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Performance of stool-based molecular tests and processing methods for paediatric tuberculosis diagnosis: a systematic review and meta-analysis

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Contributors

LC-C, EL-V, and ALG-B conceived and designed the study. LC-C and SM were in charge of the literature search, data collection, and data summarisation. LC-C performed the data analysis and wrote the first manuscript draft. All authors drafted the manuscript and provided critical feedback. All authors had full access to all data in the study and final responsibility for the decision to submit for publication. LC-C and SM have accessed and verified the data.

Declaration of interests

MB's institution received UNITAID funding for the conduct of one of the studies included in the systematic review (Marcy et al 2022[60]). All other authors declare no competing interests.

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Summary

Background: There has been a global pursuit to improve the diagnosis of tuberculosis in young children by applying diagnostic methods on accessible biospecimens such as stool. We aimed to conduct a systematic review on the accuracy of stool-based molecular tests for tuberculosis diagnosis in children and to assess the impact of the available pre-processing methods and other design characteristics.

Methods: In this systematic review and meta-analysis, we evaluated studies in children younger than 16 years with presumptive tuberculosis that were published in English, Spanish, French, and Portuguese from Jan 1, 2000, to May 3, 2024, in MEDLINE, Embase, and Embase Classic, comparing the molecular detection of *Mycobacterium tuberculosis* DNA in stool with microbiological tests on other samples or a clinical diagnosis. We did not exclude studies based on geographical location, sample size, or study design if they were reporting primary data. Two independent reviewers (LC-C and SM) screened titles, abstracts, and full-text articles for eligibility and extracted data on study characteristics, study population, and diagnostic performance. If information relevant to the main analysis was not reported in the article, the corresponding authors were contacted. Point estimates and 95% CIs were calculated for sensitivity and specificity for each study and for the different molecular tests (Xpert MTB/RIF, Xpert Ultra MTB/RIF [Cepheid, Sunnyvale, CA, USA], and other tests) versus a reference standard (culture only, any bacteriological confirmation, and tuberculosis case definition). Sensitivity and specificity were

stratified by the stool processing method. We also quantified the additionality of stool Xpert Ultra tests for tuberculosis bacteriological confirmation. The protocol was registered with PROSPERO, CRD42022341514.

Findings: A total of 4521 records were identified through the database search, one record was identified from an article bibliography, and 67 studies were retained for full-text reading. 39 studies were included in the qualitative synthesis, 35 of which were included in the meta-analyses. When using any bacteriological confirmation from a respiratory sample as the reference standard, stool Xpert sensitivity was 0·60 (95% CI 0·48–0·71), stool Xpert Ultra sensitivity was 0·73 (0·63–0·81), and sensitivity was 0·44 (0·29–0·60) for other in-house molecular methods combined. When using tuberculosis case definition as the reference standard, stool Xpert sensitivity was 0·23 (0·11–0·41), stool Xpert Ultra sensitivity was 0·38 (0·22–0·56), and sensitivity was 0·17 (0·09–0·23) for other in-house molecular methods. The addition of stool Xpert Ultra increased bacteriological confirmation of tuberculosis by 38·6% overall. Further, the utilisation of centrifuge-free simplified methods improved the sensitivity of stool Xpert Ultra when using any bacteriological confirmation as a reference standard (0·77 [0·66–0·85] for centrifuge-free methods *vs* 0·61 [0·41–0·78] for non-centrifuge-free methods).

Interpretation: This systematic review and meta-analysis supports the use of Xpert Ultra in stool samples as a diagnostic tool for paediatric tuberculosis diagnosis. Stool-based Xpert Ultra can contribute to increase the bacteriological confirmation in this population, even when respiratory specimens are also tested.

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Introduction

In 2023, children accounted for approximately 12% of the global tuberculosis incidence, 16% of the tuberculosis deaths among people negative for HIV, and 15% of deaths among people living with HIV globally; representing 1·3 million new paediatric tuberculosis cases and 191 000 childhood tuberculosis-attributable deaths.[1] Children frequently present with paucibacillary disease and are often unable to produce a spontaneous respiratory sample for microbiological confirmation of *Mycobacterium tuberculosis* infection, requiring unpleasant and resource-intensive procedures to collect samples for diagnosis including induced sputum or gastric aspirate specimen instead. Such procedures might be less feasible in low-resource settings due to specific equipment, maintenance, and training needs as well as being more operator dependent, resulting in a low diagnostic yield. Although mycobacterial culture is the accepted reference standard for assessing new tuberculosis diagnostic tests, it only confirms tuberculosis in 10–50% of children starting tuberculosis treatment.[2–6]

Due to the many challenges of diagnosing tuberculosis in children, there has been a global pursuit to increase the diagnostic yield of paediatric tuberculosis by using easy-to-collect, non-sputum-based samples, such as stool and urine, for existing diagnostic platforms such as Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA; henceforth referred to as Xpert) and Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA; henceforth referred to as Ultra), and by developing new molecular diagnostic assays to detect *M tuberculosis* in non-sputum-based

samples. Stool is a particularly promising specimen for tuberculosis diagnosis and has been shown to increase tuberculosis bacteriological confirmation in children.[7,8] Because respiratory secretions are cleared from the airway into the digestive system swallowed, then excreted, stool is a natural place to find *M tuberculosis*.

