfect. However, the evidence base is still limited and there were few studies with few children for one of the specimen types (nasopharyngeal aspirate).

For rifampicin resistance, we identified few studies with very few children with rifampicin resistance, so we are less confident.

What children do the results of this review apply to?

Children and young adolescents (birth to 14 years) who are HIV-positive or HIV-negative, with signs or symptoms of pulmonary tuberculosis. The results also apply to children with severe pneumonia or malnutrition and tuberculosis symptoms. In this review, we did not identify any studies that evaluated Xpert Ultra accuracy for tuberculous meningitis or lymph node tuberculosis.

What are the implications of this review?

The results suggest that Xpert Ultra in sputum, gastric aspirate, stool, and nasopharyngeal aspirate is an accurate method for detecting pulmonary tuberculosis and rifampicin resistance in children.

Using Xpert Ultra in sputum, gastric aspirate, stool, and nasopharyngeal aspirate, the risk of missing a diagnosis of pulmonary tuberculosis (confirmed by culture) is low, suggesting that only a small number of children will not receive that treatment. The risk of incorrectly diagnosing a child as having pulmonary tuberculosis is slightly higher. This may result in some children receiving unnecessary treatment.

How up to date is this review?

This review updates our previous review and includes evidence published up to 9 March 2021.

SUMMARY OF FINDINGS

Summary of findings 1. Xpert Ultra for pulmonary tuberculosis in children^a

Review question: what is the diagnostic accuracy of Xpert Ultra for pulmonary tuberculosis in children with signs and symptoms of pulmonary tuberculosis?

Patients/population: children with presumed pulmonary tuberculosis

Index tests: Xpert Ultra

Role: an initial test

Threshold for index tests: an automated result is provided

Reference standard: culture

Types of studies: cross-sectional and cohort studies

Setting: primary care facilities and local hospitals

Specimen	Effect (95% CI)	Number of participants (studies)	Test result	Number of results per 1000 patients tested(95% CI) ^b			Certainty of the evidence (GRADE)
				Prevalence 1%	Prevalence 10%	Prevalence 20%	
Sputum	Summary sensitivity 75.3% (64.3 to 83.8)	127 (5)	True positive	8 (6 to 8)	75 (64 to 84)	151 (129 to 168)	⊕⊕⊕⊕ High
			False negative	2 (2 to 4)	25 (16 to 36)	49 (32 to 71)	
	Summary specificity 97.1% (94.7 to 98.5)	1054 (5)	True negative	961 (938 to 975)	874 (852 to 887)	777 (758 to 788)	⊕⊕⊕⊕ High
			False positive	29 (15 to 52)	26 (13 to 48)	23 (12 to 42)	
Gastric aspirate	Summary sensitivity 70.4% (53.9 to 82.9)	120 (7)	True positive	7 (5 to 8)	70 (54 to 83)	141 (108)	⊕⊕⊕⊕ Moderate^c
			False negative	3	30	59	

				(2 to 5)	(17 to 46)	(34 to 92)	
	Summary specificity 94.1% (84.8 to 97.8)	870 (7)	True negative	932 (840 to 968)	847 (763 to 880)	753 (678 to 782)	⊕⊕⊕⊕ Moderate^d
			False positive	58 (22 to 150)	53 (20 to 137)	47 (18 to 122)	
Stool	Summary sensitivity 56.1% (39.1 to 71.7)	200 (6)	True positive	6 (4 to 7)	56 (39 to 72)	112 (78 to 143)	⊕⊕⊕⊕ Moderate^c
			False negative	4 (3 to 6)	44 (28 to 61)	88 (57 to 122)	
	Summary specificity 98.0% (93.3 to 99.4)	1232 (6)	True negative	970 (924 to 984)	882 (840 to 895)	784 (746 to 795)	⊕⊕⊕⊕ High
			False positive	20 (6 to 66)	18 (5 to 60)	16 (5 to 54)	
Nasopharyn-geal aspirate	Summary sensitivity 43.7% (26.7 to 62.2)	46 (4)	True positive	4 (3 to 6)	44 (27 to 62)	87 (53 to 124)	⊕⊕⊕⊕ Very low^{e,f}
			False negative	6 (4 to 7)	56 (38 to 73)	113 (76 to 147)	
	Summary specificity 97.5 (93.6 to 99.0)	489 (4)	True negative	965 (927 to 980)	878 (842 to 891)	780 (749 to 792)	⊕⊕⊕⊕ High
			False positive	25 (10 to 63)	22 (9 to 58)	20 (8 to 51)	

GRADE Working Group grades of evidence

High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.

Very low certainty: we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

CI: confidence interval.

^aThe results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure.

^bPrevalence levels were suggested by the WHO Global Tuberculosis Programme.

^cDowngraded one level for imprecision due to wide 95% CI.

^dDowngraded one level for inconsistency as specificity ranged from 78% to 100%, and several 95% CIs did not overlap.

^eDowngraded one level for indirectness as only two studies (50%) were of low concern regarding applicability (patients enrolled from outpatient or non-referral settings).

^fDowngraded two levels for imprecision due to wide 95% CI and because a small number of participants contributed to the analysis for sensitivity.

Summary of findings 2. Xpert Ultra for rifampicin resistance^a

Review question: what is the diagnostic accuracy of Xpert Ultra for rifampicin resistance in children with signs and symptoms of pulmonary tuberculosis?

Patients/population: children with presumed pulmonary tuberculosis

Index tests: Xpert Ultra

Role: an initial test

Threshold for index tests: an automated result is provided

Reference standard: culture-based phenotypic drug susceptibility testing and MTBDR_{plus}

Types of studies: cross-sectional and cohort studies

Setting: primary care facilities and local hospitals

Limitations: the findings are based on 3 studies. Each study included only 1 participant with rifampicin resistance

Specimen	Effect (95% CI)	Number of participants (studies)	Test result	Number of results per 1000 patients tested (95% CI) ^b			Certainty of the evidence (GRADE)
				Prevalence 2%	Prevalence 10%	Prevalence 15%	
All specimens	Sensitivity range 100% to 100%	3 (3)	True positive	20 to 20	100 to 100	150 to 150	⊕⊕⊕⊕
			False negative	0 to 0	0 to 0	0 to 0	Very low ^{c,d,e}
	Specificity range 97% to 100%	128 (3)	True negative	951 to 980	873 to 900	825 to 850	⊕⊕⊕⊕
			False positive	0 to 29	0 to 27	0 to 25	Low ^{c,d}

GRADE Working Group grades of evidence

High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.

Very low certainty: we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

CI: confidence interval.

^aThe results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure.

^bPrevalence levels were suggested by the WHO Global Tuberculosis Programme.

^cDowngraded one level for risk of bias because in one study the manner of participant selection was unclear, and in another, not all participants were included in the analysis.

^dDowngraded one level for indirectness because the three included studies took place in China, Italy, and South Africa, and applicability to other settings is uncertain.

^eDowngraded two levels for imprecision because only three participants with rifampicin resistance contributed to this analysis for the observed sensitivity.

BACKGROUND

Tuberculosis is the 13th leading cause of death and the second leading cause of death from a single infectious agent after COVID-19 (WHO Global Tuberculosis Report 2021). Globally, in 2020, an estimated 10 million people developed tuberculosis disease, including around 1.1 million children younger than 15 years of age, and 226,100 children (205,000 HIV-negative and 21,100 HIV-positive children) died from the disease (WHO Global Tuberculosis Report 2021). Globally, in 2020, 53% of HIV-negative people who died from tuberculosis were men, 32% were women, and 16% were children younger than 15 years of age. The higher proportion of children who die from tuberculosis compared with their estimated share of cases (11%) suggests poorer access to diagnosis and treatment (WHO Global Tuberculosis Report 2021). The toll on younger children is especially tragic. One systematic review that investigated tuberculosis mortality in children found higher case fatality ratios in children from birth to four years of age compared with children aged five to 14 years (Jenkins 2017). Recent epidemiological models that have been accepted and supported by the World Health Organization (WHO) suggest that there is substantial under-reporting as well as underdiagnosis of tuberculosis in children (Dodd 2017).

Tuberculosis treatment for children follows the same principles as for adults, and the same drugs are used in most cases. The standard treatment for drug-susceptible tuberculosis – both pulmonary and extrapulmonary forms – is a four-drug combination regimen of isoniazid, rifampicin, pyrazinamide, and ethambutol given daily for two months, followed by isoniazid and rifampicin given daily for an additional two to four months. Central nervous system and osteoarticular tuberculosis constitute an exception in that treatment with isoniazid and rifampicin is extended for a total of 12 months. The introduction of paediatric fixed-dose combinations with optimized dosing and taste masking has improved the efficiency of treatment (Wademan 2019). Treatment of drug-resistant tuberculosis in children generally has better outcomes than in adults (Haraus 2018). Of note, in 2020, the WHO released consolidated guidelines on the treatment of drug-resistant tuberculosis in children and adults, containing new recommendations for the treatment of child drug-resistant tuberculosis, including the use of all-oral regimens (Furin 2019; WHO Consolidated Guidelines (Module 4) 2020).

The diagnosis of child tuberculosis relies on a mix of clinical, epidemiological, radiological, and laboratory information. Child tuberculosis is typically paucibacillary (tuberculosis disease caused by a smaller number of bacteria), and young children cannot voluntarily produce sputum specimens (Marais 2005; Theart 2005). Hence, even under ideal clinical and laboratory conditions, only 30% to 40% of children with tuberculosis have bacteriological confirmation of disease (Dunn 2016). The probability of microbiological confirmation is increased in children with more severe or advanced disease (Marais 2006a; Marais 2006b). However, the diagnostic gap is perpetuated because conventional smear microscopy, which is of limited value in diagnosing child tuberculosis and is no longer recommended by the WHO for diagnosis, remains the most used and most widely available tuberculosis diagnostic method in low- and middle-income countries. Further, the clinical skills and equipment needed for sputum induction and gastric aspiration are often not available in peripheral (subdistrict and community level)

health clinics (Reid 2012). Compared with microscopy, tuberculosis culture methods have shown greater, yet highly variable, sensitivity in child tuberculosis (Chiang 2017; Frigati 2015). Unfortunately, tuberculosis culture to support diagnosis is not widely available in high-burden settings.

The development of Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), a rapid molecular diagnostic test that simultaneously detects *Mycobacterium tuberculosis* (*M tuberculosis*) complex and rifampicin resistance), was a major step towards improving detection of tuberculosis and rifampicin resistance worldwide. However, Xpert MTB/RIF sensitivity is suboptimal in people with smear-negative tuberculosis, and particularly in children, people living with HIV, and people with extrapulmonary tuberculosis (Horne 2019; Kay 2020; Kohli 2021; Zifodya 2021). To overcome these limitations, Cepheid developed Xpert MTB/RIF Ultra (Xpert Ultra), a re-engineered assay using a newly developed cartridge that is run on the same device (GeneXpert) after a software upgrade (see Index test(s)).

This Cochrane Review update assessed the accuracy of Xpert Ultra for detecting pulmonary tuberculosis, specific forms of extrapulmonary tuberculosis (i.e. tuberculous meningitis and lymph node tuberculosis), and rifampicin resistance in children presumed to have tuberculosis, using sputum, gastric aspirate, nasopharyngeal aspirate, or stool specimens.

Current WHO recommendations on the use of Xpert Ultra related to this review update are presented in Table 1 and the WHO Consolidated Guidelines (WHO Consolidated Guidelines (Module 3) 2021; WHO Consolidated Guidelines (Module 5) 2022).

Target condition being diagnosed

There are four target conditions: active pulmonary tuberculosis, tuberculous meningitis, lymph node tuberculosis, and rifampicin resistance.

Tuberculosis

Tuberculosis is an infectious disease caused by bacteria within the *M tuberculosis* complex, most commonly *M tuberculosis*. Typically disseminated through the air, *M tuberculosis* predominantly affects the lungs, causing pulmonary tuberculosis, and less typically can cause disease in other organs of the body in extrapulmonary tuberculosis forms. For this review, we limited evaluation of extrapulmonary tuberculosis to lymph node tuberculosis and tuberculous meningitis. Lymph node tuberculosis is the most common form of extrapulmonary tuberculosis in children (Marais 2006e), and tuberculous meningitis results in the highest morbidity and mortality (Marais S 2010).

The natural history of tuberculosis in children is distinct from that in adults, due to more frequent progression to primary tuberculosis disease (Marais 2004). Children younger than five years of age are at particularly high risk of progression to tuberculous disease following infection, but the risk for older children and adolescents is also higher than in adults. Overall, it is estimated that 90% of tuberculous disease in young children occurs within one year of infection (Marais 2014). In addition to age, factors such as nutritional status, immune-compromising conditions (e.g. HIV infection), bacillus Calmette-Guérin (BCG)-vaccination status, and genetic susceptibility contribute to children's risk of disease progression. Immediately following infection with *M tuberculosis*

in a child, haematogenous spread (by way of the bloodstream) can occur. The period of highest risk for presentation with tuberculous meningitis and miliary tuberculosis is one to three months following primary infection. Children between six months and two years of age are at particularly high risk of these severe forms of tuberculous disease. Approximately 50% of children in this age range progress to tuberculous disease following infection, and 20% to 40% of those children will present with disseminated disease (Marais 2004; Marais 2014). Children younger than five years of age most commonly present with hilar lymph node forms of intrathoracic tuberculous disease. Older children and adolescents more commonly manifest adult-type disease, including pleural tuberculosis and upper lobe consolidations (Marais 2004).

Laboratory confirmation of tuberculosis in children is challenging for two reasons. First, child tuberculosis most commonly represents as a primary disease process, without the formation of cavities (Marais 2006c). The number of acid-fast bacilli (the presence of acid-fast bacilli on a sputum smear or other specimen usually indicates tuberculous disease) present in forms of primary tuberculosis such as hilar lymph node or bronchial tuberculosis is substantially lower than the number present in a pulmonary cavity. Consequently, child tuberculosis is often referred to as 'paucibacillary', and it is more difficult to obtain the organisms needed to confirm disease via conventional smear (no longer recommended) or culture (Dunn 2016). Second, most children younger than six years of age lack the ability to expectorate sputum and are unable to voluntarily produce good-quality specimens. Therefore, respiratory specimens are often obtained through sputum induction. As children swallow respiratory secretions, early-morning gastric aspiration is another well-established (yet still invasive) approach to specimen collection. In one study, the yield of three consecutive morning gastric aspirates was similar to the yield of one induced sputum specimen (Zar 2005). Nasopharyngeal aspiration for respiratory specimens is a less invasive mode of specimen collection (Zar 2012). Stool has also been studied as a child tuberculosis diagnostic specimen; although sensitivity has been lower than with traditional specimens, this specimen has great appeal because collection is non-invasive and requires no training (Nicol 2014). Because laboratory diagnostics for tuberculosis perform poorly in children, algorithms involving signs, symptoms, tuberculosis exposure, HIV status, laboratory tests, and radiographic findings are commonly used to make a clinical diagnosis of child tuberculosis. However, these algorithms have been shown to perform differently across settings, and their sensitivity and specificity may be site-specific (David 2017).

Rifampicin resistance

Rifampicin-resistant tuberculosis is caused by *M tuberculosis* strains resistant to rifampicin, a critical first-line tuberculosis drug (see [Index test\(s\)](#)). These strains may be susceptible or resistant to isoniazid (i.e. multidrug-resistant (MDR) tuberculosis), or resistant to other first-line or second-line tuberculosis drugs (WHO Consolidated Guidelines (Module 4) 2020). People with drug-resistant tuberculosis can transmit the infection to others. The drugs used to treat drug-resistant tuberculosis are less potent and more toxic than the drugs used to treat drug-susceptible tuberculosis. The WHO has issued recommendations that all individuals with MDR or rifampicin-resistant tuberculosis, including those who are also resistant to fluoroquinolones, may benefit from all-oral treatment regimens (WHO Consolidated Guidelines (Module 4) 2020).

Index test(s)

The index test is Xpert Ultra (Cepheid Inc, Sunnyvale, CA, USA). Xpert Ultra is a nucleic acid amplification test (NAAT) that functions as an automated closed system that performs real-time polymerase chain reaction (PCR). Specimens are processed using Xpert Sample Reagent and are incubated for 15 minutes, after which the processed samples are pipetted into the cartridge. These tests can be run by operators (such as laboratory technicians and nurses) with minimal technical expertise. Within two hours, the test detects both live and dead *M tuberculosis* complex DNA and simultaneously recognizes mutations in the *M tuberculosis* gene encoding the beta subunit of the ribonucleic acid (RNA) polymerase (*rpoB*) gene, which is the most common site of *M tuberculosis* mutations leading to rifampicin resistance. Xpert Ultra uses the same platform (GeneXpert) as Xpert MTB/RIF. Xpert Ultra requires an uninterrupted and stable electrical power supply, temperature control, and yearly calibration of the cartridge modules. The WHO has published extensive guidance and practical information on implementing the test (WHO Operational handbook on tuberculosis 2021).

Xpert Ultra was designed to improve the sensitivity to detect *M tuberculosis* complex and reliability for detection of rifampicin resistance (WHO Operational handbook on tuberculosis 2021). To improve tuberculosis detection, Xpert Ultra incorporates two different multicopy amplification targets (IS6110 and IS1081) and a larger chamber for the PCR reaction. To improve rifampicin resistance detection, Xpert Ultra is based on melting temperature analysis. These revisions have resulted in an approximately 1-log improvement in the lower limit of detection compared with Xpert MTB/RIF, as well as improved differentiation of certain silent mutations and improved detection of rifampicin resistance in mixed infections (Chakravorty 2017; WHO Operational handbook on tuberculosis 2021). At very low bacterial loads, Xpert Ultra can give a trace result (considered a positive bacteriologic result in children and people living with HIV), though trace does not provide a result for rifampicin susceptibility or resistance. Studies have found that the increase in Xpert Ultra sensitivity for tuberculosis detection has been accompanied by a decrease in specificity, and that Xpert Ultra may be more likely to identify *M tuberculosis* DNA from prior episodes of tuberculosis, particularly in people with a trace result (Dorman 2018; Mishra 2020). Despite clear guidance in children, Xpert Ultra trace results can complicate decision-making, and clinical management of trace results is rarely straightforward.

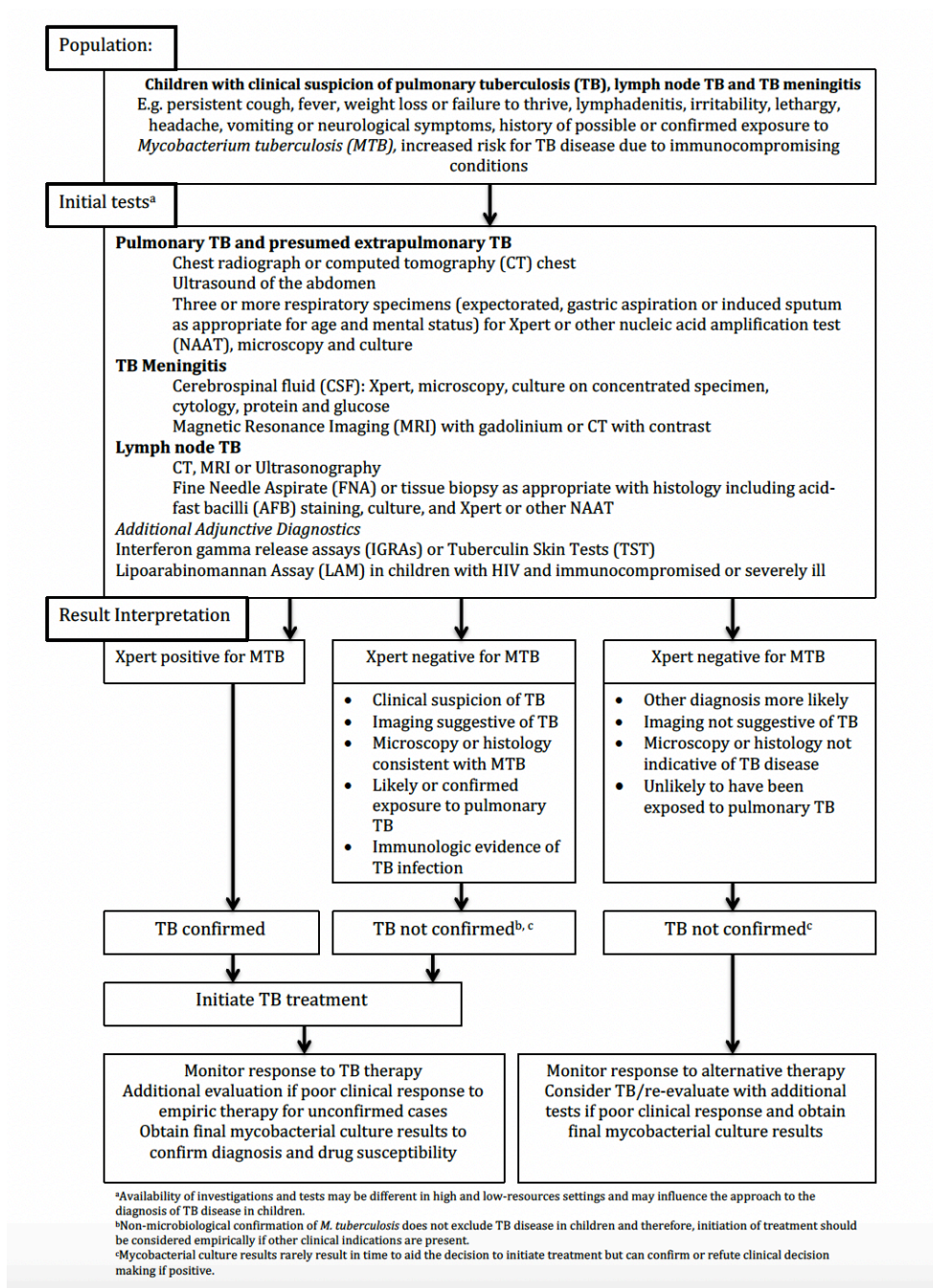
Clinical pathway

Figure 1 presents an example of the clinical pathway and placement of the index test. A careful clinical history of tuberculosis exposure and symptoms is the first step in the diagnostic pathway for child tuberculosis. Children with household or other close and persistent exposure to a person with tuberculosis are at increased risk of tuberculosis infection and resultant progression to tuberculosis disease. All children with recent exposure to tuberculosis must be evaluated for clinical symptoms and for examination findings consistent with tuberculous disease. Additional testing depends on the context but may include chest radiography and a test of tuberculosis infection. Symptoms of tuberculosis disease generally persist for longer than two weeks and are unremitting (Marais 2005). The most common symptoms are cough, fever, decreased appetite, weight loss or failure to thrive, and fatigue or reduced playfulness. Symptoms of extrapulmonary tuberculosis

are typically localized, and diagnostic findings are generally obtained from the site of disease (Figure 1). However, no symptom-based diagnostic algorithms have been validated or shown to be reliable in multiple contexts. Symptom-based diagnostic

algorithms tend to perform poorly in children younger than three years of age and in HIV-positive children: two populations at high risk for disease progression (Marais 2006d).