In 2020, WHO added stool as a recommended sample for bacteriological tuberculosis confirmation to the molecular diagnostic guidelines.[9] However, some difficulties have been encountered as stool testing is being rolled out more broadly. Stool is easier to collect but cannot always be produced on demand. Therefore, samples might need to be collected and preserved at home before testing. Although the concern about exposing the sample to contamination and the potential risk of losing samples for diagnostic workup is valid,[10] a study published in 2023 showed consistent results even after altering incubation times and temperature conditions for the stool and applying several variations to the simple one-step stool processing method.[11]

Several studies have evaluated the performance of culture[12,13] and various molecular assays such as Xpert,[14] Ultra,[12,15] and other in-house tests[16–18] on stool in children and adults. So far, molecular tests have shown more promise than culture for *M tuberculosis* detection in stool.[12,13] Simultaneously, various stool-processing techniques have been developed for the pre-analytical phase including multiple steps and requiring equipment for homogenisation, vortexing, or centrifuging.[10,19,20] But also other more simplified methods—eg, optimised sucrose flotation,[21] the two-step method,[22] or the simple one-step method[23]—have been developed. Therefore, there is considerable heterogeneity in the methods used for diagnostic accuracy and validation studies.[24] Moreover, accuracy studies do not always use the same reference standard, tests, or the type and quantity of specimens. Thus, interpretation of diagnostic accuracy indicators of the various techniques should be made with caution.

A Cochrane review of Xpert for paediatric tuberculosis diagnosis[14] found 11 publications comparing stool Xpert with the culture of respiratory samples and ten publications that also compared stool Xpert to a composite reference standard (clinical diagnosis and Xpert in respiratory samples).[14] Another review, published in 2019, of molecular detection of *M tuberculosis* in stool in children, which captured studies up to September, 2018, included several molecular tests and collected information about stool sample processing.[24] Both reviews identified a need to closer look at stool processing methods.[14,24]

Another Cochrane review, published in 2022, of Ultra for the diagnosis of tuberculosis in children included only two published studies (and four unpublished studies) assessing the performance of Ultra in stool, with different tests used as part of the reference standard.[7] Summary sensitivity of Ultra in stool was 56·1% (95% CI 39·1–71·7), and summary specificity was 98·0% (93·3–99·4).

The sensitivity of Xpert in stool samples (compared to culture of respiratory samples) based on these recent systematic reviews is very heterogeneous, ranging from 0 to 100% and from 39% to 100% for Ultra.[7,14] To homogenise the evidence on the potential for stool to be used in tuberculosis diagnosis, we aimed to conduct a systematic review and meta-analysis on the

accuracy of different stool-based molecular tests for tuberculosis diagnosis in children and assess the impact of the available pre-processing methods and other design characteristics.

Methods

Search strategy and selection criteria

In this systematic review and meta-analysis, we searched MEDLINE (PubMed and Embase), Embase, and Embase Classic to identify published literature, and MedRxiv for articles in preprint format, using the terms “tuberculosis” or “TB” or “mycobacterium tuberculosis” and “stool” or “feces” or “faeces” or “fecal” or “faecal” contained in the title and abstract including synonyms and truncated terms. We also used the following Medical Subject Headings search terms: “mycobacterium tuberculosis”, “pulmonary tuberculosis”, and “feces”. We searched for additional articles through the reference lists of relevant reviews and selected studies and sources from the WHO Portal, StoolTB Partnership’s New Diagnostics Working Group, the United States’ Centers for Disease Control and Prevention, and the International Union Against Tuberculosis and Lung Disease. We also contacted leading researchers at WHO, the Foundation for Innovative New Diagnostics, and corresponding authors of relevant articles for the original data. The search terms used for each database are in the appendix (p 8).

Predefined inclusion criteria considered studies conducted in children younger than 16 years with presumptive tuberculosis, published from database inception until May 3, 2024, reporting molecular detection of *M tuberculosis* in stool compared with culture or molecular tests in other samples or clinical diagnosis and published in English, Spanish, French, and Portuguese. We did not exclude studies based on geographical location, sample size, or study design if they were reporting primary data. Case reports, editorials, and letters not containing primary data were excluded during the review. If two studies used the same data, we selected the article that contained the largest number of participants. We also excluded articles reporting on extrapulmonary tuberculosis only and short communications from conferences where only an abstract was available. Finally, we did not exclude studies in which the molecular test on stool was not the index test or studies that were not designed as diagnostic accuracy studies, because we wanted to gather information on stool processing methods and include as many data on molecular stool diagnostics as possible.