Figure 1. Clinical pathway of Xpert Ultra in children presumed to have tuberculosis



Unfortunately, no clinical examination features are specific to pulmonary tuberculosis in children. However, examination findings in extrapulmonary tuberculosis can be quite specific when identified. Clinicians should consider medical comorbidities that increase the risk for tuberculous disease, and should modify diagnostic algorithms accordingly. HIV infection not only

significantly increases risk of tuberculosis in children, it also raises the risk of increased disease severity. HIV-positive children, especially before effective antiretroviral therapy is established, often present with advanced tuberculosis, such as disseminated disease, and have high levels of immunosuppression, further complicating diagnosis and management.

Additional diagnostic imaging studies can assist in the diagnosis of pulmonary tuberculosis and nearly all forms of extrapulmonary tuberculosis. Tests for tuberculosis infection, such as interferon gamma release assays or tuberculin skin tests, can also aid in establishing the probability of tuberculosis (disease) in a child but are not necessary to make the diagnosis. Diagnostic recommendations strongly suggest collecting appropriate specimens from suspected sites of involvement in both pulmonary and extrapulmonary tuberculosis for microbiological examination. The preferred specimen in pulmonary tuberculosis is sputum; however, in young children who cannot expectorate, the specimen is commonly obtained via a gastric aspirate or induced sputum, and stool is increasingly used. To diagnose extrapulmonary tuberculosis, sample collection targets the affected site of disease.

The purpose of Xpert Ultra testing is diagnosis of pulmonary and extrapulmonary tuberculosis and detection of rifampicin resistance. Results of Xpert Ultra can be used as a decision-making tool in the following ways.

- *M tuberculosis* detected and rifampicin resistance not detected: child would start treatment for drug-sensitive tuberculosis.
- *M tuberculosis* detected and rifampicin resistance detected: child would need further testing for drug resistance and would start treatment for drug-resistant tuberculosis according to country guidelines.
- *M tuberculosis* not detected: a negative Xpert Ultra result does not rule out tuberculosis disease; therefore, clinicians should still consider initiation of tuberculosis treatment in children with history and clinical or radiological features suggestive of tuberculosis disease despite a negative Xpert Ultra result. A negative Xpert Ultra result may also represent a true negative.

Possible consequences of a false-positive and a false-negative result may include the following.

- False positive: children (and their families) would likely experience anxiety and morbidity caused by additional testing, unnecessary treatment, and possible adverse effects; as well as missed time from school, possible stigma associated with tuberculosis or a diagnosis of drug-resistant tuberculosis, and the chance that a false-positive may halt further diagnostic evaluation for other causes of illness. Families also experience unnecessary expense, as well as the risk of missing an important alternative diagnosis.
- False negative: would imply increased risk of morbidity and mortality and delayed start of treatment.

Role of index test(s)

For tuberculosis detection, the index test would be used as an initial test, replacing standard practice (i.e. smear microscopy or culture). For detection of rifampicin resistance, the index test would replace culture-based drug susceptibility testing as the initial test.

Alternative test(s)

Here we summarize selected alternative tests.

Truenat technologies (Molbio Diagnostics, Goa, India) are rapid molecular assays that can detect tuberculosis (Truenat MTB and MTB Plus assays) and rifampicin resistance (Truenat MTB-RIF Dx assay) from sputum specimens with results reported in less than

one hour ([WHO Operational handbook on tuberculosis 2021](#)). Truenat MTB and MTB Plus assays use chip-based PCR for detection of *M tuberculosis* complex; if a result is positive, a sample of the already extracted DNA may be run on the chip-based Truenat MTB-RIF Dx assay to detect mutations associated with rifampicin resistance ([WHO Operational handbook on tuberculosis 2021](#)). The assays use portable, battery-operated devices. The WHO includes Truenat assays in the category 'molecular WHO-recommended rapid diagnostic tests that can detect tuberculosis (mWRD)' and recommends their use as follows ([WHO Consolidated Guidelines \(Module 3\) 2021](#)).

- In adults and children with signs and symptoms of pulmonary tuberculosis, the Truenat MTB or MTB Plus may be used as an initial diagnostic test for tuberculosis rather than smear microscopy/culture (conditional recommendation, moderate certainty of evidence for test accuracy).
- In adults and children with signs and symptoms of pulmonary tuberculosis and a Truenat MTB or MTB Plus positive result, Truenat MTB-RIF Dx may be used as an initial test for rifampicin resistance rather than culture and phenotypic drug susceptibility testing (conditional recommendation, very low certainty of evidence for test accuracy).

Additional alternative approaches for diagnosis of tuberculosis are still used extensively world. Main tests include examination of smear for acid-fast bacilli (tuberculosis bacteria) under a microscope (light microscopy, using the classical Ziehl-Neelsen staining technique), fluorescence microscopy, and light-emitting diode (LED)-based fluorescence microscopy (no longer recommended by the WHO for diagnosis but used for monitoring in adults). The sensitivity of smear microscopy ranges from 0% to 10% in children ([Kunkel 2016](#)). Examination of histology specimens under a microscope following a tissue biopsy targets acid-fast bacilli and granulomatous inflammation, frequently with caseous necrosis (necrotizing granulomas); however these options are seldom pursued to diagnose child tuberculosis in low-resource settings due to the invasive nature of the procedures and the technical expertise required.

Lipoarabinomannan (LAM) antigen is a lipopolysaccharide present in the mycobacterial cell wall that can be detected in the urine of people with tuberculous disease ([Bjerrum 2019](#)). This urine test offers potential advantages over sputum-based testing due to ease of sample collection. The accuracy of urinary LAM detection is improved among people living with HIV with advanced immunosuppression ([Bjerrum 2019](#); [Nicol 2014](#); [Shah 2016a](#)). One Cochrane Review found that in inpatient settings, the use of lateral flow (LF)-LAM as part of a tuberculosis diagnostic testing strategy likely reduces mortality and probably results in a slight increase in tuberculosis treatment initiation in people living with HIV ([Nathavitharana 2021](#)). The WHO recommends that LF-LAM (Alere Determine™ TB LAM Ag, Alere Inc, Waltham, MA, USA), the only product available at the time of this recommendation, should be used to assist in the diagnosis of tuberculosis disease in HIV-positive adults, adolescents, and children. The full recommendations, which differ for inpatients and outpatients, are described in the WHO Consolidated guidelines for rapid diagnostics for tuberculosis detection ([WHO Consolidated Guidelines \(Module 3\) 2021](#)). However, the evidence for LF-LAM in children is limited and is primarily extrapolated from adults. A new urinary, point-of-care LAM test, Fujifilm SILVAMP TB LAM

(FujiLAM, co-developed by FIND, Geneva, Switzerland, and Fujifilm, Tokyo, Japan), for diagnosis of tuberculosis, is currently under investigation and has the potential to increase sensitivity in children (Broger 2019).

Line probe assays are a category of molecular tests for drug-resistant tuberculosis that offer speed of diagnosis (one or two days), standardized testing, and potential for high through-put. Drawbacks are that line probe assays require skills and infrastructure only available in intermediate and central laboratories. Line probe assays for first-line drugs (which include rifampicin) include GenoType MTBDRplus assay (MTBDRplus, Bruker-Hain Lifescience, Nehren, Germany), and the Nipro NTM +MDRTB detection kit 2 (Nipro, Tokyo, Japan). These assays detect the presence of mutations associated with drug resistance to isoniazid and rifampicin. MTBDRplus is the most widely studied line probe assay. The WHO recommends that for people with a sputum smear-positive specimen or a culture isolate of *M tuberculosis* complex, commercial molecular line probe assays may be used as the initial test instead of phenotypic drug susceptibility testing to detect resistance to rifampicin and isoniazid (conditional recommendation, moderate certainty in the evidence for the test's accuracy; WHO Consolidated Guidelines (Module 3) 2021).

The quest for novel and more efficient technologies for diagnosis of tuberculosis is a cornerstone of current efforts to reduce the burden of disease worldwide. Over the past decade, unprecedented activity has focused on the development of new tools for diagnosis of extrapulmonary tuberculosis, largely supported by the engagement of global agencies. As a result, a strong pipeline of new tools for diagnosis of tuberculosis will complement the use of existing ones and will offer improved options. 'The Tuberculosis Diagnostics Pipeline Report: Advancing the Next Generation of Tools' describes tuberculosis tests in development (Branigan 2021).

Rationale

Timely and reliable diagnosis of tuberculosis in children remains challenging due to both difficulties in collecting sputum samples and the paucibacillary nature of the disease. Under-diagnosis may lead to increased morbidity, mortality, and disease transmission in this key group.

Our previously published Cochrane Review assessed the accuracy of both Xpert MTB/RIF and Xpert Ultra (Kay 2020). We limited the current review update to the diagnostic accuracy of Xpert Ultra for several reasons. Xpert Ultra has superseded Xpert MTB/RIF, and the manufacturer will be discontinuing Xpert MTB/RIF in most countries in 2023. Given the available evidence about Xpert MTB/RIF from our previous review, we therefore only updated Xpert Ultra as requested by the WHO. The Xpert MTB/RIF text and analyses are available in the last published version of the review (Kay 2020).

Regarding Xpert Ultra, in the original Cochrane Review, we identified few published studies: three studies on Xpert Ultra in sputum (697 participants) and no studies in gastric aspirate and stool specimens. In addition, we had limited data in children younger than 10 years of age, an area of considerable interest for the WHO.

In the current review update, we aimed to determine the diagnostic accuracy of Xpert Ultra for pulmonary tuberculosis, tuberculosis meningitis, lymph node tuberculosis, and rifampicin resistance in

children. Parts of the review update, particularly the analyses of gastric aspirate and stool specimens, were used to inform the 2022 WHO updated guidance on the management of tuberculosis in children and adolescents (WHO Consolidated Guidelines (Module 5) 2022; see Table 1).

OBJECTIVES

To assess the diagnostic accuracy of Xpert Ultra for detecting: pulmonary tuberculosis, tuberculous meningitis, lymph node tuberculosis, and rifampicin resistance, in children with presumed tuberculosis.

Secondary objectives

- To investigate potential sources of heterogeneity in accuracy estimates. For detection of tuberculosis, we considered age, comorbidity (HIV, severe pneumonia, and severe malnutrition), and specimen type as potential sources.
- To summarize the frequency of Xpert Ultra trace results.

METHODS

Criteria for considering studies for this review

Types of studies

We included cross-sectional studies, cohort studies, and randomized controlled trials (RCTs) from all settings. We included RCTs that evaluated use of the test for patient health outcomes but also reported sensitivity and specificity. Although we utilized RCTs for the purpose of determining the impact of the test versus a comparator (e.g. usual practice, another test) on health outcomes, the study design was interpreted as a cross-sectional design for the purpose of determining diagnostic accuracy for the index tests in this review. We included only studies from which we could extract or derive data on the index test giving true positives, false positives, true negatives, or false negatives, as assessed against the reference standards specified below. We included abstracts with sufficient data. In addition, we included ongoing studies that helped us to address the review objectives (see Data collection and analysis). For each of the ongoing studies, we recorded the stage of the study at the time of data extraction for this review (e.g. recruitment completed, recruitment completed and data cleaned, or recruitment ongoing and number (%) of the target sample size recruited) in the Characteristics of included studies table. We excluded case-control studies and case reports.

Participants

We included studies that evaluated the index tests for pulmonary or extrapulmonary tuberculosis in HIV-positive and HIV-negative children and young adolescents aged 0 to 14 years (collectively referred to as children), presumed to have tuberculosis. Studies were eligible for inclusion if they described the use of Xpert Ultra on routine respiratory specimens such as expectorated or induced sputum and gastric and nasopharyngeal specimens. Gastric specimens could be obtained via gastric aspiration, lavage, or washing, as described by study authors. In addition, we included studies evaluating stool specimens, because tuberculosis bacilli are present in swallowed sputum and are recoverable from stool samples using Xpert Ultra. We also included studies that assessed several different specimen types.

Index tests

The index test was Xpert Ultra.

Index test results are automatically generated, and the user is provided with a printable test result as follows.

- MTB (*M tuberculosis*) DETECTED HIGH; RIF (rifampicin) Resistance DETECTED.
- MTB DETECTED MEDIUM; RIF Resistance DETECTED.
- MTB DETECTED LOW; RIF Resistance DETECTED.
- MTB DETECTED VERY LOW; RIF Resistance DETECTED.
- MTB DETECTED HIGH; RIF Resistance NOT DETECTED.
- MTB DETECTED MEDIUM; RIF Resistance NOT DETECTED.
- MTB DETECTED LOW; RIF Resistance NOT DETECTED.
- MTB DETECTED VERY LOW; RIF Resistance NOT DETECTED.
- MTB DETECTED HIGH; RIF Resistance INDETERMINATE.
- MTB DETECTED MEDIUM; RIF Resistance INDETERMINATE.
- MTB DETECTED LOW; RIF Resistance INDETERMINATE.
- MTB DETECTED VERY LOW; RIF Resistance INDETERMINATE.
- MTB Trace DETECTED; RIF Resistance INDETERMINATE.
- 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).

Xpert Ultra incorporates a semi-quantitative classification for results: trace, very low, low, moderate, and high. Trace corresponds to the lowest bacterial burden for detection of *M tuberculosis* (Chakravorty 2017). Although no rifampicin resistance results are available for people with trace results, a trace-positive result is sufficient to initiate tuberculosis therapy in children or people living with HIV, according to the WHO (WHO Consolidated Guidelines (Module 3) 2021). Hence, we considered a trace result to mean *M tuberculosis* DETECTED.

Target conditions

The target conditions were active pulmonary tuberculosis; two forms of extrapulmonary tuberculosis, tuberculous meningitis and lymph node tuberculosis; and rifampicin resistance.

Reference standards

For detection of pulmonary tuberculosis, tuberculous meningitis, and lymph node tuberculosis, we included two reference standards (see below regarding stool samples).

- Culture: tuberculosis was defined as a positive culture on solid or liquid medium from a respiratory sample.
- Composite reference standard: tuberculosis was defined as a positive culture or a clinical decision, based on clinical features, to initiate treatment for tuberculosis (i.e. clinically diagnosed tuberculosis). Clinical features might include cough longer than two weeks, fever, or weight loss; pneumonia that did not improve with antibiotics; or a history of close contact with an adult who had tuberculosis.

For the composite reference standard, in the absence of information on tuberculosis treatment, we accepted a study-specific definition (i.e. a standardized definition of tuberculosis

defined by the primary study authors), if available. We also accepted the uniform research definition (Graham 2012; Graham 2015). In these situations, for the older definition (Graham 2012), we defined tuberculosis as 'confirmed, probable, and possible' and not tuberculosis as 'unlikely and not tuberculosis'. For the newer definition (Graham 2015), we defined tuberculosis as 'confirmed and unconfirmed' and not tuberculosis as 'unlikely'.

We included children with unconfirmed tuberculosis in the true-negative population when evaluating results against a culture reference standard. In contrast, we included children who were not treated for tuberculosis, or who did not meet the study research definition for tuberculosis, in the true-negative population when evaluating results against a composite reference standard.

Regarding stool specimens (used for the diagnosis of pulmonary tuberculosis), we defined the reference standard similar to MacLean 2019: (1) culture, or (2) Xpert Ultra performed on a routine respiratory specimen, such as sputum or gastric aspirate specimen. We did not include stool Xpert Ultra results in the definition of the reference standard. In addition, none of the included studies used stool culture to verify pulmonary tuberculosis. For these reasons, we thought bias due to incorporation of the index test was unlikely. Hence, tuberculosis was defined as a positive culture or a positive Xpert Ultra on a routine respiratory specimen.

Regarding stool specimens, we also included a composite reference standard as defined above.

Culture is generally considered the best reference standard for tuberculosis diagnosis. However, particularly in children with paucibacillary disease, tuberculosis is verified by culture in only 15% to 50% of cases, depending on disease severity, challenges of obtaining specimens, and resources (Graham 2015). Evaluation of multiple specimens, of the same or different types, may increase the yield of culture for confirming tuberculosis (Cruz 2012; Zar 2012). Therefore, we considered a higher-quality reference standard to be one in which more than one specimen was used to confirm tuberculosis. We considered a lower-quality reference standard to be one in which only one specimen was used for tuberculosis diagnosis. We reflected these considerations in the Quality Assessment of Studies of Diagnostic Accuracy – Revised (QUADAS-2) reference standard domain.

For rifampicin resistance, the reference standards were phenotypic drug susceptibility testing and MTBDR*plus*. MTBDR*plus* is a molecular line probe assay designed to detect the presence of multiple mutations causing resistance to isoniazid and rifampicin.

Search methods for identification of studies

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

Electronic searches

We searched the following databases up to 9 March 2021 using the search terms and strategy described in Appendix 1.

- Cochrane Infectious Diseases Group Specialized Register.
- MEDLINE (Ovid, from 1966).
- Embase (Ovid, from 1974).

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

- Cumulative Index to Nursing and Allied Health Literature (CINAHL (EBSCOHost), from 1982).
- Science Citation Index – Expanded (from 1900), Conference Proceedings Citation Index – Science (CPCI-S, from 1990), from the Web of Science (Clarivate Analytics).
- Scopus (Elsevier, from 1970).

We also searched ClinicalTrials.gov (clinicaltrials.gov), the WHO International Clinical Trials Registry Platform (ICTRP; who.int/clinical-trials-registry-platform), and the International Standard Randomized Controlled Trials Number (ISRCTN) Registry (www.isrctn.com) for trials in progress, up to 9 March 2021.

Searching other resources

We contacted researchers and experts in the field to identify additional eligible studies. This included sharing the list of included and excluded studies with the WHO Guideline Development Group on the management of tuberculosis in children and adolescents prior to a preparatory webinar for input and feedback. We also checked the references of relevant reviews and studies to identify additional studies.

Data collection and analysis

Selection of studies

We used Covidence to manage the selection of studies (Covidence). Two review authors (AWK and TN, or AWK and LFG) independently screened all titles and abstracts to identify potentially eligible studies. We then obtained the full-text articles of potentially eligible studies, and two review authors (AWK and TN, or AWK and LFG) independently assessed whether they should be included based on predefined inclusion and exclusion criteria. We resolved disagreements by discussion or by consulting a third review author (AMM or KRS), if necessary. We contacted primary study authors for clarification of methods and other information, as needed. We recorded and summarized reasons for excluding studies in the [Characteristics of excluded studies](#) table. We illustrated the study selection process in a PRISMA diagram ([Page 2021](#)).

Data extraction and management

We designed a data extraction form and piloted it on two included studies (Appendix 2). We then finalized the form based on the pilot test. Two review authors (AWK and TN or AWK and LFG) independently extracted data using this data extraction form and discussed inconsistencies to achieve consensus. We consulted a third review author (AMM or KRS) to resolve discrepancies, as needed. We entered abstracted data into Google sheets on password-protected computers. We secured the data set in a cloud storage workspace and we stored extracted data for future review updates. Selected details of data extraction are listed below.

Study details

- Number of participants after screening for exclusion and inclusion criteria
- Total number of children included in the analysis
- Specimen collection methods
- Unit of sample collection: one specimen, multiple specimens, unknown, or unclear
- Target condition(s)? – pulmonary tuberculosis, tuberculous meningitis, lymph node tuberculosis, rifampicin resistance

- For ongoing studies, we recorded the stage of the study at the time of data extraction for this review (e.g. recruitment completed, recruitment completed and data cleaned, or recruitment ongoing and number (%) of the target sample size recruited) in the [Characteristics of included studies](#) table.

Patient characteristics and setting

- Description of study population
- Age: median, mean, range
- Sex
- HIV status
- Percentage and number of HIV-positive or HIV-negative participants, if both were included in the study
- Type of respiratory specimen included: sputum, gastric aspirate or lavage, stool, nasopharyngeal aspirate
- Type of non-respiratory specimen included: cerebrospinal fluid, fine-needle aspirate, lymph node biopsy, multiple types, other, unknown
- Number of cultures performed per child to exclude tuberculosis
- Data on culture performance: number of contaminated cultures with respect to total cultures performed
- Clinical setting: outpatient, inpatient, or both
- Description of radiographic findings
- Information on tuberculosis burden in the country

We classified countries as being high-burden or not high-burden for tuberculosis, HIV-associated tuberculosis, and MDR or rifampicin-resistant tuberculosis based on the WHO classification for 2021 to 2025 ([WHO Global Tuberculosis Report 2021](#)). A country may be classified as high-burden for one, two, or all three of the high-burden categories.

We contacted the authors of all included studies for data on specific age ranges and subpopulations and for clarification on study characteristics.

Index test

- Pretreatment processing procedure for specimens used for Xpert Ultra
- Specimen condition: fresh, frozen, or both
- Numbers of true positives, false positives, false negatives, and true negatives by age group (all ages, under one year, one to four years, five to nine years, 10 to 14 years, and birth to 9 years; (see example tables in Appendix 3)
- Uninterpretable results for tuberculosis detection (invalid, error, or no result)
- Indeterminate results for detection of rifampicin resistance
- Xpert Ultra trace results

Reference standards

- Details of culture: solid or liquid
- Composite reference standard
- Rifampicin resistance: phenotypic drug susceptibility testing or MTBDR_{plus}

For each target condition and specimen type, we considered one index test result per child. Hence, the primary unit of analysis was the child. If studies evaluated more than one specimen type,

we extracted data for each specimen. Hence, a study may have contributed more than one 2 × 2 table (data set): one for each type of specimen evaluated.

Assessment of methodological quality

We assessed the methodological quality of included studies using the QUADAS-2 instrument, which we adapted for this review (Whiting 2011). The QUADAS-2 tool consists of four domains:

- patient selection;
- index test(s);
- reference standard(s); and
- flow and timing.

All domains are assessed for risk of bias, and the first three domains are assessed for concerns regarding applicability. We first developed guidance on how to appraise each signalling question within the domains and how to make the overall judgement for each domain. One review author piloted the tool with two of the included studies. We finalized the guidance based on experience gained from the pilot. Appendix 4 presents the QUADAS-2 tool with signalling questions tailored to this review. Two review authors (AK and LFG or AK and TN) independently completed QUADAS-2. We resolved disagreements through discussion or by arbitration with a third review author (KRS or AMM), when necessary. We presented results of the quality assessment in the text, in tables, and in graphs.

Statistical analysis and data synthesis

We performed descriptive analyses of the included studies and presented their key characteristics in the [Characteristics of included studies](#) table. We stratified all analyses by type of specimen and type of reference standard. We presented individual study estimates of sensitivity and specificity graphically in forest plots and in receiver operating characteristics (ROC) space using Review Manager 5 (Review Manager 2020).

When data were sufficient, we performed meta-analyses to estimate average sensitivities and specificities using a bivariate model (Chu 2006; Reitsma 2015). We used the bivariate model because the index test, Xpert Ultra, applies a common positivity criterion (Macaskill 2010). When we were unable to fit a bivariate model due to sparse data, few studies, or limited variability in specificity, we simplified the model to a univariate random-effects or fixed-effect logistic regression model to pool sensitivity and specificity separately, as appropriate given the observed data (Takwoingi 2015). We performed meta-analyses using the meqrlogit command for models that included random effects and the blogit command for fixed-effect meta-analyses in Stata version 16 (Stata 16). Meta-analysis using univariate fixed-effect or random-effects logistic regression models is not possible when all studies in a meta-analysis report 100% specificity. For such analyses, we calculated summary specificity by dividing the total number of non-cases by the total number of true negatives, and we computed the 95% confidence interval (CI) using the Wilson method (Newcombe 1998).

Approach to non-determinate and trace index test results

Non-determinate Xpert Ultra test results include 'Error', 'Invalid', and 'No Result', and may be due to an operator error, instrument, or cartridge issue. For each included study that reported the number of non-determinate results for tuberculosis detection, we

estimated the proportion of non-determinate Xpert Ultra results. As recommended by the WHO, trace results were included in the primary analyses as Xpert Ultra-positive results. For each included study that provided data on trace results, we calculated the percentage of test positives that were trace results (i.e. number of trace results/number of test positives).

Investigations of heterogeneity

We visually inspected forest and summary ROC (SROC) plots for heterogeneity. When data allowed, we evaluated sources of heterogeneity using subgroup analyses. We were unable to perform meta-regression because of the number of studies available. For tuberculosis detection, we investigated key subgroups of children: aged under 1 year, aged 1 to 4 years, aged 5 to 9 years, aged 10 to 14 years, HIV-positive, HIV-negative, with severe pneumonia, and with severe malnutrition.

Sensitivity analyses

We performed sensitivity analyses excluding data from ongoing studies in the primary analyses.

Assessment of reporting bias

We did not formally assess reporting bias using funnel plots or regression tests because these have not been reported as helpful for diagnostic test accuracy studies (Macaskill 2010).

Assessment of certainty of the evidence

We assessed certainty of the evidence using the GRADE approach for diagnostic studies (Balslem 2011; Schünemann 2008; Schünemann 2016). As recommended, we rated certainty of the evidence as high (not downgraded), moderate (downgraded one level), low (downgraded two levels), or very low (downgraded more than two levels) based on five domains: risk of bias, indirectness, inconsistency, imprecision, and publication bias. For each outcome, certainty of the evidence started as high when high-quality studies (cross-sectional or cohort studies) enrolled participants with diagnostic uncertainty. If we found a reason for downgrading, we used our judgement to classify the reason as serious (downgraded one level) or very serious (downgraded two levels).

Three review authors (AWK, TN, and KRS) discussed judgements and applied GRADE (Schünemann 2020a; Schünemann 2020b).

Risk of bias

We used QUADAS-2 to assess risk of bias.

Indirectness

We assessed indirectness in relation to the population (including disease spectrum), setting, interventions, and outcomes (accuracy measures). We also used prevalence (proportion) of the target condition in the included studies as a guide to whether there was indirectness in the population.

Inconsistency

GRADE recommends downgrading for unexplained inconsistency in sensitivity and specificity estimates. We carried out pre-specified analyses to investigate potential sources of heterogeneity and downgraded when we could not explain inconsistency in accuracy estimates. We looked at the individual point estimates in the forest

plots and judged whether they were more or less the same, as well as the CIs to see if they overlapped.

Imprecision

We considered the width of the 95% CI. In addition, we determined projected ranges for two categories of test results that have the most important consequences for patients – the number of false negatives and the number of false positives – and made judgements on imprecision from these calculations. Imprecision also depends on the number of participants included to determine sensitivity and specificity. We took note of the uncertainty around point estimates along with the number of participants providing those data. We acknowledge the judgement of imprecision is subjective.

Publication bias

We considered the comprehensiveness of the literature search and outreach to researchers in tuberculosis, the presence of only studies that produce precise estimates of high accuracy despite small sample size, and knowledge about studies that were conducted but not published.

The summary of findings tables include the following details:

- The review question and its components, population, setting, index test, and reference standards.
- Summary estimates of sensitivity and specificity with 95% CIs.

- The number of included studies and participants contributing to the estimates of sensitivity and specificity.
- Prevalences of the target condition with an explanation of why the prevalences have been chosen.
- An assessment of the certainty of the evidence (GRADE).
- Explanations for downgrading, as needed.

RESULTS

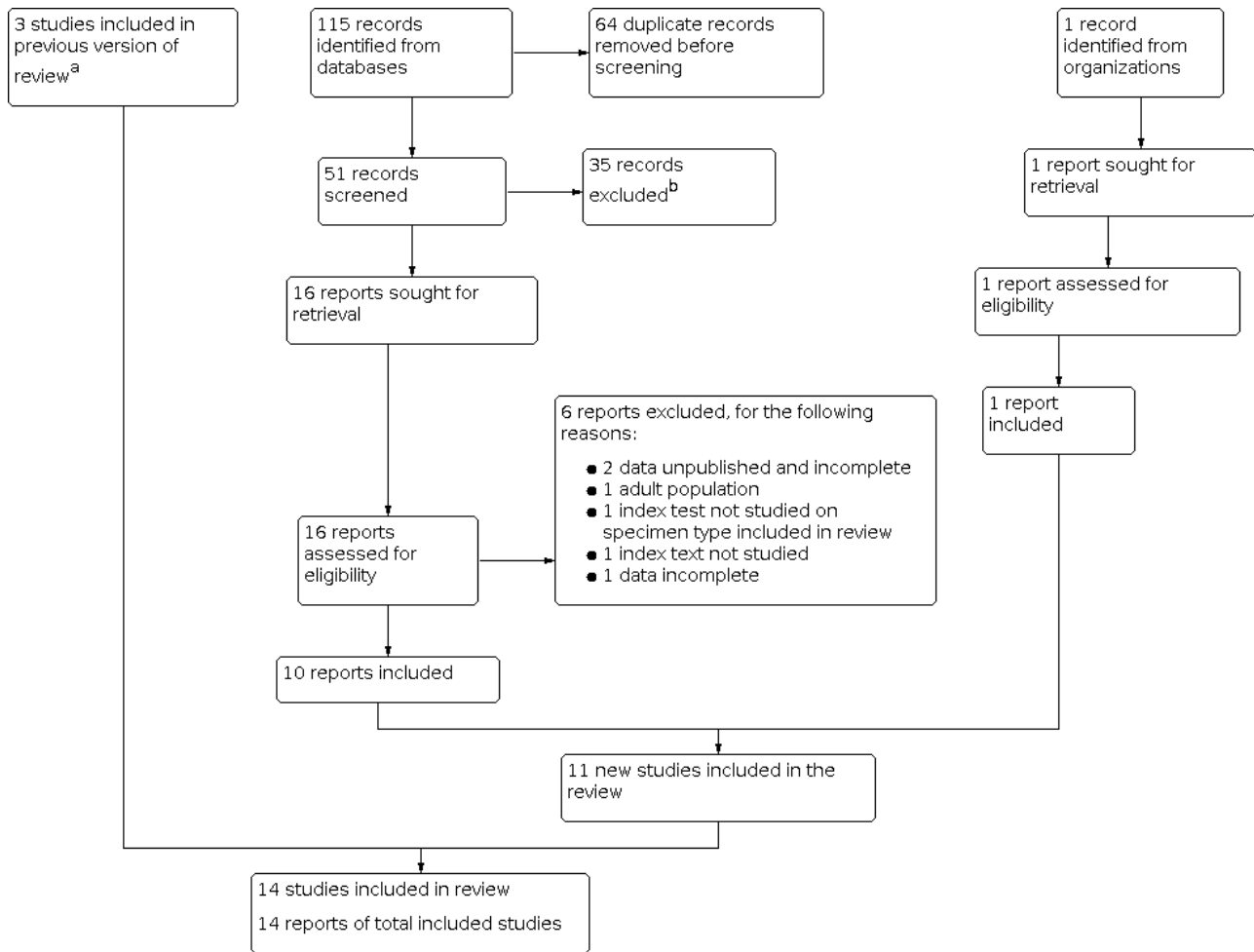
Results of the search

We identified 2174 records through database searches conducted up to 29 April 2019. An updated search to 9 March 2021 identified 115 records. We found one additional record by contacting researchers at the Foundation for Innovative New Diagnostics (FIND). After removing duplicates, we screened 51 records by title and abstract. We excluded 35 of these, leaving 16 reports, which we retrieved for full text review. We identified 14 unique studies (including one from a source outside of our database searches), integrating 11 new studies since publication of the Cochrane Review (Kay 2020). All studies were written in English. [Figure 2](#) shows the flow of studies in the review. We recorded the excluded studies, including those listed in the previous Cochrane Review (Kay 2020), with reasons for their exclusion in the [Characteristics of excluded studies](#) table.

Figure 2. Study flow diagram.

^aKay 2020.

^bStudies only evaluated Xpert MTB/RIF and other reasons: not a diagnostic study; study did not include children; case-control study; abstract; index test not studied.



Description of included studies

We describe key characteristics in the [Characteristics of included studies](#) table and [Table 2](#). All were cross-sectional or cohort studies, with the exception of one, which had an unclear study design. The studies were conducted in both inpatient and outpatient settings; seven took place in tuberculosis high-burden countries.

For pulmonary tuberculosis, 108 data sets (20,407 participants) were available for analysis; for rifampicin resistance, three data sets (131 participants) were available.

We did not identify any studies that evaluated Xpert Ultra accuracy for tuberculous meningitis or lymph node tuberculosis.

Methodological quality of included studies

Pulmonary tuberculosis

[Figure 3](#) and [Appendix 5](#) show risk of bias and applicability concerns for 14 studies that evaluated Xpert Ultra for detection of pulmonary tuberculosis.

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

	<u>Risk of Bias</u>				<u>Applicability Concerns</u>		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Barcellini 2019	+	+	+	+	+	+	+
Jaganath 2021	+	+	+	+	+	+	+
Kabir 2020	+	+	?	+	-	?	?
Liu 2021	+	+	+	-	+	+	?
NCT04121026	+	+	+	+	+	+	+
NCT04203628	+	+	+	+	+	?	+
NCT04240990	+	+	+	+	+	+	?
NCT04899076	+	+	+	+	+	?	+
Nicol 2018	+	+	+	+	+	+	+
Parigi 2021	?	+	+	+	-	+	?
Sabi 2018	+	+	+	+	+	+	+
Ssengooba 2020	+	+	+	+	+	+	+
Sun 2020	+	+	+	+	?	+	?
Zar 2019	+	+	+	+	-	+	+

- High
 ? Unclear
 + Low

In the patient selection domain, we considered 13 studies (93%) at low risk of bias because they enrolled a consecutive or random sample of eligible participants and avoided inappropriate exclusions. We considered one study (7%) at unclear risk of bias because the manner of patient sampling was unclear (Parigi 2021). With respect to applicability, we considered 10 studies (71%) of low concern because participants in these studies were evaluated in primary care facilities, in local hospitals, or in both settings (Barcellini 2019; Jaganath 2021; Liu 2021; NCT04121026; NCT04203628; NCT04240990; NCT04899076; Nicol 2018; Sabi 2018; Ssengooba 2020). We considered three studies (21%) of high concern because participants were evaluated exclusively as inpatients in tertiary care centres (Kabir 2020; Parigi 2021; Zar 2019). We considered one study of unclear concern because we were unsure about the clinical setting (Sun 2020).

In the index test domain, we considered all studies at low risk of bias. With respect to applicability, we considered eight studies (73%) to of low concern (Barcellini 2019; Jaganath 2021; Nicol 2018; Parigi 2021; Sabi 2018; Ssengooba 2020; Sun 2020; Zar 2019). We considered all studies that evaluated stool specimens of unclear concern because of the absence of an established protocol for stool processing before Xpert Ultra testing.

In the reference standard domain, we considered 13 studies (93%) at low risk of bias and one study at unclear risk of bias because the ability of the reference standard to appropriately classify child tuberculosis was uncertain (Kabir 2020). With respect to applicability, we considered nine (64%) studies of low concern because speciation was performed, confirming *M tuberculosis* instead of other mycobacterial species (Barcellini 2019; Jaganath 2021; NCT04121026; NCT04203628; NCT04899076; Nicol 2018; Sabi 2018; Ssengooba 2020; Zar 2019, and five studies of unclear concern because we could not tell whether speciation was performed (Kabir 2020; Liu 2021; NCT04240990; Parigi 2021; Sun 2020).

In the flow and timing domain, we considered 13 studies (93%) at low risk of bias because all participants were included in the

analysis. We considered one study at high risk of bias (Liu 2021) because most enrolled children were not included in the analysis.

Rifampicin resistance

In the patient selection domain, we judged two studies at low risk of bias (Liu 2021; Parigi 2021), and one study at unclear risk of bias because the manner of patient selection was not reported (Parigi 2021). Regarding applicability, in the patient selection domain we had low concern for one study (Liu 2021), and high concern for two studies because all patients were recruited from an inpatient setting (Parigi 2021; Zar 2019). In the index test and reference standard domains, we judged all studies at low risk of bias and of low concern regarding applicability. In the flow and timing domain, we judged one study at high risk of bias because not all participants were included in the analysis (Liu 2021).

Findings

I. Detection of pulmonary tuberculosis

Due to little observed variability in specificity and in the volume of analyses, we chose to present only forest plots, as such plots were more informative than corresponding SROC plots.

Xpert Ultra for pulmonary tuberculosis

Xpert Ultra in sputum specimens

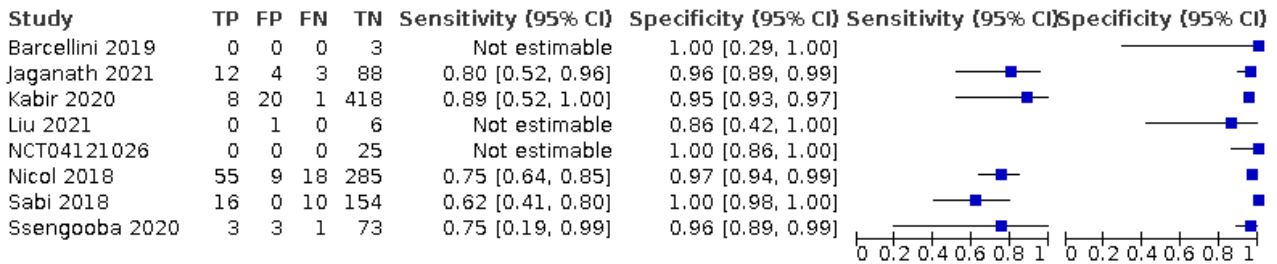
Culture reference standard

Eight studies (1216 participants) evaluated Xpert Ultra in sputum specimens against culture (Barcellini 2019; Jaganath 2021; Kabir 2020; Liu 2021; NCT04121026; Nicol 2018; Sabi 2018; Ssengooba 2020). Xpert Ultra sensitivity ranged from 62% to 89%, and specificity from 86% to 100% (Figure 4). Three studies did not contribute data to the meta-analysis because sensitivity was not estimable (Barcellini 2019; Liu 2021; NCT04121026). In the remaining five studies (1181 participants), Xpert Ultra summary sensitivity was 75.3% (95% CI 64.3 to 83.8), and summary specificity was 97.1% (95% CI 94.7 to 98.5) (Table 3).

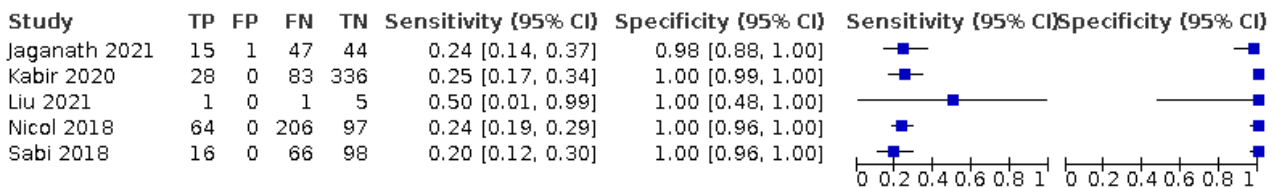
Figure 4. Forest plots of Xpert Ultra sensitivity and specificity in sputum for pulmonary tuberculosis in children by type of specimen and reference standard. The squares represent the sensitivity and specificity of each study, the black lines their confidence intervals (CIs).

FN: false negative; FP: false positive; TN: true negative; TP: true positive.

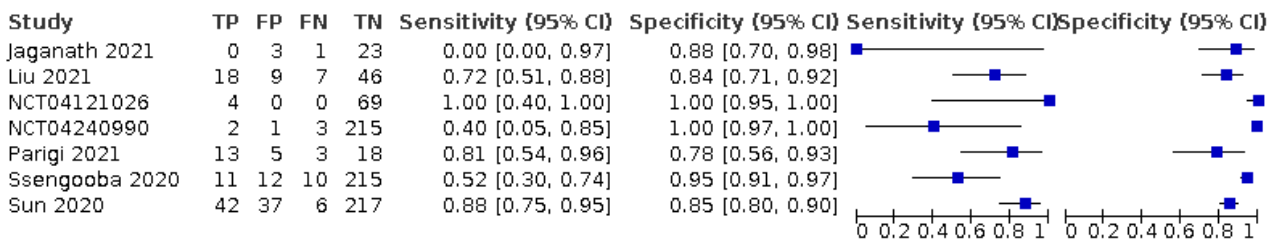
Xpert Ultra, sputum, all ages, culture



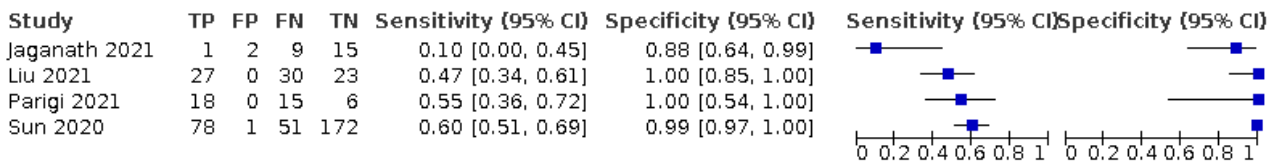
Xpert Ultra, sputum, all ages, composite reference standard



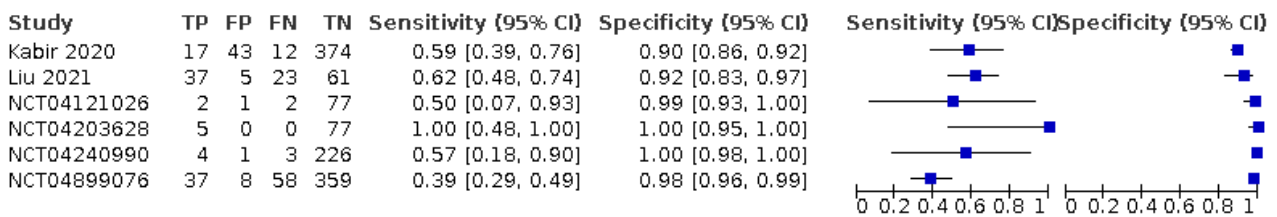
Xpert Ultra, gastric aspirate, all ages, culture



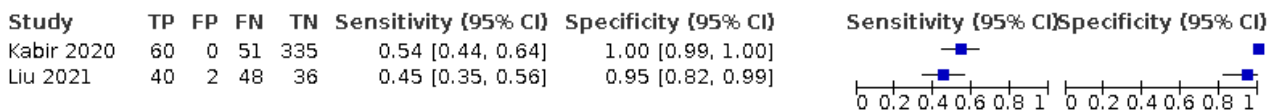
Xpert Ultra, gastric aspirate, all ages, composite reference standard



Xpert Ultra, stool, all ages, culture



Xpert Ultra, stool, all ages, composite reference standard



Xpert Ultra, nasopharyngeal aspirate, all ages, culture

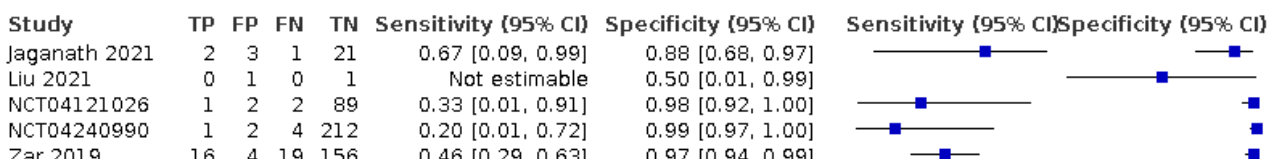
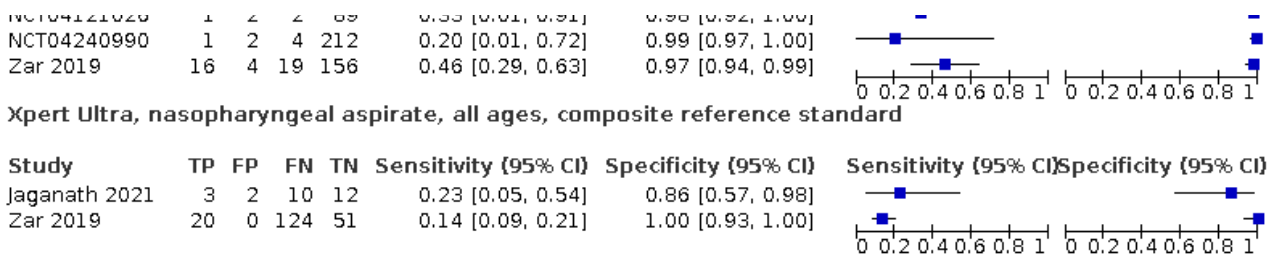


Figure 4. (Continued)



Composite reference standard

Five studies (1108 participants) evaluated Xpert Ultra in sputum specimens against a composite reference standard (Jaganath 2021; Kabir 2020; Liu 2021; Nicol 2018; Sabi 2018). Xpert Ultra sensitivity ranged from 20% to 50%, and specificity from 98% to 100% (Figure 4). Xpert Ultra summary sensitivity was 23.5% (95% CI 20.1 to 27.3), and summary specificity was 99.8% (95% CI 98.8 to 100.0) (Table 3).