We used Rayyan free online software to manage the selection of the studies. Two independent reviewers (LC-C and SM) screened titles and abstracts for eligibility. Any discrepancies were solved by agreement whenever possible. If there was no agreement, a senior author (ALG-B) was consulted for the final decision. The same two independent reviewers (LC-C and SM) performed a full-text reading of the selected articles, assessing full eligibility criteria. An additional search was conducted by identifying the bibliography of the retrieved publications.

Data analysis

Variables including first author, year of publication, study period, study design, participants or population, index test, reference test, country, sociodemographic data, disease status,

sample collection and sample processing methods, number of eligible participants, final number of participants, total number of positives by index and reference test, number of true positives, number of true negatives, number of false positives, and number false negatives, were independently extracted by two reviewers (LC-C and SM) and included in a database created with Microsoft Excel version 16.48. If some information relevant to the main analysis was not reported in the article, the corresponding authors were contacted.

Extracted data were analysed using Stata (version 17). Point estimates and 95% CIs were calculated for sensitivity and specificity for each study and displayed in forest plots for the different molecular tests stratified by the stool processing method. All analyses were performed by molecular diagnostic index test (Xpert, Ultra, and others) and reference standard (culture only, any bacteriological confirmation, and tuberculosis case definition) using Stata's metadta[25] command for obtaining pooled sensitivity and specificity estimates. For the reference standard any bacteriological confirmation, we included laboratory confirmation using culture or molecular detection in a respiratory sample. For tuberculosis case definition, we included definite tuberculosis and probable tuberculosis using the criteria by Graham and colleagues (2012),[26] confirmed tuberculosis and unconfirmed tuberculosis using the criteria by Graham and colleagues (2015),[27] and start of tuberculosis treatment in the absence of information of any classification. Specificity was calculated from the whole sample in cohort and cross-sectional studies and from the control group in case–control studies. All meta-analyses were performed fitting a random-effects model.

The influence of the stool processing method and other sources of heterogeneity were assessed using an extra stratification whenever there were at least two studies in each subcategory. We summarised stool processing methods on their level of complexity, carrying out subanalyses between centrifuge-free versus non-centrifuge-free methods, and two-step and simple one-step methods versus more complex methods.

The estimates included in the data summarisation and analysis were those conducted per patient (not per sample), and a minimum of one sample per index test and one sample per reference test were considered as complete records. We included non-determinate results when these were reported. When more than one sample was collected for the reference standard, we considered it positive when any of the samples were positive. When the same index test was repeated twice in different samples, we reported the results of the first sample to homogenise data extraction across studies, because most of them only used one test.

In the interest of quantifying the advantage of adding stool Ultra to the tests already used for bacteriological confirmation, we analysed the additionality in tuberculosis confirmation with stool Ultra. This analysis was performed by calculating the additional number of children among those diagnosed with tuberculosis who were confirmed bacteriologically by stool Ultra and negative on all other microbiological tests. Childrennot meeting the tuberculosis case definition but positive on stool Ultra were considered false positives.

Heterogeneity was assessed by comparing the study designs and the different techniques used for stool processing and molecular testing in each of the studies. We assessed the extent of heterogeneity among studies visually with forest plots with 95% prediction regions and

statistically by τ^2 and I^2 statistics that account for the mean–variance relationship across studies.[28]

Two authors (LC-C and SM) conducted a risk of bias assessment at the level of the individual study using the QUADAS-2 revised tool for diagnostic accuracy studies (University of Bristol, Bristol, UK).[29] This systematic review followed the PRISMA 2020 guidelines[30] and was registered with PROSPERO, CRD42022341514.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing the report.

Results

A total of 4521 records were identified through the databases search strategy and one published paper from the bibliography of the selected articles. After title and abstract revision, 67 studies were retained for full-text reading. 28 articles did not meet inclusion criteria upon full-text reading (figure 1; appendix pp 2–3). 39 studies were included in the qualitative synthesis (table 1). Of these studies, eight were case–control studies,[16–18,31–35] 22 were cross-sectional studies,[12,22,23,36–54] seven were cohort studies,[10,15,19,55–58] and two used data from clinical trials.[59,60] No additional papers were identified in the grey literature.