Xpert Ultra in gastric aspirate specimens

Culture reference standard

Seven studies (990 participants) evaluated Xpert Ultra in gastric aspirate specimens against culture (Jaganath 2021; Liu 2021; NCT04121026; NCT04240990; Parigi 2021; Ssenooba 2020; Sun 2020). Xpert Ultra sensitivity ranged from 0% to 100%, and specificity from 78% to 100% (Figure 4). The low sensitivity in Jaganath 2021 could be due to having only one culture positive case in the study. Xpert Ultra summary sensitivity was 70.4% (95% CI 53.9 to 82.9), and summary specificity was 94.1% (95% CI 84.8 to 97.8) (Table 3).

Composite reference standard

Four studies (448 participants) evaluated Xpert Ultra in gastric aspirate specimens against a composite reference standard (Jaganath 2021; Liu 2021; Parigi 2021; Sun 2020). Xpert Ultra sensitivity ranged from 10% to 60%, and specificity from 88% to 100% (Figure 4). Xpert Ultra summary sensitivity was 46.5% (95% CI 29.7 to 64.1), and summary specificity was 98.4% (95% CI 91.4 to 99.7) (Table 3).

Xpert Ultra in stool specimens

Culture reference standard

Six studies (1432 participants) evaluated Xpert Ultra in stool specimens against culture (Kabir 2020; Liu 2021; NCT04121026; NCT04203628; NCT04240990; NCT04899076). Xpert Ultra sensitivity ranged from 39% to 100%, and specificity from 90% to 100% (Figure 4). Xpert Ultra summary sensitivity was 56.1% (95% CI 39.1 to 71.7), and summary specificity was 98.0% (95% CI 93.3 to 99.4) (Table 3).

Composite reference standard

Two studies (572 participants) evaluated Xpert Ultra in stool specimens against a composite reference standard (Kabir 2020; Liu

2021). In Kabir 2020, Xpert Ultra sensitivity was 54% (95% CI 44 to 64), and specificity was 100% (95% CI 99 to 100); while in Liu 2021, Xpert Ultra sensitivity was 45% (95% CI 35 to 56), and specificity was 95% (95% CI 82 to 99) (Figure 4). Xpert Ultra summary sensitivity was 50.3% (95% CI 43.3 to 57.1), and summary specificity was 99.5% (95% CI 97.9 to 99.9) (Table 3).

Xpert Ultra in nasopharyngeal aspirate specimens

Culture reference standard

Five studies (537 participants) evaluated Xpert Ultra in nasopharyngeal aspirate against culture (Jaganath 2021; Liu 2021; NCT04121026; NCT04240990; Zar 2019). Xpert Ultra sensitivity ranged from 20% to 67%, and specificity from 50% to 99% (Figure 4). In Liu 2021, only one participant did not have tuberculosis, and sensitivity was not estimable. In the remaining four studies (535 participants), Xpert Ultra summary sensitivity was 43.7% (95% CI 26.7 to 62.2), and summary specificity was 97.5% (95% CI 93.6 to 99.0) (Table 3).

Composite reference standard

Two studies (222 participants) evaluated Xpert Ultra in nasopharyngeal aspirate against a composite reference standard (Jaganath 2021; Zar 2019). In Jaganath 2021, Xpert Ultra sensitivity was 23% (95% CI 5 to 54), and specificity was 86% (95% CI 57 to 98); while in Zar 2019, Xpert Ultra sensitivity was 14% (95% CI 9 to 21), and specificity was 100% (95% CI 93 to 100) (Figure 4). Xpert Ultra summary sensitivity was 50.0% (95% CI 31.0 to 69.0), and summary specificity was 98.2% (95% CI 95.4 to 99.3) (Table 3).

Investigations of heterogeneity

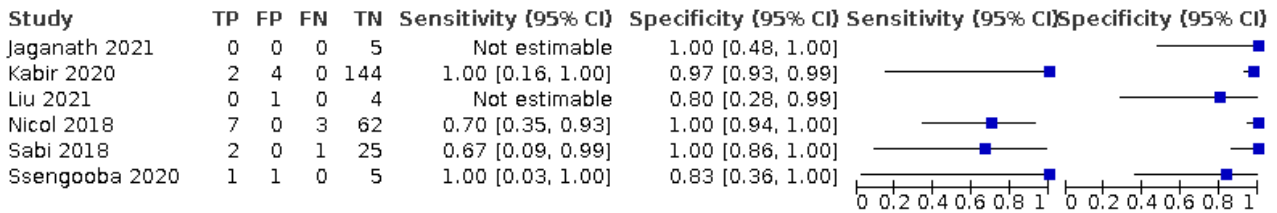
Xpert Ultra accuracy by age group

The analyses for Xpert Ultra sensitivity and specificity by specimen type and age group are based on a small number of studies. For sensitivity and specificity estimates for individual studies, refer to Figure 5 (sputum and gastric aspirate) and Figure 6 (stool and nasopharyngeal aspirate). For summary sensitivity and specificity estimates by specimen type and age group, see Table 4. We describe several key analyses below.

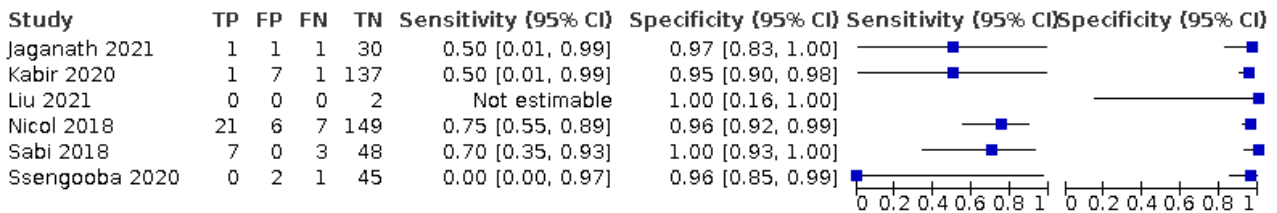
Figure 5. Forest plots of Xpert Ultra sensitivity and specificity in sputum and gastric aspirate for pulmonary tuberculosis by age group, culture reference standard. The squares represent the sensitivity and specificity of each study, the black lines their confidence intervals (CIs).

FN: false negative; FP: false positive; TN: true negative; TP: true positive.

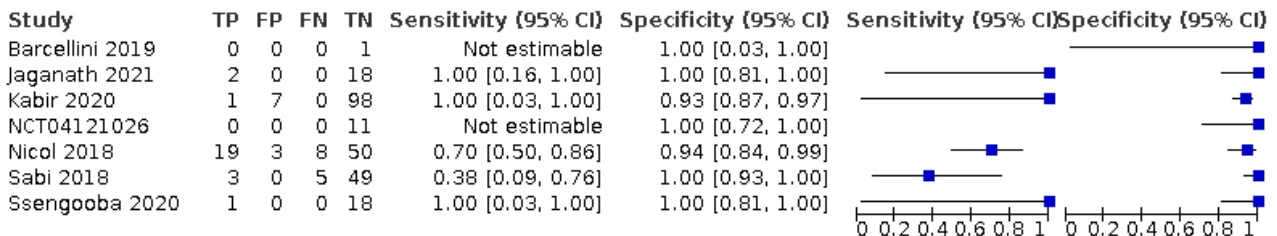
Xpert Ultra, sputum, < 1 year, culture



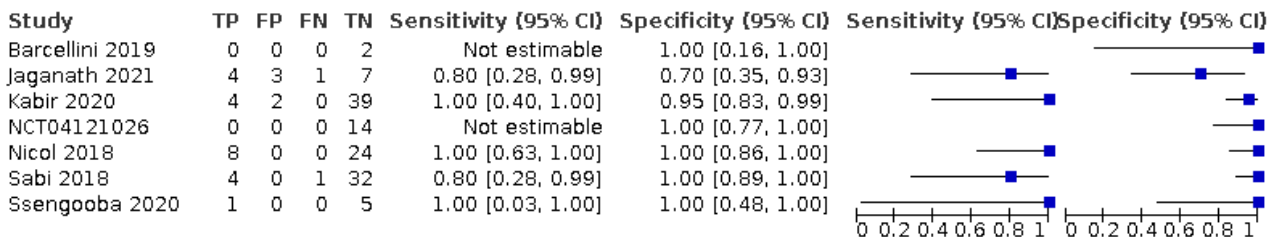
Xpert Ultra, sputum, 1-4 years, culture



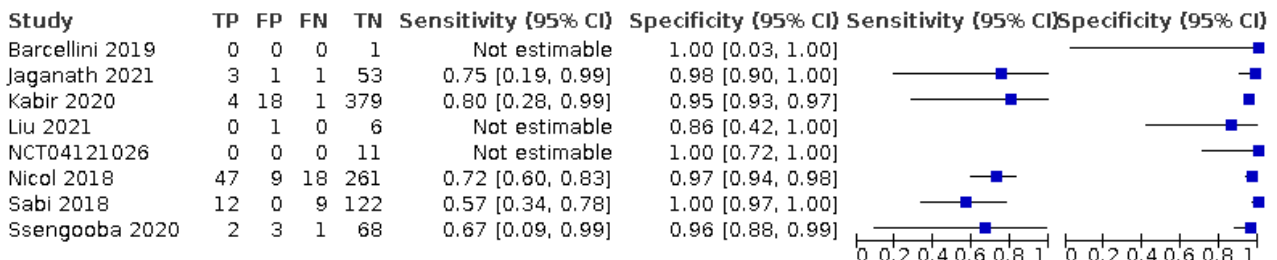
Xpert Ultra, sputum, 5-9 years, culture



Xpert Ultra, sputum, 10-14 years, culture



Xpert Ultra, sputum, 0-9 years, culture



Xpert Ultra, gastric aspirate, < 1 year, culture

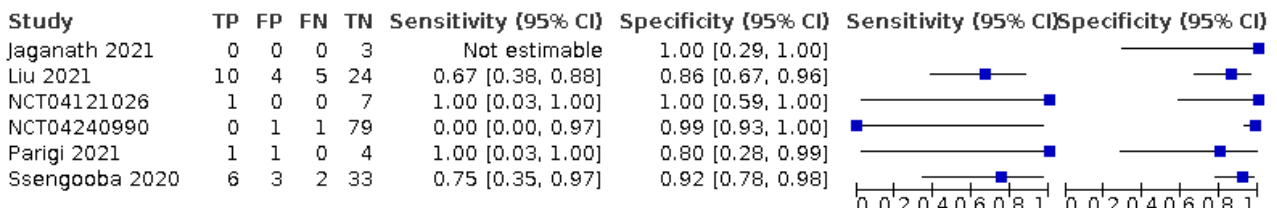


Figure 5. (Continued)

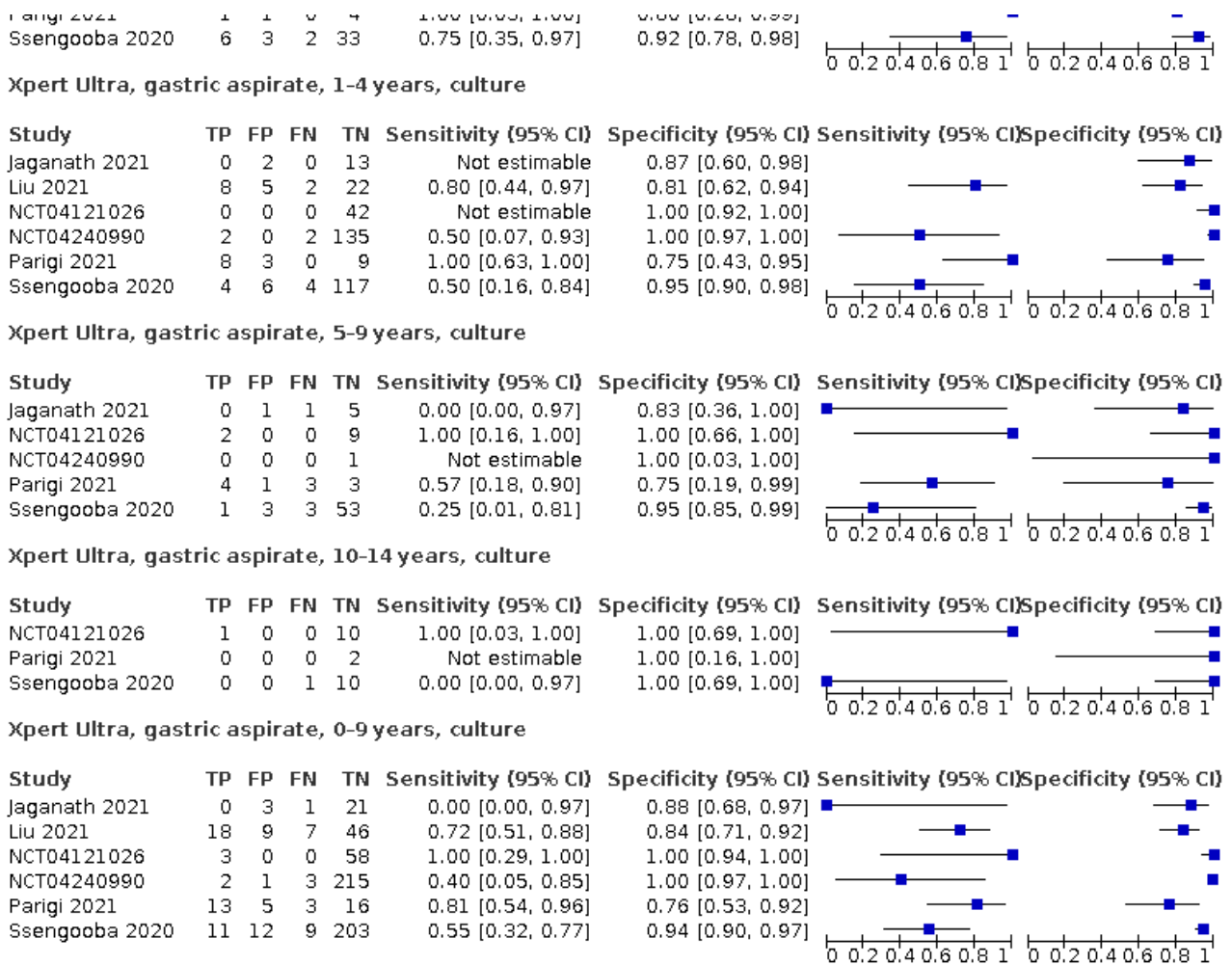


Figure 6. Forest plots of Xpert Ultra sensitivity and specificity in stool and nasopharyngeal specimens for pulmonary tuberculosis by age group, culture reference standard. The squares represent the sensitivity and specificity of each study, the black lines their confidence intervals (CIs).

FN: false negative; FP: false positive; TN: true negative; TP: true positive.

Xpert Ultra, stool, < 1 year, culture

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Kabir 2020	5	12	1	132	0.83 [0.36, 1.00]	0.92 [0.86, 0.96]		
Liu 2021	15	1	7	26	0.68 [0.45, 0.86]	0.96 [0.81, 1.00]		
NCT04121026	1	0	0	7	1.00 [0.03, 1.00]	1.00 [0.59, 1.00]		
NCT04240990	0	1	2	85	0.00 [0.00, 0.84]	0.99 [0.94, 1.00]		

Xpert Ultra, stool, 1-4 years, culture

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Kabir 2020	2	17	7	120	0.22 [0.03, 0.60]	0.88 [0.81, 0.93]		
Liu 2021	7	2	9	22	0.44 [0.20, 0.70]	0.92 [0.73, 0.99]		
NCT04121026	0	0	0	41	Not estimable	1.00 [0.91, 1.00]		
NCT04240990	4	0	1	140	0.80 [0.28, 0.99]	1.00 [0.97, 1.00]		

Xpert Ultra, stool, 5-9 years, culture

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Kabir 2020	4	11	3	87	0.57 [0.18, 0.90]	0.89 [0.81, 0.94]		
Liu 2021	6	1	4	9	0.60 [0.26, 0.88]	0.90 [0.55, 1.00]		
NCT04121026	1	1	1	17	0.50 [0.01, 0.99]	0.94 [0.73, 1.00]		
NCT04240990	0	0	0	1	Not estimable	1.00 [0.03, 1.00]		

Xpert Ultra, stool, 10-14 years, culture

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Kabir 2020	6	3	0	35	1.00 [0.54, 1.00]	0.92 [0.79, 0.98]		
Liu 2021	9	1	3	4	0.75 [0.43, 0.95]	0.80 [0.28, 0.99]		
NCT04121026	0	0	1	10	0.00 [0.00, 0.97]	1.00 [0.69, 1.00]		

Xpert Ultra, stool, 0-9 years, culture

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Kabir 2020	11	40	12	339	0.48 [0.27, 0.69]	0.89 [0.86, 0.92]		
Liu 2021	28	4	20	57	0.58 [0.43, 0.72]	0.93 [0.84, 0.98]		
NCT04121026	2	1	1	65	0.67 [0.09, 0.99]	0.98 [0.92, 1.00]		
NCT04203628	3	0	0	71	1.00 [0.29, 1.00]	1.00 [0.95, 1.00]		
NCT04240990	4	1	3	226	0.57 [0.18, 0.90]	1.00 [0.98, 1.00]		
NCT04899076	23	8	48	313	0.32 [0.22, 0.45]	0.98 [0.95, 0.99]		

Xpert Ultra, nasopharyngeal aspirate, < 1 year, culture

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Jaganath 2021	0	1	0	3	Not estimable	0.75 [0.19, 0.99]		
Liu 2021	0	1	0	0	Not estimable	0.00 [0.00, 0.97]		
NCT04121026	0	0	0	7	Not estimable	1.00 [0.59, 1.00]		
NCT04240990	0	1	1	81	0.00 [0.00, 0.97]	0.99 [0.93, 1.00]		
Zar 2019	2	1	5	36	0.29 [0.04, 0.71]	0.97 [0.86, 1.00]		

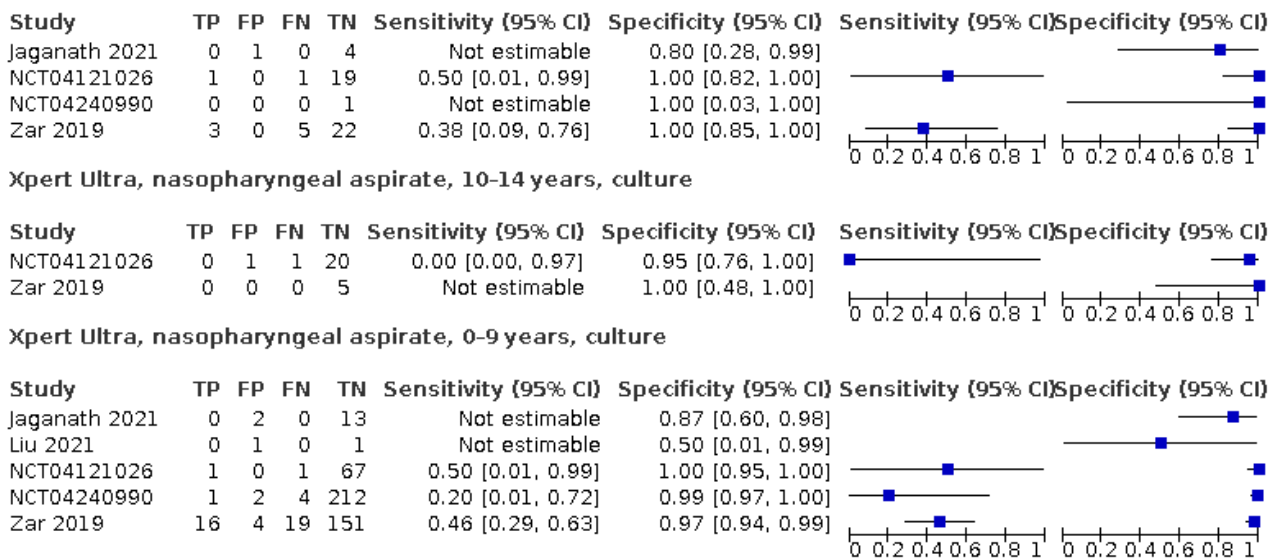
Xpert Ultra, nasopharyngeal aspirate, 1-4 years, culture

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Jaganath 2021	0	0	0	6	Not estimable	1.00 [0.54, 1.00]		
Liu 2021	0	0	0	1	Not estimable	1.00 [0.03, 1.00]		
NCT04121026	0	0	0	41	Not estimable	1.00 [0.91, 1.00]		
NCT04240990	1	1	3	130	0.25 [0.01, 0.81]	0.99 [0.96, 1.00]		
Zar 2019	11	3	9	93	0.55 [0.32, 0.77]	0.97 [0.91, 0.99]		

Xpert Ultra, nasopharyngeal aspirate, 5-9 years, culture

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Jaganath 2021	0	1	0	4	Not estimable	0.00 [0.00, 0.99]		

Figure 6. (Continued)



Sputum specimens, 10 to 14 years

In children aged 10 to 14 years, seven studies (151 participants) evaluated Xpert Ultra in sputum against culture (Barcellini 2019; Jaganath 2021; Kabir 2020; NCT04121026; Nicol 2018; Sabi 2018; Ssengooba 2020). Two studies did not contribute data to the meta-analysis because sensitivity was not estimable (Barcellini 2019; NCT04121026). In the remaining five studies (135 participants), Xpert Ultra summary sensitivity was 91.9% (95% CI 68.7 to 98.3), and summary specificity was 97.7% (95% CI 77.2 to 99.8).