For the quantitative analysis, we excluded four additional studies[34,36,44,54] that did not contain sufficient data for analysis after contacting the authors for additional information. 35 studies were included in the meta-analyses: 20 in the meta-analysis using culture as the reference standard,[10,12,15,17–19,23,24,31,34,36–41,43,45,47,51,59] 32 in the meta-analysis using any type of bacteriological confirmation as the reference standard,[12,13,15–19,22–24,31–34,36,38,41–43,45–52,55,56,58–60] and 14 in the meta-analysis using tuberculosis case definition as the reference standard.[13–17,33,37–39,45–47,56,58]

The pooled sensitivity of stool Xpert was 0·69 (95% CI 0·51–0·82) when compared against culture on respiratory samples, 0·60 (0·48–0·71) when compared with any bacteriological confirmation on respiratory samples, and 0·23 (0·11–0·41) when using the tuberculosis case definition as reference standard. Pooled specificity was above 0·99 in all three scenarios (figure 2; appendix p 4).

For stool Ultra, pooled sensitivity was 0·80 (95% CI 0·62–0·90) when compared against culture on respiratory samples, 0·73 (0·63–0·81) when compared with any bacteriological confirmation on respiratory samples, and 0·38 (0·22–0·56) when using tuberculosis case definition as reference standard (figure 2; appendix p 4). Pooled specificity ranged from 0·93 (0·87–0·97) when using culture on respiratory samples as the reference standard to 1·00 (0·90–1·00) when using any bacteriological confirmation.

For other molecular methods, pooled sensitivity was 0·47 (95% CI 0·20–0·76) against culture on respiratory samples, 0·44 (0·29–0·60) against bacteriological confirmation on respiratory samples, and 0·17 (0·09–0·23) when using tuberculosis case definition as the

reference standard. Specificity point estimates were above 0·95 in all three scenarios (appendix p 4).

Our risk-of-bias assessment identified as the primary area of concern the study flow and timing category, because several analyses could not be conducted on the entire study sample due to the absence of stool specimens for some participants. The second area of concern was patient selection, whereby case–control studies were categorised as having a high risk of bias unless they were nested within a cohort or explicitly mentioned the selection of controls from the same population.

In terms of the reference standard-related risk of bias, studies were considered to have a high risk if they solely used Xpert or culture on respiratory samples as the reference standard, without incorporating any clinical diagnostic classification. This decision was made to prevent potential overestimation of sensitivity.

Concerns regarding applicability were minimal, because we aimed to assess the performance of any moleculartest on a stool sample for diagnosing pulmonary tuberculosis in children without imposing restrictions on the setting, disease presentation, reference standard, or the specific molecular test used on a stool sample. For studies in which applicability-related concerns were not classified as low, the main reasons were insufficient information or the inability to accurately assess the methods employed for participant selection, stool processing, testing, or other details necessary for proper classification. A more comprehensive description of how the QUADAS-2 tool was applied can be found in the appendix (pp 6, 9–10).

The heterogeneity in sensitivity estimates was found to be higher when using Xpert as the index test, when any bacteriological confirmation and tuberculosis case definition were used as reference standards compared with culture as reference standard. Other molecular tests showed a similar level of heterogeneity when using culture and any bacteriological confirmation as reference standards. By contrast, sensitivity estimates for Ultra had low heterogeneity when reference standards were culture (τ^2=0·09) and any bacteriological confirmation (τ^2=0·24). The I^2 statistic was below 50% in both scenarios (figure 2; appendix p 4). However, when the reference standard was tuberculosis case definition, heterogeneity was found high (I^2=90·58, τ^2=0·67). Notably, for most specificity estimates, the I^2 statistic was below 50%, with the exception of comparing stool Ultra with culture (I^2=71·26) and any bacteriological confirmation (I^2=50·66) as the reference standards.

16 of 39 studies used centrifuge-free stool processing methods. From those, two used the Banada method, five used the simple one-step method, one used the two-step method, and eight used different variations of homogenisation and debris separation techniques (appendix p 5). When using stool Xpert as an index test and any bacteriological confirmation as a reference standard, the studies using centrifuge-free stool processing had almost the same sensitivity (0·63, 95% CI 0·43–0·79) as the studies using non-centrifuge-free processing methods (0·59, 0·44–0·72; figure 3A). When grouping the simple one-step and two-step methods together sensitivity was 1·00 (0·00–1·00), whereas for more complex processing methods, sensitivity was 0·56 (0·45–0·66; figure 3B). For stool Ultra as an index test and

any bacteriological confirmation as the reference standard, sensitivity was 0·61 (0·41–0·78) when non-centrifuge-free methods were used versus 0·77 (0·66–0·85) when centrifuge-free methods were used (figure 3C); for studies using simple one-step, sensitivity was 0·83 (0·68–0·92), whereas for studies using non-simple one-step methods sensitivity was 0·68 (0·57–0·77; figure 3D).