Gastric aspirate specimens, under one year

In children under one year of age, six studies (185 participants) evaluated Xpert Ultra on gastric aspirate specimens against culture (Jaganath 2021; Liu 2021; NCT04121026; NCT04240990; Parigi 2021; Ssengooba 2020). One study did not contribute data to the meta-analysis because sensitivity was not estimable (Jaganath 2021). In the remaining five studies (182 participants), Xpert Ultra summary sensitivity was 67.3% (95% CI 43.5 to 84.6), and summary specificity was 94.0% (95% CI 84.7 to 97.8).

Gastric aspirate specimens, one to four years

In children aged one to four years, six studies (384 participants) evaluated Xpert Ultra on gastric aspirate specimens against culture (Jaganath 2021; Liu 2021; NCT04121026; NCT04240990; Parigi 2021; Ssengooba 2020). Two studies did not contribute data to the meta-analysis because sensitivity was not estimable (Jaganath 2021;

NCT04121026). In the remaining four studies (327 participants), Xpert Ultra summary sensitivity was 71.5% (95% CI 40.0 to 90.4), and summary specificity was 94.0% (95% CI 73.8 to 98.9).

Stool specimens, under one year

In children under one year of age, four studies (295 participants) evaluated Xpert Ultra in stool against culture (Kabir 2020; Liu 2021; NCT04121026; NCT04240990). Xpert Ultra summary sensitivity was 65.2% (95% CI 33.7 to 87.3), and summary specificity was 96.2% (95% CI 88.9 to 98.7).

Stool specimens, one to four years

In children aged one to four years, four studies (372 participants) evaluated Xpert Ultra in stool against culture (Kabir 2020; Liu 2021; NCT04121026; NCT04240990). One study did not contribute data to the meta-analysis because sensitivity was not estimable (NCT04121026). In the remaining three studies (331 participants), Xpert Ultra summary sensitivity was 43.3% (95% CI 27.1 to 61.2), and summary specificity was 97.1% (95% CI 74.8 to 99.7).

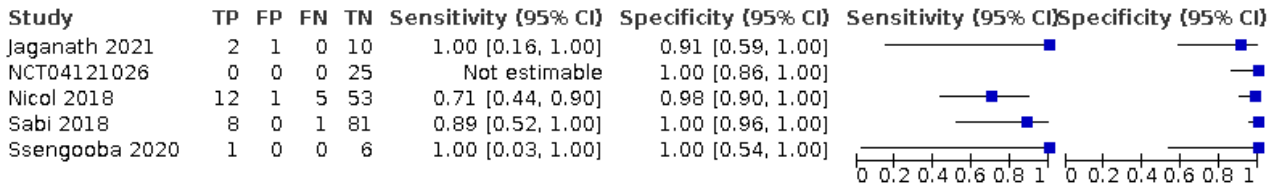
Xpert Ultra accuracy by HIV status

We identified few studies that determined Xpert Ultra accuracy for pulmonary tuberculosis by specimen type in HIV-positive and HIV-negative children. For sensitivity and specificity estimates for individual studies, refer to Figure 7 and for summary sensitivity and specificity, to Table 5. We describe two analyses below.

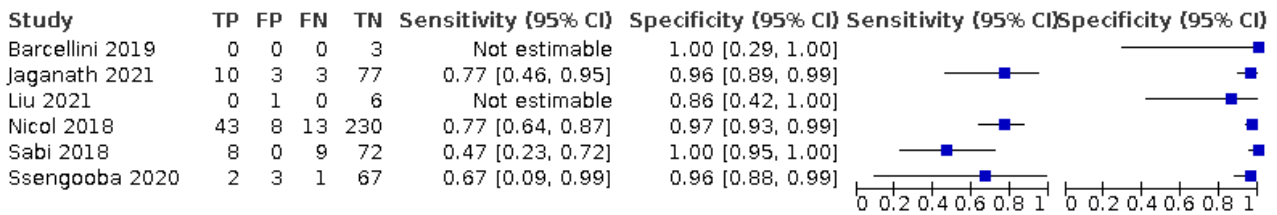
Figure 7. Forest plots of Xpert Ultra sensitivity and specificity for pulmonary tuberculosis by specimen type and HIV status, culture reference standard. The squares represent the sensitivity and specificity of each study, the black lines their confidence intervals (CIs).

FN: false negative; FP: false positive; TN: true negative; TP: true positive.

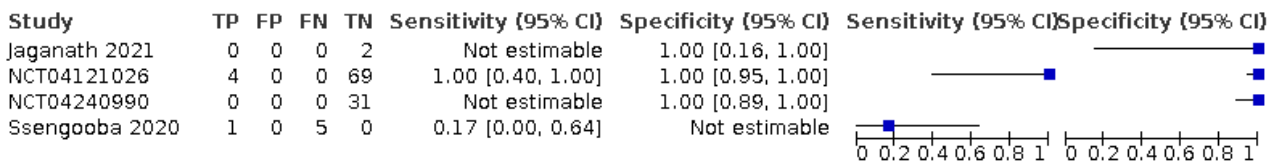
Xpert Ultra, sputum, HIV-positive, all ages, culture



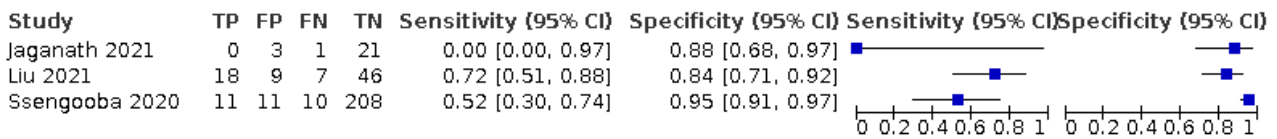
Xpert Ultra, sputum, HIV-negative, all ages, culture



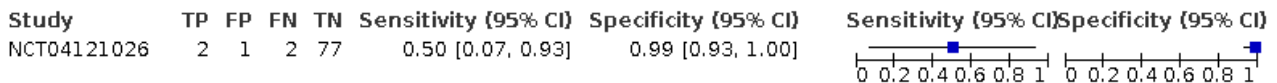
Xpert Ultra, gastric aspirate, HIV-positive, all ages, culture



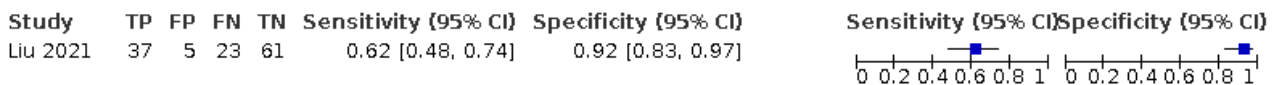
Xpert Ultra, gastric aspirate, HIV-negative, all ages, culture



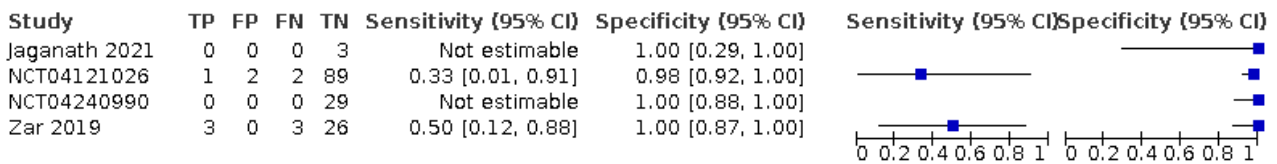
Xpert Ultra, stool, HIV-positive, all ages, culture



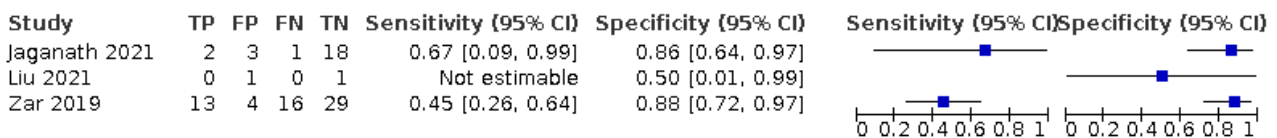
Xpert Ultra, stool, HIV-negative, all ages, culture



Xpert Ultra, nasopharyngeal aspirate, HIV-positive, all ages, culture



Xpert Ultra, nasopharyngeal aspirate, HIV-negative, culture



Sputum specimens, HIV-positive

Five studies (206 participants) evaluated Xpert Ultra in sputum against culture in HIV-positive children aged birth to 14 years (Jaganath 2021; Nicol 2018; NCT04121026; Sabi 2018; Ssengooba 2020). One study did not contribute data to the meta-analysis because sensitivity was not estimable (NCT04121026). In the remaining four studies (181 participants), Xpert Ultra summary sensitivity was 79.5% (95% CI 59.6 to 91.1), and summary specificity was 98.7% (95% CI 93.9 to 99.7).

Sputum specimens, HIV-negative

Six studies (559 participants) evaluated Xpert Ultra in sputum against culture in HIV-negative children aged birth to 14 years

(Barcellini 2019; Jaganath 2021; Liu 2021; Nicol 2018; Sabi 2018; Ssengooba 2020). Two studies did not contribute data to the meta-analysis because sensitivity was not estimable (Barcellini 2019; Liu 2021). In the remaining four studies (549 participants), Xpert Ultra summary sensitivity was 69.6% (95% CI 53.3 to 82.1), and summary specificity was 97.3% (95% CI 94.5 to 98.7).

Xpert Ultra accuracy in other comorbid conditions

We identified few studies that determined Xpert Ultra accuracy for pulmonary tuberculosis by specimen type in children with severe pneumonia or severe malnutrition. For sensitivity and specificity estimates for individual studies, refer to Figure 8, and for summary sensitivity and specificity, to Table 5. We describe two analyses below.

Figure 8. Forest plots of Xpert Ultra sensitivity and specificity for pulmonary tuberculosis by specimen type and comorbidity, culture reference standard. The squares represent the sensitivity and specificity of each study, the black lines their confidence intervals (CIs).

FN: false negative; FP: false positive; TN: true negative; TP: true positive.

Xpert Ultra, sputum, severe pneumonia, all ages, culture

Study	TP	FP	FN	TN	Sensitivity {95% CI}	Specificity {95% CI}	Sensitivity {95% CI}	Specificity {95% CI}
Jaganath 2021	1	0	0	1	1.00 [0.03, 1.00]	1.00 [0.03, 1.00]		
Nicol 2018	3	0	0	8	1.00 [0.29, 1.00]	1.00 [0.63, 1.00]		

Xpert Ultra, sputum, severe malnutrition, all ages, culture

Study	TP	FP	FN	TN	Sensitivity {95% CI}	Specificity {95% CI}	Sensitivity {95% CI}	Specificity {95% CI}
Jaganath 2021	1	0	0	9	1.00 [0.03, 1.00]	1.00 [0.66, 1.00]		
Kabir 2020	4	10	0	173	1.00 [0.40, 1.00]	0.95 [0.90, 0.97]		
Nicol 2018	6	0	1	36	0.86 [0.42, 1.00]	1.00 [0.90, 1.00]		
Sabi 2018	3	0	1	19	0.75 [0.19, 0.99]	1.00 [0.82, 1.00]		
Ssengooba 2020	0	0	1	3	0.00 [0.00, 0.97]	1.00 [0.29, 1.00]		

Xpert Ultra, gastric aspirate, severe malnutrition, all ages, culture

Study	TP	FP	FN	TN	Sensitivity {95% CI}	Specificity {95% CI}	Sensitivity {95% CI}	Specificity {95% CI}
Jaganath 2021	0	1	0	6	Not estimable	0.86 [0.42, 1.00]		
NCT04121026	1	0	0	22	1.00 [0.03, 1.00]	1.00 [0.85, 1.00]		
NCT04240990	2	1	3	215	0.40 [0.05, 0.85]	1.00 [0.97, 1.00]		
Ssengooba 2020	1	0	0	7	1.00 [0.03, 1.00]	1.00 [0.59, 1.00]		

Xpert Ultra, stool, severe malnutrition, all ages, culture

Study	TP	FP	FN	TN	Sensitivity {95% CI}	Specificity {95% CI}	Sensitivity {95% CI}	Specificity {95% CI}
Kabir 2020	10	19	4	153	0.71 [0.42, 0.92]	0.89 [0.83, 0.93]		
NCT04121026	1	0	0	22	1.00 [0.03, 1.00]	1.00 [0.85, 1.00]		
NCT04240990	4	1	3	226	0.57 [0.18, 0.90]	1.00 [0.98, 1.00]		

Xpert Ultra, nasopharyngeal aspirate, severe pneumonia, all ages, composite reference standard

Study	TP	FP	FN	TN	Sensitivity {95% CI}	Specificity {95% CI}	Sensitivity {95% CI}	Specificity {95% CI}
Zar 2019	0	0	4	0	0.00 [0.00, 0.60]	Not estimable		

Xpert Ultra, nasopharyngeal aspirate, severe malnutrition, all ages, culture

Study	TP	FP	FN	TN	Sensitivity {95% CI}	Specificity {95% CI}	Sensitivity {95% CI}	Specificity {95% CI}
Jaganath 2021	0	0	0	2	Not estimable	1.00 [0.16, 1.00]		
NCT04121026	0	0	0	22	Not estimable	1.00 [0.85, 1.00]		
NCT04240990	1	2	4	212	0.20 [0.01, 0.72]	0.99 [0.97, 1.00]		
Zar 2019	2	1	2	19	0.50 [0.07, 0.93]	0.95 [0.75, 1.00]		

Sputum specimens, severe malnutrition

Five studies (267 participants) evaluated Xpert Ultra in sputum against culture in children with severe malnutrition (Jaganath 2021; Kabir 2020; Nicol 2018; Sabi 2018; Ssengooba 2020). Xpert Ultra summary sensitivity was 83.2% (95% CI 54.2 to 95.5), and summary specificity was 98.5% (95% CI 62.6 to 100).

Stool specimens, severe malnutrition

Three studies (443 participants) evaluated Xpert Ultra in stool against culture in children with severe malnutrition (Kabir 2020; NCT04121026; NCT04240990). Xpert Ultra summary sensitivity was 68.2% (46.6 to 84.0) and summary specificity was 98.5% (95% CI 84.2 to 99.9).

Sensitivity analyses

We did not perform a sensitivity analysis for sputum specimens because the only ongoing study (NCT04121026) had no tuberculosis cases and so was not included in the primary analysis. Excluding ongoing studies, meta-analyses were possible for gastric aspirate specimens (5 studies) and stool specimens (2 studies). As expected, we observed differences owing to the number of studies

included in the primary analyses and the sensitivity analyses (Table 6).

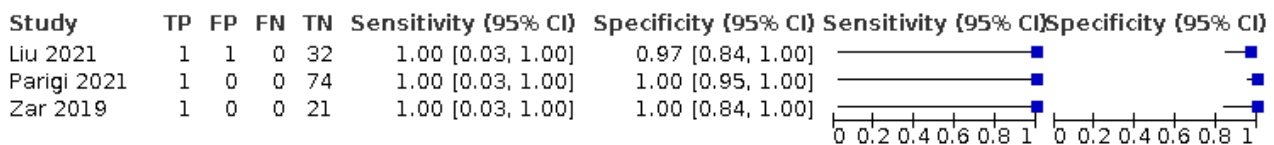
Xpert Ultra trace results

Of the 14 included studies, 13 (93%) reported the number of Xpert Ultra-positive results that were trace results. In these 13 studies, of the total Xpert Ultra-positive results, the proportion (expressed as a percentage) of Ultra trace results ranged from 0% to 67% in studies evaluating sputum; 25% to 67% in studies evaluating gastric aspirate; 0% to 45% in studies evaluating nasopharyngeal aspirate; and 0% to 80% in studies evaluating stool (Table 2).

Detection of rifampicin resistance

We identified three studies that evaluated Xpert Ultra for rifampicin resistance (Liu 2021; Parigi 2021; Zar 2019). Each study included only one child with rifampicin resistance (true positive). In Liu 2021, Xpert Ultra sensitivity was 100% (95% CI 3 to 100), and specificity was 97% (95% CI 84 to 100); in Parigi 2021, Xpert Ultra sensitivity was 100% (95% CI 3 to 100), and specificity was 100% (95% CI 95 to 100); and in Zar 2019, Xpert Ultra sensitivity was 100% (95% CI 3 to 100), and sensitivity was 100% (95% CI 84 to 100) (Figure 9).

Figure 9. Forest plots of Xpert Ultra sensitivity and specificity for rifampicin resistance. The squares represent the sensitivity and specificity of each study, the black lines their confidence intervals (CIs). TP: true positive; FP: false positive; FN: false negative; TN: true negative.



Inconclusive index test results

Non-determinate results for detection of tuberculosis

The percentage of non-determinate results ranged from 0% to 11% of Xpert Ultra tests performed in sputum and from 1% to 10% of Xpert Ultra tests performed in stool. Non-determinate results were not reported or could not be disaggregated from other specimen types for Xpert Ultra tests performed in gastric aspirate and nasopharyngeal aspirate (Table 2).

Indeterminate results for detection of rifampicin resistance

Indeterminate results for detection of rifampicin resistance were common owing to lack of rifampin resistance results with trace results on Xpert Ultra. As a percentage of positive results, trace results represented 0% to 67% for sputum, 26% to 67% for gastric aspirate, 0% to 80% for stool and 0% to 45% for nasopharyngeal aspirate.

DISCUSSION

Summary of main results

This systematic review update summarizes the current literature on the diagnostic accuracy of Xpert Ultra for pulmonary tuberculosis, tuberculous meningitis, lymph node tuberculosis, and rifampicin resistance. Our previously published Cochrane Review assessed the accuracy of both Xpert MTB/RIF and Xpert Ultra (Kay 2020). We

limited the current review update to Xpert Ultra for several reasons. Xpert Ultra has superseded Xpert MTB/RIF and the manufacturer will be discontinuing Xpert MTB/RIF in most countries in 2023. Given the available evidence about Xpert MTB/RIF from our previous review, we therefore only updated Xpert Ultra, as requested by the WHO. The Xpert MTB/RIF text and analyses are available in the last published version of the review (Kay 2020).

In this review update, we identified 14 unique studies, integrating 11 new studies since publication of the previous Cochrane Review (Kay 2020). We did not identify any studies that evaluated Xpert Ultra accuracy for tuberculous meningitis or lymph node tuberculosis. The main results from the review are summarized in Table 3, Summary of findings 1, and Summary of findings 2.

Xpert Ultra accuracy for detection of pulmonary tuberculosis (culture reference standard)

See Summary of findings 1

Sputum, 5 studies

Xpert Ultra summary sensitivity verified by culture was 75.3% (95% CI 64.3 to 83.8; 127 participants; high-certainty evidence), and specificity was 97.1% (95% CI 94.7 to 98.5; 1054 participants; high-certainty evidence).

Gastric aspirate, 7 studies

Xpert Ultra summary sensitivity verified by culture was 70.4% (95% CI 53.9 to 82.9; 120 participants; moderate-certainty evidence), and specificity was 94.1% (95% CI 84.8 to 97.8; 870 participants; moderate-certainty evidence).

Stool, 6 studies

Xpert Ultra summary sensitivity verified by culture was 56.1% (95% CI 39.1 to 71.7; 200 participants; moderate-certainty evidence), and specificity was 98.0% (95% CI 93.3 to 99.4; 1232 participants; high certainty-evidence).

Nasopharyngeal aspirate, 4 studies

Xpert Ultra summary sensitivity verified by culture was 43.7% (95% CI 26.7 to 62.2; 46 participants; very low-certainty evidence), and specificity was 97.5% (95% CI 93.6 to 99.0; 489 participants; high-certainty evidence).

Interpretation of results

In theory, for a population of 1000 children:

- where 100 have pulmonary tuberculosis in sputum (by culture):
 - 101 would be Xpert Ultra-positive, and of these, 26 (26%) would not have pulmonary tuberculosis (false positive); and
 - 899 would be Xpert Ultra-negative, and of these, 25 (3%) would have tuberculosis (false negative).
- where 100 have pulmonary tuberculosis in gastric aspirate (by culture):
 - 123 would be Xpert Ultra-positive, and of these, 53 (43%) would not have pulmonary tuberculosis (false positive); and
 - 877 would be Xpert Ultra-negative, and of these, 30 (3%) would have tuberculosis (false negative).
- where 100 have pulmonary tuberculosis in stool (by culture):
 - 74 would be Xpert Ultra-positive, and of these, 18 (24%) would not have pulmonary tuberculosis (false positive); and
 - 926 would be Xpert Ultra-negative, and of these, 44 (5%) would have tuberculosis (false negative).
- where 100 have pulmonary tuberculosis in nasopharyngeal aspirate (by culture):
 - 66 would be Xpert Ultra-positive, and of these, 22 (33%) would not have pulmonary tuberculosis (false positive); and
 - 934 would be Xpert Ultra-negative, and of these, 56 (6%) would have tuberculosis (false negative).

Xpert Ultra accuracy for detection of pulmonary tuberculosis (composite reference standard)

See [Table 3](#).

Sputum, 5 studies

Xpert Ultra summary sensitivity by composite reference standard was 23.5% (95% CI 20.1 to 27.3; 527 participants) and summary specificity was 99.8% (95% CI 98.8 to 100; 581 participants).

Gastric aspirate, 4 studies

Xpert Ultra summary sensitivity by composite reference standard was 46.5% (95% CI 29.7 to 64.1; 229 participants) and summary specificity was 98.4% (95% CI 91.4 to 99.7; 219 participants).

Stool, 2 studies

Xpert Ultra summary sensitivity by composite reference standard was 50.3% (95% CI 43.3 to 57.1; 199 participants) and summary specificity was 99.5% (95% CI 97.9 to 99.9; 373 participants).

Nasopharyngeal aspirate, 2 studies

Xpert Ultra summary sensitivity by composite reference standard was 50.0% (95% CI 31.0 to 69.0; 24 participants) and summary specificity was 98.2% (95% CI 95.4 to 99.3; 198 participants).

Xpert Ultra accuracy for detection of rifampicin resistance

See [Summary of findings 2](#).

Xpert Ultra sensitivity was 100% (3 studies, 3 participants; very low-certainty evidence), and specificity range was 97% to 100% (3 studies, 128 participants; low-certainty evidence)

Trace results

See [Table 2](#).

Xpert Ultra trace results, regarded as positive in children by WHO standards, were common. Xpert Ultra specificity remained high in children, despite the frequency of trace results.

Xpert Ultra on different types of specimens

Overall, this review adds to the existing body of evidence on the diagnostic accuracy of Xpert Ultra in children. Most notable are the new data on performance of different specimen types that are now being introduced to improve access to diagnostic testing for tuberculosis in children. These findings provide new evidence to shape the development of global practice guidelines for the diagnosis of tuberculosis in children.

Specifically, our review demonstrated differing sensitivities in the different types of specimens. We think that these findings may in part be attributable to differences in the clinical setting and in the quality of the reference standard, mainly the number of cultures used. With respect to the clinical setting, it is more common to collect gastric aspirate specimens in inpatient settings; further, these settings tend to include a higher number of children with advanced disease, which often has a higher microbiological yield ([Marais 2006b](#)). Thus, the higher sensitivity against a culture and composite reference standard for gastric aspirate specimens may in part be due to the inpatient setting and higher likelihood of advanced disease. The diagnostic accuracy for Xpert Ultra in sputum and nasopharyngeal aspirate specimens was similar to that presented in the prior review, and the findings would be unlikely to change the current recommendations for these samples ([WHO Consolidated Guidelines \(Module 3\) 2021](#)).

Against a composite reference standard, we found that Xpert Ultra had a sensitivity that ranged from 23.5% to 50.3% and a specificity of greater than 98.2%. These sensitivity estimates were higher than those reported for Xpert MTB/RIF against a composite reference standard in [Kay 2020](#) and may reflect the increased sensitivity

of Xpert Ultra compared to Xpert MTB/RIF. In adults, Xpert Ultra trace results may be more likely to reflect false-positive results, particularly in people with prior tuberculosis (Dorman 2018). Xpert Ultra trace results were common, with the proportion reported in 13 of the 14 studies and recorded in Table 2. Existing guidance in children suggests that trace results should be treated as true-positive results (WHO Consolidated Guidelines (Module 3) 2021); the test remained highly specific despite the high proportion of trace results.

We found the sensitivity of stool Xpert Ultra to be slightly lower at 56.1% (95% CI 39.1% to 71.7%) than that of sputum Xpert Ultra at 75.3% (95% CI 64.3% to 83.8%) or gastric aspirate Xpert Ultra at 70.4% (95% CI 53.9% to 82.9%). Nonetheless, stool is a promising specimen for diagnosis because, unlike sputum or gastric aspirates, it is non-invasive. Its greatest benefit may be seen in children younger than five years of age owing to the challenges of collecting specimens through sputum induction and gastric aspiration in this population. The sensitivity of stool was 65.2% (95% CI 33.7% to 87.3%) in children younger than one year of age and 43.3% (95% CI 27.1% to 61.2%) in children aged one to four, suggesting the performance is comparable in younger children. We again noted the lack of standardized procedures for processing stool, with each study using a different approach, and could not evaluate diagnostic accuracy by stool processing procedure.

Subgroup analyses

In subgroup analyses, we were limited by the paucity of data and could not compare diagnostic accuracy in HIV-positive and HIV-negative children. Similar to our prior findings with Xpert MTB/RIF (Kay 2020), the limited data did not suggest Xpert Ultra had lower sensitivity in HIV-positive children. The sensitivity of Xpert Ultra in sputum in HIV-positive children of all ages was 79.5% (59.6% to 91.1%), compared with 69.6% (53.3% to 82.1%) in HIV-negative children.

In children with severe malnutrition, the sensitivity of Xpert Ultra was 83.2% (54.2% to 95.5%) in sputum and 68.2% (46.6% to 84.0%) in stool. These estimates were higher than in all children, suggesting that children with severe malnutrition, who, in most settings, are also at high risk for tuberculosis, may represent an ideal population for Xpert Ultra testing. However, these analyses, as well as the analyses in children with severe pneumonia, are based on a small number of studies and participants, so we advise caution in interpretation of the results.

Regarding age group, we found that Xpert Ultra sensitivity in sputum was higher in children aged 10 to 14 years (91.9%, 95% CI 68.7 to 98.3) compared to children of all ages, (75.3%, 95% CI 64.3 to 83.8). We did not find large decreases in the sensitivity of Xpert Ultra in children younger than five years of age in sputum, gastric aspirates or stool compared with all ages. This is an important finding and suggests that Xpert Ultra may perform more comparably in younger children than Xpert MTB/RIF, which has shown decreased sensitivity in younger children (Kay 2020).

Strengths and weaknesses of the review

Completeness of evidence

The data set resulted from comprehensively searching numerous databases, including non-English studies, handsearching references of included studies, and contacting study authors for

additional evidence. We included all identified studies, as well as ongoing studies, from which we could obtain accuracy data. However, we acknowledge that we may have missed some studies despite the comprehensive search and outreach to study authors. We searched for studies for the two most common forms of extrapulmonary tuberculosis (tuberculous meningitis and lymph node tuberculosis) but did not identify studies for these conditions. The review does not include an evaluation of the accuracy of Xpert Ultra in less common forms of child tuberculosis.

Accuracy of the reference standards used

In a systematic review of diagnostic test accuracy studies, the reference standard is the best available test to determine the presence or absence of the target condition. In this review, we included two reference standards: culture and a composite reference standard. Although culture is the best available microbiological reference standard, it is not a perfect reference standard for tuberculosis disease in children owing to the paucibacillary nature of the disease in this population. Some studies performed only one culture and others more than one culture to verify tuberculosis. We considered multiple cultures to be a higher-quality reference standard. We also evaluated the accuracy of Xpert Ultra against a composite reference standard. The accuracy of composite reference standards is also variable and limited, but may reflect the paucibacillary nature of childhood tuberculosis, which is not taken into account when culture positivity is the reference standard for comparison. For all specimen types, Xpert Ultra sensitivity was lower and specificity similar against a composite reference standard compared with culture. If data on tuberculosis treatment were not provided, we accepted the uniform research definitions or the definition used by the primary study authors (study-specific definition) for the composite reference standard. Therefore, clinical characteristics and component tests in the composite reference standard differed across studies, and these differences may have contributed to variation in accuracy estimates.

Quality assessment and quality of reporting of the included studies

We considered risk of bias to be low for the patient selection, index test, and flow and timing domains, and low or unclear for the reference standard domain, because some studies collected only a single specimen for culture. In general, studies were fairly well reported. When data were unclear, or when we needed additional information, we corresponded with all primary study authors. Although the quality of the studies was good, for some analyses by age group and comorbidity, the numbers of studies and participants enrolled were small, limiting our ability to draw definitive conclusions in these circumstances.

Comparison with other systematic reviews

We are aware of two previously published systematic reviews that estimated the diagnostic accuracy of Xpert Ultra for pulmonary tuberculosis and rifampicin resistance in children (Signorino 2022; Zhang 2020).

In Signorino 2022, Xpert Ultra accuracy results were: summary sensitivity 74% (95% CI 66 to 81) and specificity 97% (95% CI 95 to 98) in sputum; summary sensitivity 87% (95% CI 76 to 94) and specificity 85% (95% CI 81 to 89) in gastric aspirate; summary sensitivity 73% (95% CI 59 to 85) and specificity 87% (95% CI 84

to 90) in stool; and summary sensitivity 46% (95% CI 29 to 63) and specificity 97% (95% CI 94 to 99) in nasopharyngeal aspirates. These results were similar to ours with the exception of stool and gastric aspirate, which showed higher sensitivity in [Signorino 2022](#) than in our review. For the accuracy estimates in gastric specimens, [Signorino 2022](#) included only two studies ([Parigi 2021](#); [Sun 2020](#)), whereas we included these two plus another five studies. The accuracy estimates for [Parigi 2021](#) and [Sun 2020](#) the two included studies were similar in both reviews. For stool specimens, [Signorino 2022](#) again included only two studies ([Kabir 2020](#); [Liu 2021](#)), whereas we included these two plus another four studies. The sensitivity estimates for [Kabir 2020](#) and [Liu 2021](#) were higher in [Signorino 2022](#) than in our review; this was likely attributable to the different reference standard for stool. [Signorino 2022](#) used culture on a respiratory specimen as the reference standard for stool, while we used either culture or Xpert Ultra on a respiratory specimen. This difference in reference standard likely also contributed to the lower specificity in [Signorino 2022](#).

[Zhang 2020](#) included only two studies with children, both focused on sputum specimens.

Another review compared the diagnostic accuracy of Xpert Ultra and Xpert MTB/RIF for detection of pulmonary tuberculosis and rifampicin resistance in adults ([Zifodya 2021](#)). For detection of rifampicin resistance, Xpert Ultra summary sensitivity was 94.9% (95% credible interval 88.9 to 97.9), and specificity was 99.1% (95% credible interval 97.7 to 99.8) (5 studies, 921 participants in total, 240 with rifampicin resistance).

Applicability of findings to the review question

To assess the applicability of findings to the review question, we considered QUADAS-2 domains for patient selection, index test, and reference standard. With respect to the patient selection domain, we considered three studies (21%) of high concern because participants were evaluated exclusively as inpatients in tertiary care centres. Studies that take place in referral settings may include patients whose condition is more advanced or more difficult to diagnose than patients seen at lower levels of the health system. With respect to the index test, we considered most studies of low concern regarding applicability. However, we considered applicability of the index test in stool to be unclear, as currently, there is not a standardized protocol for stool testing with Xpert Ultra. With respect to the reference test domain, we considered most studies of low concern regarding applicability.

AUTHORS' CONCLUSIONS

Implications for practice

Xpert Ultra sensitivity (defined by culture) for pulmonary tuberculosis was variable across different specimen types, including sputum, gastric aspirate, stool, and nasopharyngeal aspirate. The highest sensitivity was seen with sputum, followed by gastric aspirate, and the lowest in nasopharyngeal aspirate. Xpert Ultra specificity was high in all specimen types. Sensitivity for Xpert Ultra in stool was lower than in sputum or gastric aspirate, but higher than in nasopharyngeal aspirate. However, the evidence base is still limited, and findings may be imprecise and vary by study setting. Additional data are needed on the differential performance of Xpert Ultra by specimen type to guide recommendations for diagnostic algorithms in children. The sensitivity of Xpert Ultra was

not dramatically reduced in children aged 0 to 4 years, which differs from our prior findings ([Kay 2020](#)), and may represent an advance in the diagnosis of young children with Xpert Ultra compared with Xpert MTB/RIF. Subgroup analyses were limited, but data from sputum and stool specimens in children with severe malnutrition suggest that Xpert Ultra performs well, with a sensitivity that was markedly higher than in all children for both specimens. This high risk group would benefit from access to Xpert Ultra testing to complement treatment for severe malnutrition.

Although we found Xpert Ultra to be accurate for detection of rifampicin resistance, the results were based on a very small number of studies that included only three children with rifampicin resistance. Findings should, therefore, be interpreted with caution.

Evidence in this review is based mainly on culture as the reference standard, and we calculated Xpert Ultra accuracy on the assumption that the reference standard is 100% sensitive and specific. Although culture is acceptable, it is an imperfect reference standard for child tuberculosis. Without a more accurate reference standard, with a limit of detection low enough to detect paucibacillary tuberculosis, the accuracy of novel diagnostic tests for tuberculosis in children will remain difficult to estimate. Despite the presence of a negative Xpert Ultra result, clinicians will still need to consider tuberculosis treatment in children with a high suspicion of tuberculosis or at high risk of a poor outcome. The percentage of non-determinate results ranged from 0% to 11% in the studies of Xpert Ultra, and tended to be slightly higher in stool specimens. This increased percentage of non-determinate results should be considered when using stool as a diagnostic specimen.

The evidence from [Kay 2020](#) informed Module 3 of the WHO Consolidated Guidelines on Tuberculosis ([WHO Consolidated Guidelines \(Module 3\) 2021](#)), and the current review update informed module 5 ([WHO Consolidated Guidelines \(Module 5\) 2022](#)). Specific recommendations from those guidelines, with implications for practice, are presented in [Table 1](#).

Implications for research

There are several areas for which additional research regarding the diagnostic accuracy of molecular tests in children is necessary. There is a need for:

- data to evaluate how Xpert Ultra impacts patient-important outcomes in children and how Xpert Ultra diagnostic accuracy changes when multiple specimen types are evaluated;
- studies that evaluate the accuracy of Xpert Ultra for detecting extrapulmonary tuberculosis in children. This is particularly relevant given the encouraging results regarding Xpert Ultra performance in cerebrospinal fluid obtained from adults ([Kohli 2021](#));
- more research to identify an improved reference standard that accurately defines tuberculosis in children;
- accurate tests performed at the point of care;
- additional operational and qualitative research to determine the best approach to less invasive specimen collection;
- implementation studies on a method of suction for nasopharyngeal aspiration that is appropriate for low-skill or low-resource environments;
- additional operational research concerning the use of stool as a diagnostic specimen. These studies should address

integration into normal diagnostic clinical pathways, definition of laboratory protocols – including processing methods – that successfully balance ease of implementation and diagnostic performance, and the impact of stool testing on patient-important outcomes;

- qualitative research identifying child and family preferences for and acceptability of comparative diagnostic approaches and specimen collection procedures.

We underscore the continued urgent need to develop new tools that accurately diagnose tuberculosis in children. Ideally, these new tools will be rapid, affordable, feasible, and acceptable to children and their parents.

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Editorial and peer-reviewer contributions

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- Contact Editors: Professor Gerry Davies (CIDG); Dr Danielle van der Windt (DTA).
- Sign-off Editor (final editorial decision): Professor Paul Garner (CIDG).
- Managing Editor (collated Editors' comments, provided editorial guidance to authors, edited the article): Dr Deirdre Walshe (CIDG).
- Copy Editor (copy editing and production): Julia Turner.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Barcellini 2019

Study characteristics

Patient Sampling	Cross-sectional, consecutive enrolment
Patient characteristics and setting	<p>Presenting signs and symptoms: screening cohort but 891 participants had at least one positive TB symptom, only 3 of whom were children</p> <p>Age: median 10 years</p> <p>Sex, female: 33%</p> <p>HIV infection: 0%</p> <p>Sample size included for analysis: 3</p> <p>Clinical setting: outpatient</p> <p>Laboratory level where index test was performed: central reference laboratory</p> <p>Country: Italy</p> <p>World Bank income classification: high income</p> <p>TB high-burden country: no</p> <p>TB/HIV high-burden country: no</p> <p>MDR-TB high-burden country: no</p> <p>Prevalence of TB cases in the study: 0/3 (0%)</p>
Index tests	Xpert Ultra in sputum
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (liquid and solid culture)
Flow and timing	Index and reference tests were collected within pre-specified time period
Comparative	
Notes	This was a nested diagnostic evaluation within a screening study. Only 3 children screened positive, and of those, all were evaluated.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	

Barcellini 2019 (Continued)

Are there concerns that the included patients and setting do not match the review question? Low concern

DOMAIN 2: Index Test (Xpert Ultra)

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

If a threshold was used, was it pre-specified? Yes

Could the conduct or interpretation of the index test have introduced bias? Low risk

Are there concerns that the index test, its conduct, or interpretation differ from the review question? Low concern

DOMAIN 3: Reference Standard

Is the reference standards likely to correctly classify the target condition? Yes

Were the reference standard results interpreted without knowledge of the results of the index tests? Yes

Could the reference standard, its conduct, or its interpretation have introduced bias? Low risk

Are there concerns that the target condition as defined by the reference standard does not match the question? Low concern

DOMAIN 4: Flow and Timing

Was there an appropriate interval between index test and reference standard? Yes

Did all patients receive the same reference standard? Yes

Were all patients included in the analysis? Yes

Could the patient flow have introduced bias? Low risk

Jaganath 2021
Study characteristics

Patient Sampling Prospective cohort, consecutive enrolment

Patient characteristics and setting Presenting signs and symptoms: cough for ≥ 1 week and ≥ 2 of the following: unexplained weight loss or failure to thrive; unexplained fever for ≥ 1 week; unexplained lethargy or reduced playfulness for ≥ 1 week; an abnormal CXR; or contact with an individual with pulmonary TB
 Age: median 3.9 (IQR 1.5–7) years
 Sex, female: 47.9%
 HIV infection: 13%

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Jaganath 2021 (Continued)

Sample size included for analysis: 213
 Clinical setting: outpatient and inpatient
 Laboratory level where index test was performed: academic hospital
 Country: Uganda
 World Bank income classification: low income
 TB high-burden country: no
 TB/HIV high-burden country: yes
 MDR-TB high-burden country: no
 Prevalence of TB cases in the study: 23 (10.8%) confirmed TB, 88 (41.3%) unconfirmed TB

Index tests	Xpert Ultra in sputum, gastric, and nasopharyngeal specimens
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (liquid and solid culture) and composite reference standard
Flow and timing	Index and reference tests were collected within pre-specified time period
Comparative	
Notes	The exclusions accounted for a low percentage of the total study population

Methodological quality

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

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Jaganath 2021 (Continued)

Were the reference standard results interpreted without knowledge of the results of the index tests? Yes

Could the reference standard, its conduct, or its interpretation have introduced bias? Low risk

Are there concerns that the target condition as defined by the reference standard does not match the question? Low concern

DOMAIN 4: Flow and Timing

Was there an appropriate interval between index test and reference standard? Yes

Did all patients receive the same reference standard? Yes

Were all patients included in the analysis? No

Could the patient flow have introduced bias? Low risk

Kabir 2020
Study characteristics

Patient Sampling Cross-sectional, consecutive enrolment

Patient characteristics and setting Presenting signs and symptoms: persistent, nonremitting cough for > 2 weeks not responding to conventional antibiotics; persistent documented fever (> 38°C/100.4°F) for > 2 weeks; documented weight loss or not gaining weight adequately during the past 3 months; and fatigue, reduced playfulness, and decreased activity
 Age: 0–4 years: 296 (66.2%) children, 5–9 years: 105 (23.5%) children, 10–14 years: 46 (10.3%) children
 Sex, female: 193 (42%)
 HIV infection: not reported
 Sample size included for analysis: 447
 Clinical setting: exclusively inpatient tertiary/specialized hospital
 Laboratory level where index test was performed: academic hospital
 Country: Bangladesh
 World Bank income classification: lower middle income
 TB high-burden country: yes
 TB/HIV high-burden country: no
 MDR-TB high-burden country: yes
 Prevalence of TB cases in the study: 29 (6.5%) confirmed by reference standard, 72 (16.1%) confirmed by reference standard or index test, 39 (8.9%) diagnosed by composite reference standard, 111 (24.8%) total cases by microbiologic or composite reference standard

Index tests Xpert Ultra in sputum and stool

Target condition and reference standard(s) Pulmonary TB; microbiological reference standard (solid culture) and composite reference standard

Flow and timing Index and reference tests were collected within pre-specified time period

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Kabir 2020 (Continued)

Comparative

Notes	Only 7 participants were excluded from the main analysis because they could not provide stool.
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Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (Xpert Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Unclear
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Unclear		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Unclear
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	No		

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Kabir 2020 (Continued)

Could the patient flow have introduced bias?

Low risk

Liu 2021
Study characteristics

Patient Sampling	Prospective cohort, consecutive enrolment
Patient characteristics and setting	<p>Presenting signs and symptoms: pulmonary lesions in chest imaging (X-ray or CT scan) including inflammatory infiltration, nodules, cavities, and mediastinal lymphadenopathy, irrespective of close TB exposure history or immunologic evidence (tuberculin skin test or interferon gamma release assay)</p> <p>Age: mean 4.15 (SD 4.16) years</p> <p>Sex, female: 55/126 (44%)</p> <p>HIV infection: 0/126 (0%)</p> <p>Sample size included for analysis: 311</p> <p>Clinical setting: both inpatient and outpatient</p> <p>Laboratory level where index test was performed: intermediate laboratory</p> <p>Country: China</p> <p>World Bank income classification: upper middle income</p> <p>TB high-burden country: yes</p> <p>TB/HIV high-burden country: yes</p> <p>MDR-TB high-burden country: yes</p> <p>Prevalence of TB cases in the study: 53.2% confirmed TB, 16.7% unconfirmed</p>
Index tests	Xpert Ultra in sputum, gastric, stool, and nasopharyngeal specimens
Target condition and reference standard(s)	<p>Pulmonary TB; microbiological reference standard (liquid culture performed on a sputum or gastric specimen)</p> <p>Rifampicin resistance; MGIT drug susceptibility testing</p>
Flow and timing	Index and reference tests were collected within pre-specified time period
Comparative	
Notes	<p>Most enrolled children were not included in the analysis. Participants needed to be willing to provide a stool sample for the purposes of the study and have routine testing respiratory samples, including Xpert, culture, or smear for acid-fast bacilli. Participants with a definite diagnosis of TB were excluded. Other exclusion criteria included inadequate or invalid sample for the parallel assays, incomplete clinical data or indeterminate clinical diagnosis, and history of anti-TB therapy</p>

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Liu 2021 (Continued)

Did the study avoid inappropriate exclusions?	Yes	
Could the selection of patients have introduced bias?		Low risk
Are there concerns that the included patients and setting do not match the review question?		Low concern
DOMAIN 2: Index Test (Xpert Ultra)		
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes	
If a threshold was used, was it pre-specified?	Yes	
Could the conduct or interpretation of the index test have introduced bias?		Low risk
Are there concerns that the index test, its conduct, or interpretation differ from the review question?		Low concern
DOMAIN 3: Reference Standard		
Is the reference standards likely to correctly classify the target condition?	Unclear	
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes	
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk
Are there concerns that the target condition as defined by the reference standard does not match the question?		Unclear
DOMAIN 4: Flow and Timing		
Was there an appropriate interval between index test and reference standard?	Yes	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	No	
Could the patient flow have introduced bias?		High risk

NCT04121026
Study characteristics

Patient Sampling	Prospective cohort, consecutive enrolment
Patient characteristics and setting	Presenting signs and symptoms: ≥ 1 criteria among the following: persistent cough > 2 weeks; persistent fever > 2 weeks; recent failure to

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

NCT04121026 (Continued)

thrive (documented clear deviation from a previous growth trajectory in the last 3 months or Z score weight/age < 2); failure of broad spectrum antibiotics for treatment of pneumonia; suggestive CXR features or history of contact with a TB case and any of several symptoms listed above with a shorter duration (< 2 weeks)
 Age: median 5.46 (IQR 2.02–9.86) years
 Sex, female: 47.60%
 HIV infection: 100% (all participants HIV positive)
 Sample size included for analysis: 124
 Clinical setting: both inpatient and outpatient
 Laboratory level where index test was performed: central reference laboratory
 Countries: Côte d'Ivoire, Uganda, Mozambique, and Zambia
 World Bank income classification: Côte d'Ivoire, Zambia: lower middle income; Mozambique, Uganda: low income
 TB high-burden country: only Mozambique and Zambia
 TB/HIV high-burden country: only Mozambique, Uganda, and Zambia
 MDR-TB high-burden country: only Mozambique
 Prevalence of TB cases in the study: 5%
 Stage of study when the data were provided: recruitment ongoing

Index tests	Xpert Ultra in sputum, gastric aspirate, stool, and nasopharyngeal aspirate
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (multiple liquid cultures)
Flow and timing	
Comparative	
Notes	

Methodological quality

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

NCT04121026 (Continued)

Could the conduct or interpretation of the index test have introduced bias?