Seven studies (n=2465 participants) were included in the additionality analysis. 210 (8·5%) of 2465 participants had bacteriologically confirmed tuberculosis by respiratory sample. Tuberculosis was confirmed in 291 (11·8%) when adding stool Ultra to the tests completed. Thus, the addition of stool Ultra resulted in a 38·6% increased bacteriological confirmation rate (table 2; appendix p 7).

Discussion

In this systematic review and meta-analysis, we estimated boththe accuracy of molecular tests instool for thediagnosis of pulmonary tuberculosis in children and assessed the impact of the available pre-processing methods. We found a considerable increase in sensitivity in stool Ultra compared with stool Xpert. For stool Xpert, the sensitivity against a positive culture from a respiratory sample was approximately 69%, with a sensitivity of 60% when any bacteriological confirmation was used as the reference standard. However, stool Ultra identified approximately 73% of children that were bacteriologically confirmed via respiratory samples.

These findings are consistent with previous publications which show that Ultra has a higher sensitivity than Xpert in respiratory samples, as Ultra has a lower limit of detection.61 This effect was even more pronounced in studies directly comparing both Xpert and Ultra on stool against the same reference standard, in which Ultra demonstrated approximately 50% higher sensitivity.[12,33]

Due to the absence of a perfect reference standard for tuberculosis in children, the lower specificity estimates of stool Ultra compared with stool Xpert must be cautiously interpreted. There is potential misclassification of stool Ultra positives as false positives if clinical diagnosis is not considered. When clinical diagnosis serves as the reference standard, stool Ultra confirms around 38·6% of children classified as having the disease, representing a considerable improvement in bacteriological yield for children diagnosed with tuberculosis without bacteriological confirmation. Moreover, incorporating stool Ultra for bacteriological confirmation in children with presumptive tuberculosis increases the confirmation rate by 38%, suggesting that stool Ultra should be included among the standard tools for bacteriological confirmation, both in clinical and research settings.

There were at least 11 centrifuge-free and 24 more complex stool-processing methods employed in reviewed studies, and it was challenging to summarise them all. Generally, the processing methods seem to have simplified over time, as 14 of the 16 included studies from 2020 onwards used centrifuge-free methods (appendix p 5). As a result, seven of the nine studies included in the analysis testing stool Ultra used centrifuge-free processing methods.

When accounting for centrifuge-free stool processing methods, no differences in stool Xpert accuracy were found. When considering the two-step and simple one-step methods as a differentiated group, there was an increased sensitivity on the point estimate. Nevertheless, the modest subgroup sample size was associated to wide confidence intervals. Hence, it was not possible to definitively conclude that these methods increased sensitivity for stool Xpert.

When using stool Ultra as an index test and any bacteriological confirmation as a reference standard, processing the stool with centrifuge-free methods increased the absolute sensitivity by 16% (from 0·61 to 0·77). The difference was 15% when the studies were divided between those who used simple one-step method versus those who did not use it (from 0·68 to 0·83).

Another important finding is the reduced heterogeneity of stool Ultra sensitivity compared with stool Xpert estimates as shown by the I^2 and τ^2 statistics when using culture or any bacteriological confirmation as reference standard (figure 2). This finding might be related to the more homogeneous study designs and implementation of the techniques in recent years, but the lower limit of DNA detection in Ultra might make it a more robust test that is less dependent on the sample collection and processing methods. Due to the complexity of summarising the data given the observed methods and sources of variability, we recommend future studies adopt standardised study designs, including reference standards and case definitions, to facilitate reliable direct comparisons and conclusions.

This study had several limitations. First, we were unable to conduct meta-regression when culture was used as a reference standard for both Xpert and Ultra as index tests, because the number of studies in each subcategory was small. Second, despite our efforts to accurately classify the stool processing methods, we had to combine diverse methods within each category, blurring the potential effects of each processing method in diagnostic accuracy. Third, to ensure a more standardised analysis, we included data from complete records (complete samples in this case), which could have resulted in overestimated sensitivity compared with more programmatic conditions. Fourth, we had to consider all respiratory samples together for the reference standard (including gastric aspirate) despite having slightly different sensitivities due to the heterogeneity in the methods across studies and the data from many studies that combined both sample collection methods. Fifth, we were not able to perform an age stratified analysis to consider children younger than 5 years, because not all studies contained such stratification, and the estimates would represent a substantially smaller sample size. Finally, the results of this meta-analysis cannot be extrapolated for all types of tuberculosis, because we limited the analysis to diagnose pulmonary tuberculosis only.

A relevant strength in the analysis was the use of the metadta command on STATA 17, because heterogeneity was accounted for by fitting the data into a random effects model, something that is particularly advantageous when heterogenous accuracy estimates and variances within the studies are expected.[26]

In conclusion, the results of this systematic review and meta-analysis indicate that stool Ultra confirmed tuberculosis in up to 38% of children classified as having tuberculosis and 73% of children with bacteriologically confirmed tuberculosis, showing a higher sensitivity

than Xpert. Moreover, the addition of stool Ultra to the sputum-based tests improves bacteriological confirmation by 38·6%. These findings support the WHO recommendation to use Ultra as the initial confirmatory test for paediatric tuberculosis investigation on sputum, nasopharyngeal aspirate, gastric aspirate, or stool.[62,63] We also encourage the incorporation of stool Ultra as part of the microbiological composite reference standard to be used in paediatric diagnostic evaluations. Our findings on the use of centrifuge-free methods for stool processing are encouraging, because they do not negatively affect sensitivity estimates for Ultra testing and might even enhance them in some scenarios. Although we did not investigate the feasibility and cost-effectiveness of adopting these methods, it is recommended for future research as stool Ultra has been recently accepted as a valid sample for microbiological confirmation by several African and Asian National Tuberculosis Programmes[62,63] and centrifuge-free processing methods bring the opportunity to further expand its use in resource-challenged settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data sharing

Data presented in this systematic review has been obtained from published studies, which are cited in the references. A copy of the database used for data collection and analysis is available upon request to the corresponding author.

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Research in context

Evidence before this study

Before this systematic review and meta-analysis, we conducted a search on MEDLINE for previous systematic reviews with the terms “stool”, “faeces”, “feces”, “tuberculosis”, “TB”, “tuberculo*”, “children”, “child”, “pediatric”, “paediatric”, and “diagnostics” published between Jan 1, 2018, and Nov 1, 2023. We did not apply any language restrictions to this search. We found six previous systematic reviews containing accuracy estimates of molecular tests in stool to diagnose pulmonary tuberculosis in children. Two reviews were the latest Cochrane reviews on Xpert MTB/RIF (Xpert) and Xpert MTB/RIF Ultra (Ultra) diagnostics from 2020 and 2022 without a focus on stool diagnostics, three reviews focused on stool diagnostics only included Xpert (MacLean et al 2019, Gebre et al 2020, and Segala et al 2023), and one review included both Xpert and other molecular diagnostics (Mesman et al 2019). Due to the relatively recent release in 2017 of the Ultra cartridge, only the most recent Cochrane review included accuracy estimates for this diagnostic test. Stool Xpert sensitivity against respiratory sample culture or Xpert ranged from 0·53 (95% CI 0·39–0·67) in Mesman et al to 0·68 (0·52–0·79) in Segala et al. For Ultra, the sensitivity was 0·56 (0·39–0·72) in the 2022 Cochrane review. All reviews highlighted the heterogeneity of the study methods and mentioned the need to further analyse these sources of variability, especially in the stool processing methods.

Added value of this study

We conducted a systematic review and meta-analysis, which included 39 studies conducted in 22 countries across the Americas, Asia, and Africa. The review included 6835 children with presumptive tuberculosis or with clinical criteria for tuberculosis screening. To our knowledge, this systematic review is the most up to date on this topic, with many studies using the Ultra test, the current frontline test for tuberculosis diagnosis in countries with the highest tuberculosis burden. Consequently, our meta-analysis provides the most current estimates for Ultra’s sensitivity and specificity on stool samples. Furthermore, to our knowledge, our study is the first to detect, classify, and analyse the various sources of heterogeneity related to study design, stool processing methods, and reference standard and index tests, which previous systematic reviews had identified as potential sources of variability.

Implications of all the available evidence

The WHO tuberculosis paediatric guidelines have recently included stool-based molecular diagnostics as a valid tool for diagnosing tuberculosis in children. Our study further supports this approach by providing new evidence on the diagnostic accuracy of Ultra in stool and its additive yield (when other existing tests are negative). Furthermore, our study supports the use of simplified stool processing methods, because the evidence suggests no difference in the sensitivity of the molecular test compared to more complex and labour-intensive stool processing methods. Lastly, our study highlights the need for researchers in the field to standardise the reference standard (number and type of samples

collected and tests performed) and tuberculosis case definitions for diagnostic accuracy studies in the field of paediatric tuberculosis.