Low risk

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

Low concern

DOMAIN 3: Reference Standard

Is the reference standards likely to correctly classify the target condition? Yes

Were the reference standard results interpreted without knowledge of the results of the index tests? Yes

Could the reference standard, its conduct, or its interpretation have introduced bias?

Low risk

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

Low concern

DOMAIN 4: Flow and Timing

Was there an appropriate interval between index test and reference standard? Yes

Did all patients receive the same reference standard? Yes

Were all patients included in the analysis? Yes

Could the patient flow have introduced bias?

Low risk

NCT04203628
Study characteristics

Patient Sampling

Prospective cohort, consecutive enrolment

Patient characteristics and setting

Presenting signs and symptoms: clinical suspicion of active pulmonary TB, irrespective of extrapulmonary disease (CXR suggestive of TB; or weight loss or failure to thrive within 3 months not solely due to inadequate feeding, or another non-TB cause; or any cough with loss of weight; or cough alone \geq 14 days or persistent (> 1 week) and unexplained fever) or microbiological confirmation of active TB disease referred from non-study health facilities

Age: median 1.5 (IQR 0.9–3.8) years

Sex, female: 47%

HIV infection: 4/111 (3.6%)

Sample size included for analysis: 111

Clinical setting: outpatient and inpatient

Laboratory level where index test was performed: academic hospital

Countries: Uganda and Zambia

World Bank income classification: low income

TB high-burden country: yes

TB/HIV high-burden country: yes

MDR-TB high-burden country: no

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

NCT04203628 (Continued)

Prevalence of TB cases in the study: 3 (5%) confirmed TB

Stage of study when the data were provided: recruitment ongoing

Index tests	Xpert Ultra in stool
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (multiple liquid cultures)
Flow and timing	Index and reference tests were performed within the pre-specified time period
Comparative	
Notes	The exclusions accounted for a low percentage of the total study population

Methodological quality

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

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

52

NCT04203628 (Continued)

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

Low concern

DOMAIN 4: Flow and Timing

Was there an appropriate interval between index test and reference standard? Yes

Did all patients receive the same reference standard? Yes

Were all patients included in the analysis? No

Could the patient flow have introduced bias?

Low risk

NCT04240990
Study characteristics

Patient Sampling Prospective cohort, consecutive enrolment

 Patient characteristics and setting
 Presenting signs and symptoms: severe acute malnutrition defined as weight-for-height Z score (WHZ) < -3 SD or mid-upper arm circumference < 115 mm (in children > 6 months) or clinical signs of bilateral pitting oedema. Usual criteria for hospitalization of children with severe acute malnutrition recommended by the WHO include: medical complications including sepsis and dehydration, severe oedema, poor appetite, and presentation of ≥ 1 'Integrated Management of Childhood Illness' danger signs (unable to drink or breastfeed; vomiting everything; > 1 or prolonged convulsions lasting > 15 min; lethargic or unconscious; convulsing now)
 Age: median 1.21 (IQR 0.83–1.60) years
 Sex, female: 40.50%
 HIV infection: 14.80%
 Sample size included for analysis: 257
 Clinical setting: exclusively inpatient tertiary/specialized hospital
 Laboratory level where index test was performed: central reference laboratory
 Countries: Uganda, Zambia
 World Bank income classification: Zambia: lower middle income; Uganda: low income
 TB high-burden country: only Zambia
 TB/HIV high-burden country: yes
 MDR-TB high-burden country: no
 Prevalence of TB cases in the study: 3%
 Stage of study when the data were provided: recruitment ongoing

Index tests Xpert Ultra in gastric aspirate, stool, and nasopharyngeal aspirate

Target condition and reference standard(s) Pulmonary TB; microbiological reference standard (multiple liquid cultures)

Flow and timing

Comparative

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

53

NCT04240990 (Continued)

Notes

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (Xpert Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Unclear
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

NCT04899076

Study characteristics

Patient Sampling	Prospective cohort, consecutive enrolment and referral
Patient characteristics and setting	<p>Presenting signs and symptoms: clinical suspicion of active pulmonary TB, irrespective of extrapulmonary disease (CXR suggestive of TB; or weight loss or failure to thrive within 3 months not solely due to inadequate feeding, or another non-TB cause; or any cough with loss of weight; or cough alone \geq 14 days or persistent (> 1 week) and unexplained fever) or microbiological confirmation of active TB disease referred from non-study health facilities</p> <p>Age: mean 4.62 years Sex, female: 48.5% HIV infection: 19.7% Sample size included for analysis: 486 Clinical setting: outpatient and inpatient Laboratory level where index test was performed: academic hospital Countries: India, Uganda, South Africa World Bank income classification: India: lower middle income; Uganda: low income; South Africa: upper middle income TB high-burden country: only India and South Africa TB/HIV high-burden country: yes MDR-TB high-burden country: only India and South Africa Prevalence of TB cases in the study: 70 (17.8%) confirmed TB</p> <p>Stage of study when the data were provided: recruitment completed</p>
Index tests	Xpert Ultra in stool
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (liquid culture)
Flow and timing	
Comparative	
Notes	Children had cultures and Xpert Ultra performed in gastric aspirate and sputum specimens as a reference standard

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (Xpert Ultra)			

NCT04899076 (Continued)

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

Nicol 2018
Study characteristics

Patient Sampling	Prospective cohort, manner of selection not reported, with analysis of frozen specimens
Patient characteristics and setting	Presenting signs and symptoms: cough lasting > 2 weeks and ≥ 1 of the following: household TB contact in previous 3 months; weight loss or failure to gain weight in previous 3 months; positive tuberculin skin test; or chest radiograph suggestive of pulmonary TB Age: median 33 (IQR 15–74) months Sex, female: 49% HIV infection: 19% Sample size included for analysis: 367 Clinical setting: both inpatient and outpatient Laboratory level where index test was performed: research laboratory Country: South Africa

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Nicol 2018 (Continued)

 World Bank income classification: middle income
 TB high-burden country: yes
 TB/HIV high-burden country: yes
 MDR-TB high-burden country: yes
 Prevalence of TB cases in the study: 20%

Index tests	Xpert Ultra in sputum
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (MGIT) and composite reference standard
Flow and timing	Index and reference tests were performed within pre-specified time period
Comparative	
Notes	The index test was performed on frozen specimens from a previously enrolled cohort

Methodological quality

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

Nicol 2018 (Continued)

Could the reference standard, its conduct, or its interpretation have introduced bias?

Low risk

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

Low concern

DOMAIN 4: Flow and Timing

Was there an appropriate interval between index test and reference standard?

Yes

Did all patients receive the same reference standard?

Yes

Were all patients included in the analysis?

Yes

Could the patient flow have introduced bias?

Low risk

Parigi 2021
Study characteristics

Patient Sampling

Unclear

Patient characteristics and setting

Presenting signs and symptoms: clinical suspicion of pulmonary TB
 Age: median 81.8 months
 Sex, female: not available
 HIV infection: not available
 Sample size included for analysis: 67
 Clinical setting: exclusively inpatient tertiary/specialized hospital
 Laboratory level where index test was performed: academic hospital
 Country: Italy
 World Bank income classification: high income
 TB high-burden country: no
 TB/HIV high-burden country: no
 MDR-TB high-burden country: no
 Prevalence of TB cases in the study: 82.6%

Index tests

Xpert Ultra in gastric specimen

Target condition and reference standard(s)

Pulmonary TB; culture type not specified and composite reference standard
 Rifampicin resistance; drug susceptibility testing

Flow and timing

Index and reference tests were performed within pre-specified time period

Comparative

Notes

Methodological quality
Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Parigi 2021 (Continued)

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Unclear risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (Xpert Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Unclear
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Sabi 2018
Study characteristics

Patient Sampling	Prospective cohort, consecutive enrolment, with analysis of frozen specimens
Patient characteristics and setting	<p>Presenting signs and symptoms: 1 of the following symptoms: persistent non-remitting cough > 14 days not responding to antibiotics; repeated episodes of fever within the last 14 days not responding to antibiotics, after malaria has been excluded; weight loss or failure to thrive during previous 3 months; signs and symptoms suggestive of extrapulmonary TB</p> <p>Age: median 65 (IQR 18–120) months Sex, female: 43% HIV infection: 52% Sample size included for analysis: 215 Clinical setting: both inpatient and outpatient Laboratory level where index test was performed: academic hospital Country: Tanzania World Bank income classification: lower middle income TB high-burden country: no TB/HIV high-burden country: yes MDR-TB high-burden country: no Prevalence of TB cases in the study: 13%</p>
Index tests	Xpert Ultra in sputum
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (solid (LJ) and liquid (MGIT) culture) and composite reference standard
Flow and timing	Index and reference tests were collected within pre-specified time period
Comparative	
Notes	Xpert Ultra was performed on frozen specimens

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (Xpert Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Sabi 2018 (Continued)

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

Ssengooba 2020
Study characteristics

Patient Sampling	Prospective cohort, consecutive enrolment, with analysis of frozen specimens
Patient characteristics and setting	Presenting signs and symptoms: symptomatic but non-severe TB including extrathoracic lymph node TB; intrathoracic uncomplicated (hilar) lymph node TB; minimal or no parenchymal abnormality on CXR; smear-negative on gastric aspirate/other respiratory sample Age: median 2.8 (IQR 1.2–5.3) years Sex, female: 52% HIV infection: 8.5% Sample size included for analysis: 398 Clinical setting: unclear Laboratory level where index test was performed: academic hospital Country: Uganda World Bank income classification: low income TB high-burden country: no TB/HIV high-burden country: yes

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Ssengooba 2020 (Continued)

	MDR-TB high-burden country: no Prevalence of TB cases in the study: 18.7% with confirmed pulmonary TB by the reference tests
Index tests	Xpert Ultra in sputum and gastric aspirate
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (solid (LJ) and liquid (MGIT) culture)
Flow and timing	Index and reference tests were collected within pre-specified time period
Comparative	
Notes	Symptomatic children who provided samples in the trial screening process but were not subsequently enrolled in the clinical trial were also eligible for inclusion in this substudy. A low percentage (~10%) of enrolled participants were not included in the analysis

Methodological quality

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

Ssengooba 2020 (Continued)

Could the reference standard, its conduct, or its interpretation have introduced bias?

Low risk

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

Low concern

DOMAIN 4: Flow and Timing

Was there an appropriate interval between index test and reference standard? Yes

Did all patients receive the same reference standard? Yes

Were all patients included in the analysis? No

Could the patient flow have introduced bias?

Low risk

Sun 2020
Study characteristics

Patient Sampling	Prospective cohort, consecutive enrolment, with analysis of frozen specimens
Patient characteristics and setting	<p>Presenting signs and symptoms: thought to have TB based on cough lasting > 2 weeks, weight loss, malnutrition, HIV, TB contact, or positive chest radiograph in accordance with the China and WHO guidelines</p> <p>Age: median 6.4 (IQR 2.1–10.7) years Sex, female: 44% HIV infection: not available Sample size included for analysis: 302 Clinical setting: unclear Laboratory level where index test was performed: unclear Country: China World Bank income classification: upper middle income TB high-burden country: yes TB/HIV high-burden country: yes MDR-TB high-burden country: yes Prevalence of TB cases in the study: 16% with confirmed pulmonary TB by the reference tests, 43% meeting a composite definition for TB</p>
Index tests	Xpert Ultra in gastric specimens
Target condition and reference standard(s)	Pulmonary TB; microbiological reference standard (solid (LJ) and liquid (MGIT) culture) and composite reference standard
Flow and timing	Index and reference tests were collected within pre-specified time period
Comparative	
Notes	

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

63

Sun 2020 (Continued)

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Unclear
DOMAIN 2: Index Test (Xpert Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Unclear
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	No		
Could the patient flow have introduced bias?		Low risk	

Zar 2019

Study characteristics

Patient Sampling	Cohort, consecutive, prospective with analysis of frozen specimens
Patient characteristics and setting	<p>Presenting signs and symptoms: cough lasting > 2 weeks and at least 1 of the following: household TB contact in previous 3 months, weight loss or failure to gain weight in previous 3 months, positive tuberculin skin test, or chest radiograph suggestive of pulmonary TB</p> <p>Age: median 23.3 (IQR 13.5–47.3) months</p> <p>Sex, female: not reported</p> <p>HIV infection: 16%</p> <p>Sample size included for analysis: 195</p> <p>Clinical setting: inpatient</p> <p>Laboratory level where index test was performed: intermediate</p> <p>Country: South Africa</p> <p>World Bank income classification: upper middle income</p> <p>TB high-burden country: yes</p> <p>TB/HIV high-burden country: yes</p> <p>MDR-TB high-burden country: yes</p> <p>Prevalence of TB cases in the study: 21%</p>
Index tests	Xpert Ultra in nasopharyngeal aspirate
Target condition and reference standard(s)	<p>Pulmonary TB; microbiological reference standard (MGIT) and composite reference standard</p> <p>Rifampicin resistance; LPA (MTBDRplus)</p>
Flow and timing	Index and reference tests were collected within pre-specified time period
Comparative	
Notes	Xpert Ultra test was performed on frozen specimens

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (Xpert Ultra)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Zar 2019 (Continued)

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

CXR: chest X-ray; IQR: interquartile range; LJ: Löwenstein-Jensen; LPA: line probe assay; MDR-TB: multidrug-resistant tuberculosis; MGIT: mycobacteria growth indicator tube; SD: standard deviation; TB: tuberculosis.

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Ali 2017	Unable to separate paediatric data from adult data
Atashi 2017	Adult population
Atehortúa Muñoz 2017	Not a diagnostic accuracy study
Azevedo 2018	Adult population
Ballif 2015	Not a diagnostic accuracy study
Banada 2016	Case-control study
Biadlegne 2014	Unable to separate paediatric data from adult data
Bojang 2016	Unable to separate paediatric data from adult data

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

Study	Reason for exclusion
Che 2017	Adult population
Cox 2014	Adult population
Cross 2014	Unable to separate paediatric data from adult data
Diallo 2016	Unable to separate paediatric data from adult data
DiNardo 2016	Not a diagnostic accuracy study
DiNardo 2018	Index test not studied
Ejeh 2018	Unable to separate paediatric data from adult data
Gautam 2018	Unable to separate paediatric data from adult data
Gelalcha 2017	Unable to separate paediatric data from adult data
Geleta 2015	Adult population
Ghariani 2015	Unable to separate paediatric data from adult data
Giang 2015	Unable to extract data by sample type
Guajardo-Lara 2018	Insufficient data
Gulla 2019	Not a diagnostic accuracy study
Hakim 2017	Not a diagnostic accuracy study
Helb 2010	Adult population
Horo 2017	Unable to separate paediatric data from adult data
Huh 2014	Adult population
Kuyinu 2018	Inappropriate reference standard
Lopez 2019	Index text not studied
Lu J 2017	Screening for clinical tuberculosis before enrolment
Lu Y 2018	Adult population
Malik 2018	Not a diagnostic accuracy study
Marcy 2018	Duplicate data for Marcy 2016
Masenga 2017	Unable to separate paediatric data from adult data
Mekonnen 2015	Adult population
Memon 2018	Clinical diagnosis of tuberculosis established at enrolment
Metaferia 2018	Inappropriate reference standard

Study	Reason for exclusion
Mijovic 2018	Not a diagnostic accuracy study
Modi 2016	Index test not studied
Mulenga 2015	Index test not studied
Naidoo 2016	Not a diagnostic accuracy study
Nair 2016	Not a diagnostic accuracy study
Nansumba 2016	Index test not studied
Nataprawira 2016	Not a diagnostic accuracy study
NCT03831906	Data is unpublished and incomplete
NCT04038632	Data is unpublished and incomplete
Ncube 2017	Not a diagnostic accuracy study
Nduba 2015	Index text not studied
Ngabonziza 2016	Adult population
Nicol 2020	Index text not studied
Ntinginya 2012	Not a diagnostic accuracy study
Opota 2019	Adult population
Pandey 2017	Unable to separate paediatric data from adult data
Pink 2016	Unable to separate paediatric data from adult data
Planting 2014	Not a diagnostic accuracy study
Raizada 2014	Inappropriate reference standard
Raizada 2015a	Inappropriate reference standard
Raizada 2015b	Inappropriate reference standard
Raizada 2018a	Inappropriate reference standard
Raizada 2018b	Inappropriate reference standard
Rathour 2019	Screening for clinical tuberculosis before enrolment
Rebecca 2018	Case-control study
Rivera 2017	Not a diagnostic accuracy study
Sabi 2016	Not a diagnostic accuracy study
Sachdeva 2015	Not a diagnostic accuracy study

Study	Reason for exclusion
Sanchini 2014	Not a diagnostic accuracy study
Sander 2019	Adult population
Sanjuan-Jimenez 2015	Adult population
Schumacher 2016	Not a diagnostic accuracy study
Scott 2014	Unable to separate paediatric data from adult data
Shah 2016b	Insufficient data
Shah 2018	Not a diagnostic accuracy study
Shah 2019	Case-control study
Sharma 2015	Unable to separate paediatric data from adult data
Sieiro 2018	Unable to separate paediatric data from adult data
Singh 2015	Clinical diagnosis of tuberculosis established at enrolment
Singh 2016	Adult population
Solomons 2016	Not a diagnostic accuracy study
Sun 2019	Index test not studied on specimen type included in review
Sureshbabu 2016	Unable to separate paediatric data from adult data
Tadesse 2015	Unable to separate paediatric data from adult data
Tafur 2018	Not a diagnostic accuracy study
Tang 2017	Adult population
Theron 2011	Adult population
Triasih 2015	Not a diagnostic accuracy study
Ullah 2017	Unable to separate paediatric data from adult data
Walters 2012	Insufficient data
Walters 2017	Index text not studied
Walters 2018	Not a diagnostic accuracy study
Wang 2020	Adult population
Yadav 2020	Insufficient data
Zhang 2016	Insufficient data

Characteristics of ongoing studies [ordered by study ID]

ChiCTR1800015075

Study name	Diagnostic accuracy of Xpert MTB/RIF Ultra assay on diagnosing paediatric pulmonary tuberculosis
Target condition and reference standard(s)	Pulmonary tuberculosis
Index and comparator tests	Xpert Ultra
Starting date	1 January 2018
Contact information	Xuhui Liu; liuxuhui@shaphc.org
Notes	

ADDITIONAL TABLES

Table 1. Current World Health Organization (WHO) diagnostic recommendations in children

WHO Consolidated Guidelines (Module 3) 2021^a

In children with signs and symptoms of pulmonary tuberculosis, Xpert Ultra should be used as the initial diagnostic test for tuberculosis and detection of rifampicin resistance in sputum or nasopharyngeal aspirate, rather than smear microscopy/culture and phenotypic drug susceptibility testing (strong recommendation, low certainty of evidence for test accuracy in sputum; very low certainty of evidence for test accuracy in nasopharyngeal aspirate).

In children with signs and symptoms of tuberculous meningitis, Xpert MTB/RIF or Xpert Ultra should be used in cerebrospinal fluid (CSF) as an initial diagnostic test for tuberculous meningitis rather than smear microscopy/culture (strong recommendation, moderate certainty of evidence for test accuracy for Xpert MTB/RIF; low certainty of evidence for test accuracy for Xpert Ultra).

In children with signs and symptoms of extrapulmonary tuberculosis, Xpert Ultra may be used in lymph node aspirate and lymph node biopsy as the initial diagnostic test rather than smear microscopy/culture (conditional recommendation, low certainty of evidence).

In children with presumed pulmonary tuberculosis and an initial Xpert Ultra-negative result, in settings with a pretest probability of 5% or more, the WHO recommends a repeat Xpert Ultra test (for a total of two tests). Sputum and nasopharyngeal aspirate specimens may be used (conditional recommendation, very low certainty of evidence for test accuracy).

WHO Consolidated Guidelines (Module 5) 2022^b

In children aged below 10 years with signs and symptoms of pulmonary tuberculosis, Xpert MTB/RIF Ultra should be used in gastric aspirate or stool specimens as the initial diagnostic test for tuberculosis and the detection of rifampicin resistance, rather than smear microscopy/culture and phenotypic drug susceptibility testing.

^aThe findings from [Kay 2020](#) informed development of the guidelines.

^bThe findings from this review update informed development of the guidelines.

Table 2. Key characteristics of included studies

Study	Reference standard	Study design	HIV status	Clinical setting	High tuberculosis burden	Type of specimens	Xpert Ultra non-determinate ^a % (number/total)	Xpert Ultra trace ^b % (number)
Barcellini 2019	Culture	Cross-sectional	Negative	Outpatient	No	Sputum	None	None
Jaganath 2021	Culture, composite	Cohort	Both	Both	No	Sputum, gastric, nasopharyngeal	Not reported	Sputum: 12% (2); gastric: 67% (2); nasopharyngeal: 40% (2)
Kabir 2020	Culture ^c , composite	Cross-sectional	Yes	Inpatient	Yes	Sputum, stool	< 1% (1/446)	Sputum: 39% (11); stool: 80% (48)
Liu 2021	Culture ^c	Cohort	Negative	Both	Yes	Sputum, gastric, stool, nasopharyngeal	Not reported	Sputum: 0%; gastric: 30% (8); stool: 38.% (16); nasopharyngeal: 0%
NCT04121026	Culture	Cohort	Positive	Both	Yes	Sputum, gastric, stool, nasopharyngeal	4% (5/114)	Sputum: 0%; gastric: 25% (1); nasopharyngeal: 0%; stool: 0%
NCT04203628	Culture	Cohort	Both	Both	Yes	Stool	3% (2/76)	Stool: 40% (2)
NCT04240990	Culture	Cohort	Both	Inpatient	Yes	Gastric, stool, nasopharyngeal	1% (2/237)	Stool: 60% (3)
NCT04899076	Culture ^c	Cohort	Both	Both	Yes	Stool	10% (42/434)	Stool: 39% (12)
Nicol 2018	Culture, composite	Cohort	Both	Inpatient	Yes	Sputum	11% (50/453)	Sputum: 26% (8)
Parigi 2021	Culture, composite	Unclear	Not reported	Inpatient	No	Gastric	Not reported	NA
Sabi 2018	Culture, composite	Cohort	Both	Both	Yes	Sputum	0% (0/215)	Sputum: 19% (3)

Table 2. Key characteristics of included studies (Continued)

Ssengooba 2020	Culture	Cohort	Both	Unclear	No	Sputum, gastric	Not reported	Sputum: 67% (4); gastric: 57% (13)
Sun 2020	Culture, composite	Cohort	Not reported	Unclear	Yes	Gastric	Not reported	Gastric: 26% (20)
Zar 2019	Culture, composite	Cohort	Both	Inpatient	Yes	Nasopharyngeal	Not reported	Nasopharyngeal: 45% (9)

^aNon-determinate results are Error, Invalid, or No Result.