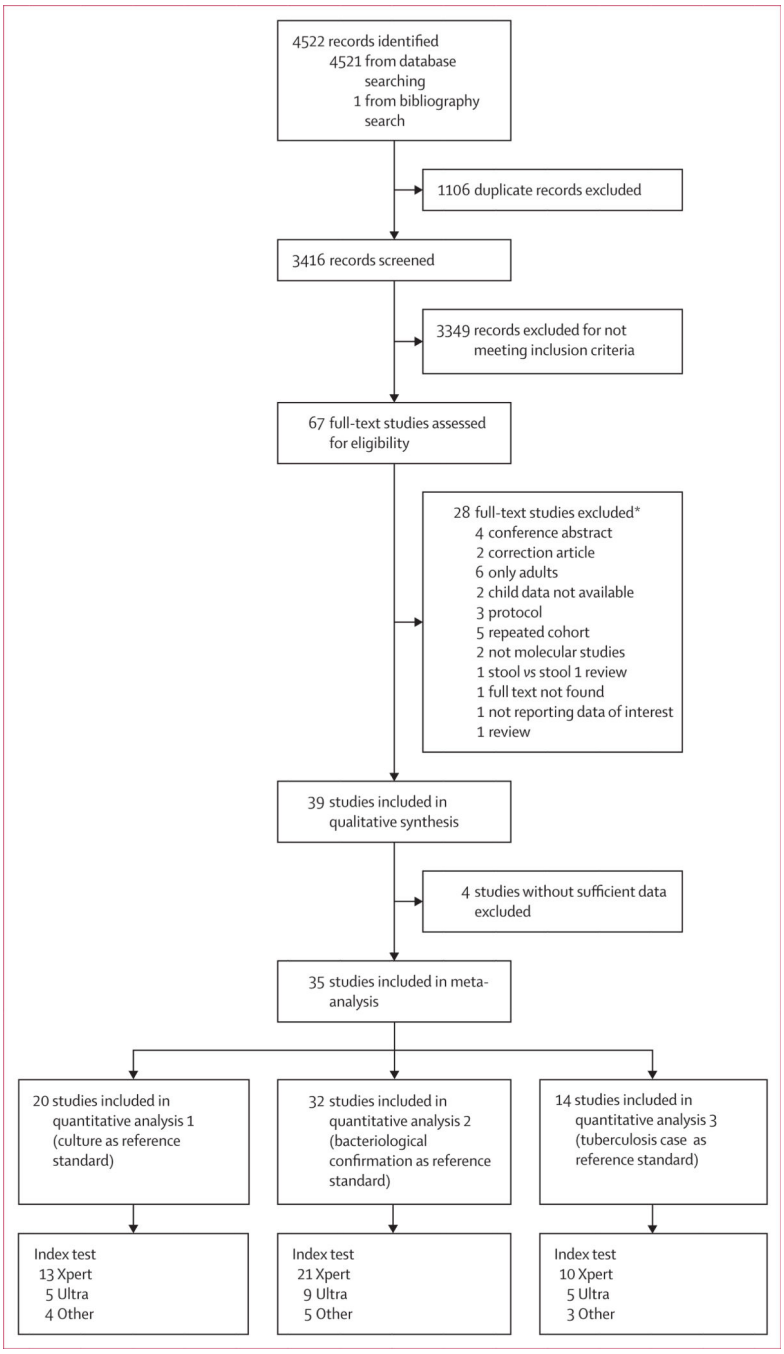
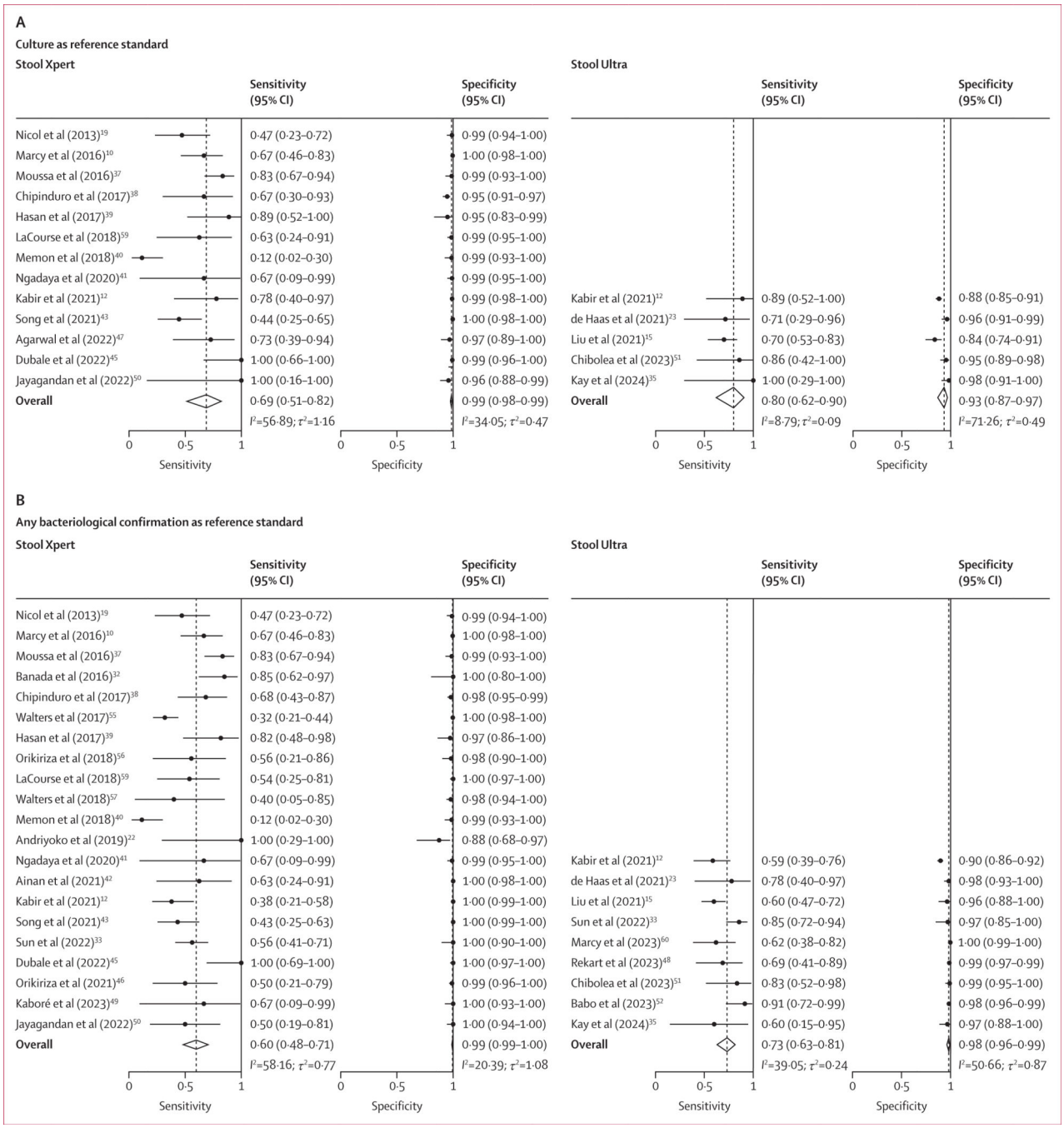