^bCalculated as percentage of total number of positive tests.

^cFor stool, Xpert on respiratory specimens was accepted as part of the reference standard.

Table 3. Xpert Ultra summary sensitivity and specificity for pulmonary tuberculosis, by type of specimen

Analysis group	Reference standard	Studies	Number of children (TB cases)	Summary sensitivity % (95% CI)	Summary specificity % (95% CI)	Positive predictive value % (95% CI) ^a	Negative predictive value % (95% CI) ^a
Sputum	Culture	5	1181 (127)	75.3 (64.3 to 83.8)	97.1 (94.7 to 98.5)	74.4 (61.9 to 84.0)	97.3 (95.9 to 98.1)
Sputum	Composite	5	1108 (527)	23.5 (20.1 to 27.3)	99.8 (98.8 to 100)	93.8 (68.1 to 99.1)	92.1 (91.8 to 92.5)
Gastric aspirate	Culture	7	990 (120)	70.4 (53.9 to 82.9)	94.1 (84.8 to 97.8)	56.9 (34.9 to 76.6)	96.7 (94.6 to 97.8)
Gastric aspirate	Composite	4	448 (229)	46.5 (29.7 to 64.1)	98.4 (91.4 to 99.7)	76.9 (31.6 to 96.0)	94.3 (92.1 to 95.9)
Stool	Culture	6	1432 (200)	56.1 (39.1 to 71.7)	98.0 (93.3 to 99.4)	75.3 (45.5 to 91.7)	95.2 (93.2 to 96.8)
Stool	Composite	2	572 (199)	50.3 (43.3 to 57.1)	99.5 (97.9 to 99.9)	91.2 (72.2 to 97.7)	94.7 (93.9 to 95.4)
Nasopharyngeal aspirate	Culture	4	535 (46)	43.7 (26.7 to 62.2)	97.5 (93.6 to 99.0)	65.8 (45.3 to 81.7)	93.9 (91.8 to 95.5)
Nasopharyngeal aspirate	Composite	2	222 (24)	50.0 (31.0 to 69.0)	98.2 (95.4 to 99.3)	75.9 (52.4 to 90.0)	94.6 (92.2 to 96.4)

CI: confidence interval; TB: tuberculosis.

^aPredictive values were determined at a pretest probability of 10%.

Table 4. Xpert Ultra summary sensitivity and specificity for pulmonary tuberculosis, by type of specimen and age group

Analysis group	Reference standard	Studies	Number of children (TB cases)	Summary sensitivity % (95% CI)	Summary specificity % (95% CI)	Positive predictive value % (95% CI) ^a	Negative predictive value % (95% CI) ^a
Sputum specimen							
< 1 year	Culture	4	257 (16)	75.0 (49.2 to 90.3)	97.9 (95.1 to 99.1)	80.0 (61.7 to 90.9)	97.2 (93.8 to 98.8)
<1 year	Composite	5	260 (103)	15.5 (9.74 to 23.9)	100 (97.6 to 100) ^b	— ^c	— ^c
1–4 years	Culture	5	468 (43)	69.8 (54.6 to 81.6)	96.2 (93.9 to 97.7)	67.3 (55.0 to 77.6)	96.7 (94.8 to 97.8)
1–4 years	Composite	4	420 (213)	20.7 (15.7 to 26.6)	100 (98.2 to 100) ^b	— ^c	— ^c
5–9 years	Culture	5	282 (39)	66.7 (50.7 to 79.6)	96.8 (87.6 to 99.2)	70.0 (35.8 to 90.7)	96.4 (94.3 to 97.6)
5–9 years	Composite	4	263 (134)	25.4 (16.6 to 36.8)	100 (97.1 to 100) ^b	— ^c	— ^c
10–14 years	Culture	5	135 (23)	91.9 (68.7 to 98.3)	97.7 (77.2 to 99.8)	81.7 (26.8 to 98.2)	99.1 (95.9 to 99.8)
10–14 years	Composite	4	129 (62)	40.3 (28.9 to 52.9)	100 (94.6 to 100) ^b	— ^c	— ^c
0–9 years	Culture	5	1012 (98)	69.7 (58.1 to 79.3)	97.2 (94.5 to 98.6)	73.4 (58.7 to 84.2)	96.7 (95.3 to 97.6)
0–9 years	Composite	5	943 (450)	21.1 (17.6 to 25.1)	100 (99.2 to 100) ^b	— ^c	— ^c
Gastric aspirate specimen							
< 1 year	Culture	5	182 (26)	67.3 (43.5 to 84.6)	94.0 (84.7 to 97.8)	55.4 (31.5 to 77.1)	96.3 (93.1 to 98.0)
1–4 years	Culture	4	327 (30)	71.5 (40.0 to 90.4)	94.0 (73.8 to 98.9)	57.1 (25.1 to 84.1)	96.8 (92.5 to 98.6)
1–4 year	Composite	3	72 (52)	50.0 (36.7 to 63.3)	100 (83.9 to 100) ^b	— ^c	— ^c
0–9 years	Culture	6	659 (70)	63.6 (47.7 to 77.0)	94.9 (83.8 to 98.5)	57.9 (31.0 to 80.9)	95.9 (94.1 to 97.2)
0–9 years	Composite	3	142 (101)	47.5 (38.0 to 57.2)	100 (91.4 to 100) ^b	100 (32.9 to 100)	94.5 (93.0 to 95.5)

Table 4. Xpert Ultra summary sensitivity and specificity for pulmonary tuberculosis, by type of specimen and age group (Continued)

Stool specimen								
< 1 year	Culture	4	295 (31)	65.2 (33.7 to 87.3)	96.2 (88.9 to 98.7)	65.3 (40.2 to 84.0)	96.2 (91.5 to 98.3)	
< 1 year	Composite	2	199 (65)	50.8 (38.8 to 62.6)	100 (97.2 to 100) ^b	— ^c	— ^c	
1–4 years	Culture	3	331 (30)	43.3 (27.1 to 61.2)	97.1 (74.8 to 99.7)	62.7 (13.2 to 94.9)	93.9 (91.8 to 95.5)	
1–4 years	Composite	2	186 (62)	42.8 (28.4 to 58.6)	99.3 (77.2 to 100)	87.0 (14.1 to 99.6)	93.9 (92.2 to 95.3)	
5–9 years	Culture	3	145 (19)	57.9 (35.6 to 77.4)	89.7 (83.0 to 93.9)	38.4 (24.7 to 54.3)	95.0 (91.8 to 97.0)	
5–9 years	Composite	2	126 (47)	46.8 (33.2 to 60.9)	100 (95.4 to 100) ^b	— ^c	— ^c	
10–14 years	Composite	2	61 (25)	72.0 (51.8 to 86.0)	97.5 (38.9 to 100)	76.4 (5.50 to 99.5)	96.9 (94.3 to 98.4)	
0–9 years	Culture	6	1279 (154)	52.8 (35.0 to 69.9)	98.0 (93.4 to 99.4)	74.1 (55.2 to 96.6)	94.9 (92.7 to 96.6)	
0–9 years	Composite	2	511 (174)	47.1 (39.8 to 54.6)	99.7 (97.9 to 100)	94.6 (71.2 to 99.2)	94.4 (93.7 to 95.1)	
1–4 years	Culture	2	251 (24)	50.0 (31.0 to 69.0)	98.2 (95.4 to 99.3)	75.9 (52.4 to 90.0)	94.6 (92.2 to 96.4)	
Nasopharyngeal aspirate specimen								
0–9 years	Culture	3	478 (42)	42.6 (26.0 to 61.1)	98.6 (96.7 to 99.4)	77.7 (58.5 to 89.5)	93.9 (91.8 to 95.5)	
0–9 years	Composite	2	205 (148)	14.2 (9.43 to 20.8)	98.5 (74.3 to 99.9)	50.5 (04.6 to 95.7)	91.2 (90.5 to 91.8)	

CI: confidence interval; TB: tuberculosis.

^aPredictive values were determined at a pre-test probability of 10%.

^bMeta-analysis using univariate fixed-effect or random-effects logistic regression models is not possible when all studies in a meta-analysis report 100% specificity. Therefore, the summary specificity was calculated by dividing the total number of non-cases by the total number of true negatives.

^cCould not be determined. It was not possible to compute likelihood ratios post-estimation because different models were fitted separately for sensitivity and specificity.

Table 5. Xpert Ultra summary sensitivity and specificity by type of specimen and comorbidity

Analysis group	Reference standard	Studies	Number of children (TB cases)	Summary sensitivity % (95% CI)	Summary specificity % (95% CI)	Positive predictive value % (95% CI) ^a	Negative predictive value % (95% CI) ^a
Sputum specimen							

Table 5. Xpert Ultra summary sensitivity and specificity by type of specimen and comorbidity (Continued)

HIV-positive, all ages	Culture	4	181 (29)	79.5 (59.6 to 91.1)	98.7 (93.9 to 99.7)	87.5 (57.7 to 97.3)	97.7 (95.2 to 98.9)
HIV-positive, all ages	Composite	3	174 (114)	21.1 (14.5 to 29.5)	100 (94.0 to 100) ^b	— ^c	— ^c
HIV-positive, 0–9 years	Composite	2	66 (51)	23.5 (13.9 to 37.0)	100 (79.6 to 100) ^b	— ^c	— ^c
HIV-negative, all ages	Culture	4	549 (89)	69.6 (53.3 to 82.1)	97.3 (94.5 to 98.7)	74.2 (59.1 to 85.1)	96.7 (94.7 to 97.9)
HIV-negative, all ages	Composite	4	483 (301)	24.3 (19.7 to 29.4)	100 (97.9 to 100) ^b	— ^c	— ^c
HIV-negative, 0–9 years	Culture	3	399 (56)	73.2 (60.2 to 83.2)	96.5 (93.9 to 98.0)	69.9 (56.5 to 80.6)	97.0 (95.4 to 98.0)
HIV-negative, 0–9 years	Composite	3	337 (232)	21.1 (16.3 to 26.8)	100 (96.5 to 100) ^b	— ^c	— ^c
Severe malnutrition, all ages	Culture	5	267 (17)	83.2 (54.2 to 95.5)	98.5 (62.6 to 100)	86.1 (14.3 to 99.6)	98.1 (94.1 to 99.4)
Severe malnutrition, all ages	Composite	4	263 (110)	21.8 (15.1 to 30.5)	100 (97.6 to 100) ^b	— ^c	— ^c
Severe malnutrition, 0–9 years	Culture	4	228 (11)	81.8 (49.3 to 95.4)	95.9 (92.2 to 97.8)	68.6 (52.2 to 81.5)	97.9 (93.2 to 99.4)
Severe malnutrition, 0–9 years	Composite	3	224 (91)	19.8 (12.8 to 29.2)	100 (97.2 to 100) ^b	— ^c	— ^c
Gastric aspirate specimen							
HIV-negative, all ages	Culture	3	345 (47)	61.7 (47.2 to 74.4)	90.8 (82.4 to 95.4)	42.7 (26.7 to 60.4)	95.5 (93.7 to 96.9)
HIV-negative, 0–9 years	Culture	3	325 (46)	63.0 (48.4 to 75.6)	90.5 (82.5 to 95.1)	42.5 (27.4 to 59.1)	95.6 (93.8 to 97.0)
Stool specimen							
Severe malnutrition, all ages	Culture	3	443 (22)	68.2 (46.6 to 84.0)	98.5 (84.2 to 99.9)	83.5 (29.4 to 98.4)	96.6 (93.8 to 98.0)

Table 5. Xpert Ultra summary sensitivity and specificity by type of specimen and comorbidity (Continued)

Severe malnutrition, 0–9 years	Culture	3	428 (19)	63.2 (40.3 to 81.3)	98.5 (84.1 to 99.9)	82.3 (27.7 to 98.3)	96.1 (93.1 to 97.7)
Nasopharyngeal aspirate specimen							
HIV-negative, all ages	Composite	2	186 (132)	21.1 (8.5 to 43.5)	100 (93.4 to 100) ^b	— ^c	— ^c
HIV-negative, 0–9 years	Composite	2	184 (132)	21.1 (8.5 to 43.5)	100 (93.1 to 100) ^b	— ^c	— ^c

CI: confidence interval; TB: tuberculosis.

^aPredictive values were determined at a pre-test probability of 10%.

^bMeta-analysis using univariate fixed-effect or random-effects logistic regression models is not possible when all studies in a meta-analysis report 100% specificity. Therefore, the summary specificity was calculated by dividing the total number of non-cases by the total number of true negatives.

^cCould not be determined. It was not possible to compute likelihood ratios post-estimation because different models were fitted separately for sensitivity and specificity.

Table 6. Sensitivity analyses

Analysis group	Reference standard	Studies	Number of children (TB cases)	Summary sensitivity % (95% CI)	Summary specificity % (95% CI)	Positive predictive value % (95% CI) ^a	Negative predictive value % (95% CI) ^a
Gastric aspirate specimen	Culture	7	990 (120)	70.4 (53.9 to 82.9)	94.1 (84.8 to 97.8)	56.9 (34.9 to 76.6)	96.7 (94.6 to 97.8)
Gastric aspirate specimen	Culture	5	696 (111)	73.4 (57.3 to 85.5)	88.1 (81.6 to 92.6)	40.8 (29.6 to 53.1)	96.8 (94.5 to 98.1)
Stool specimen	Culture	6	1432 (200)	56.1 (39.1 to 71.7)	98.0 (93.3 to 99.4)	75.3 (45.5 to 91.7)	95.2 (93.2 to 96.8)
Stool specimen	Culture	2	572 (89)	60.7 (50.2 to 70.2)	90.1 (87.1 to 92.4)	40.4 (33.1 to 48.2)	95.3 (94.0 to 96.4)
Nasopharyngeal specimen	Culture	4	535 (46)	43.7 (26.7 to 62.2)	97.5 (93.6 to 99.0)	65.8 (45.3 to 81.7)	93.9 (91.8 to 95.5)
Nasopharyngeal specimen^b	Culture	2	222 (38)	—	—	—	—

Sensitivity analyses are indicated in bold.

CI: confidence interval; TB: tuberculosis.

^aPredictive values were determined at a pre-test probability of 10%.

^bMeta-analysis was not performed due to paucity of data and heterogeneity, which precluded the use of univariate fixed-effect logistic regression as performed for the meta-analysis of the two studies for stool specimen.

WHAT'S NEW

Date	Event	Description
31 August 2022	New search has been performed	The previous published review version assessed the accuracy of both Xpert MTB/RIF and Xpert Ultra. The authors limited this review update to Xpert Ultra, which has superseded Xpert MTB/RIF. The Xpert MTB/RIF text and analyses are available in the previous published review version.
31 August 2022	New citation required and conclusions have changed	The date of search was updated to 9 March 2021. The authors included 14 unique studies, integrating 11 new studies since the previous published review version.

HISTORY

Protocol first published: Issue 6, 2019

CONTRIBUTIONS OF AUTHORS

AWK, TN, and LFG assessed articles for inclusion and extracted data. AMM and KRS resolved disagreements.

AWK, TN, and KRS entered data in [Review Manager 2020](#).

AWK, AMM, KRS, and YT analysed the data and interpreted the analyses. In particular, YT performed statistical analyses.

SEV, KV, AB, and TM reviewed the protocol and co-ordinated the presentation of the findings to a WHO Guideline Development Group.

AWK, LFG, AMM, KRS, and YT drafted the manuscript.

TN, SEV, KV, AB, TM, ME, and AKD provided critical comments on the manuscript.

All review authors read and approved the final manuscript draft.

DECLARATIONS OF INTEREST

AK has conducted prior primary research on tuberculosis diagnostics. The Baylor College of Medicine Children's Foundation-Swaziland, where Dr Kay is based, received a discount from Cepheid on Xpert MTB/RIF Ultra cartridges for a tuberculosis case finding programme. The Baylor College of Medicine Children's Foundation-Eswatini is separate from Baylor College of Medicine (AK's employer).

TN has no known conflicts of interest.

SEV is a Medical Officer at the World Health Organization Global Tuberculosis Programme, which commissioned this review update for the 2022 WHO consolidated guidelines on the management of tuberculosis in children and adolescents.

KV is a WHO staff member.

AB works as a technical officer at the WHO Global Tuberculosis Programme, which commissioned this review update for the 2022 WHO consolidated guidelines on the management of tuberculosis in children and adolescents.

TM is a consultant to the WHO.

LGF has no known conflicts of interest.

ME is a CIDG Editor, and has no known conflicts of interest.

AKD has conducted prior primary research on tuberculosis diagnostics and has no known conflicts of interest. She works for UNICEF, and in her role as child health specialist is sometimes involved in developing recommendations for diagnostic approaches to detect childhood illnesses.

AMM has conducted prior primary research on tuberculosis diagnostics and has no known conflicts of interest. She has undertaken work as an independent contractor for Janssen Global Services, Medscape Independent Contractor, and Oxford Immunotec Inc.

Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children (Review)

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KRS has received financial support for the preparation of systematic reviews and educational materials, consultancy fees from the Foundation for Innovative New Diagnostics (FIND) (for the preparation of systematic reviews), honoraria, and travel support to attend WHO guidelines meetings. KRS is a CIDG and DTA Editor.

YT is a Cochrane Editorial Board Member; a CIDG and DTA Editor; and a Statistical Editor for the Cochrane Bone Joint and Muscle Trauma Group.

The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions or policies of the institutions with which they are affiliated.

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- World Health Organization, Switzerland

WHO Registration 2021/1090755-0

Purchase Order 202638664

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Differences between review and review update

Title

The title has been updated to reflect the fact that tuberculosis clinicians and researchers are shifting away from latent and active as descriptors of tuberculosis; instead, we have used tuberculosis disease.

Scope of the review

Our previously published Cochrane Review assessed the accuracy of both Xpert MTB/RIF and Xpert Ultra ([Kay 2020](#)). We limited the current review update to the diagnostic accuracy of Xpert Ultra for several reasons. Xpert Ultra has superseded Xpert MTB/RIF and the manufacturer will be discontinuing Xpert MTB/RIF in most countries in 2023. Additionally, the WHO specifically requested an updated review on Xpert Ultra to inform gaps in data in children and adolescents. Further, we considered guidance on when to update systematic reviews. An update is suggested if the question is topical for decision-making for practice, policy, or research priorities, or if the new data will change the findings or credibility of the original review ([Garner 2016](#)). We did not believe that an update on Xpert MTB/RIF met these criteria. The Xpert MTB/RIF text and analyses are available in the last published version of the review ([Kay 2020](#)).

Objectives

We added a secondary objective: to summarize the frequency of Xpert Ultra trace results. In investigations of heterogeneity, we focused on the age, HIV status, and other comorbid conditions of participants. The prior review evaluated other possible sources of heterogeneity, such as smear and tuberculosis burden, with more included studies on Xpert MTB/RIF ([Kay 2020](#)).

Types of studies

We included abstracts with sufficient data. We included ongoing studies that helped us to address the review objectives and recorded the stage of the study at the time data were extracted.

Reference standard

We defined the microbiological reference standard as culture only and did not include smear microscopy, which is less accurate. In addition, we clarified the reference standards as follows. For stool, we accepted as a reference standard a positive result by Xpert Ultra in a

[Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children \(Review\)](#)

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sputum specimen. For the composite reference standard, when information about tuberculosis treatment was not available, we accepted the uniform research definition (Graham 2012; Graham 2015). In these situations, using the older definition (Graham 2012), we defined tuberculosis as (1) confirmed, probable, and possible cases; and (2) non-tuberculosis. For the newer definition (Graham 2015), we used the categories tuberculosis confirmed and not confirmed. In cases where a study-specific definition for the composite reference standard was applied, this was accepted as well. We added MTBDR*plus*, a WHO-recommended test, as a reference standard for rifampicin resistance.

Assessment of methodological quality

Using QUADAS-2, we judged all studies that evaluated stool as being of unclear concern, because there is no established technique for stool processing prior to performing Xpert Ultra.

Inconclusive results

We had planned to estimate the summary proportion of non-determinate Xpert Ultra results; however there were few non-determinate results reported. We have summarized these results in Table 2.

Sensitivity analyses

We had planned to explore the effects of risk of bias items and study characteristics on summary estimates of Xpert Ultra accuracy by excluding the following studies:

1. studies that used consecutive or random selection of participants;
2. studies in which the reference standard results were interpreted without knowledge of the index test results; and
3. studies that included only untreated participants.

We did not perform these sensitivity analyses because all studies satisfied criteria for analyses 1 and 2, and data were insufficient for analysis 3.

INDEX TERMS

Medical Subject Headings (MeSH)

*Antibiotics, Antitubercular [therapeutic use]; Cross-Sectional Studies; *HIV Infections [drug therapy]; Microbial Sensitivity Tests; *Mycobacterium tuberculosis [genetics]; Rifampin [pharmacology]; Sensitivity and Specificity; Sputum [microbiology]; *Tuberculosis, Lymph Node [diagnosis] [drug therapy]; *Tuberculosis, Meningeal [cerebrospinal fluid] [diagnosis] [drug therapy]; *Tuberculosis, Pulmonary [diagnosis] [drug therapy] [microbiology]

MeSH check words

Adolescent; Child; Humans