Figure 1: Study selection

*Reasons for exclusions are listed in the appendix (pp 2–3).



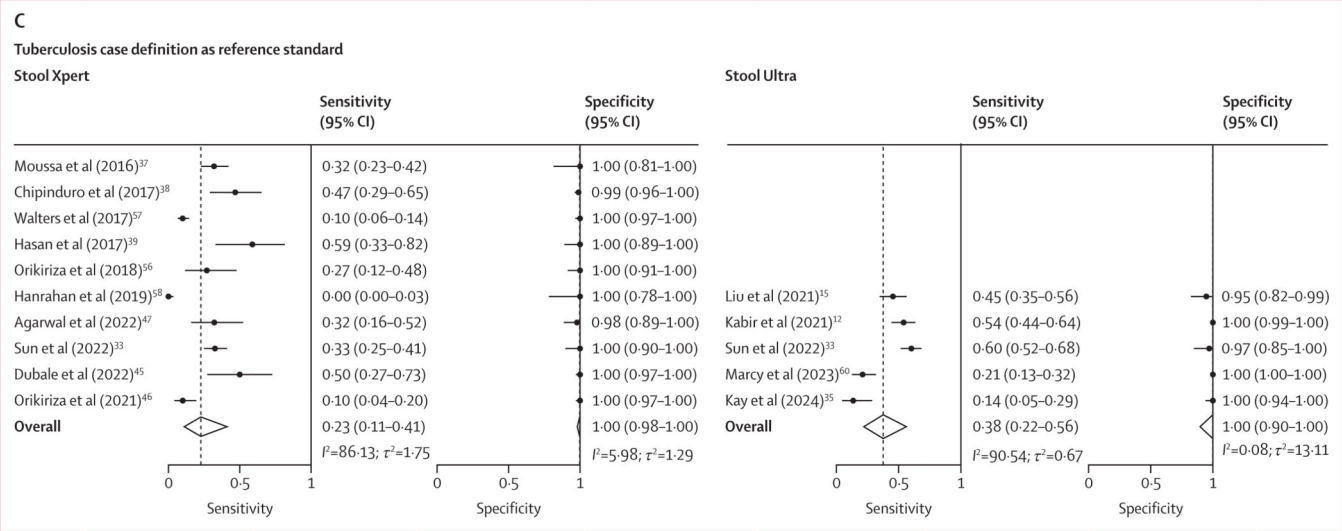


Figure 2: Forest plots for meta-analyses of sensitivity and specificity of stool Xpert and stool Ultra by reference standard

Culture (A), any bacteriological confirmation (B), and tuberculosis case definition (C) as reference standards.

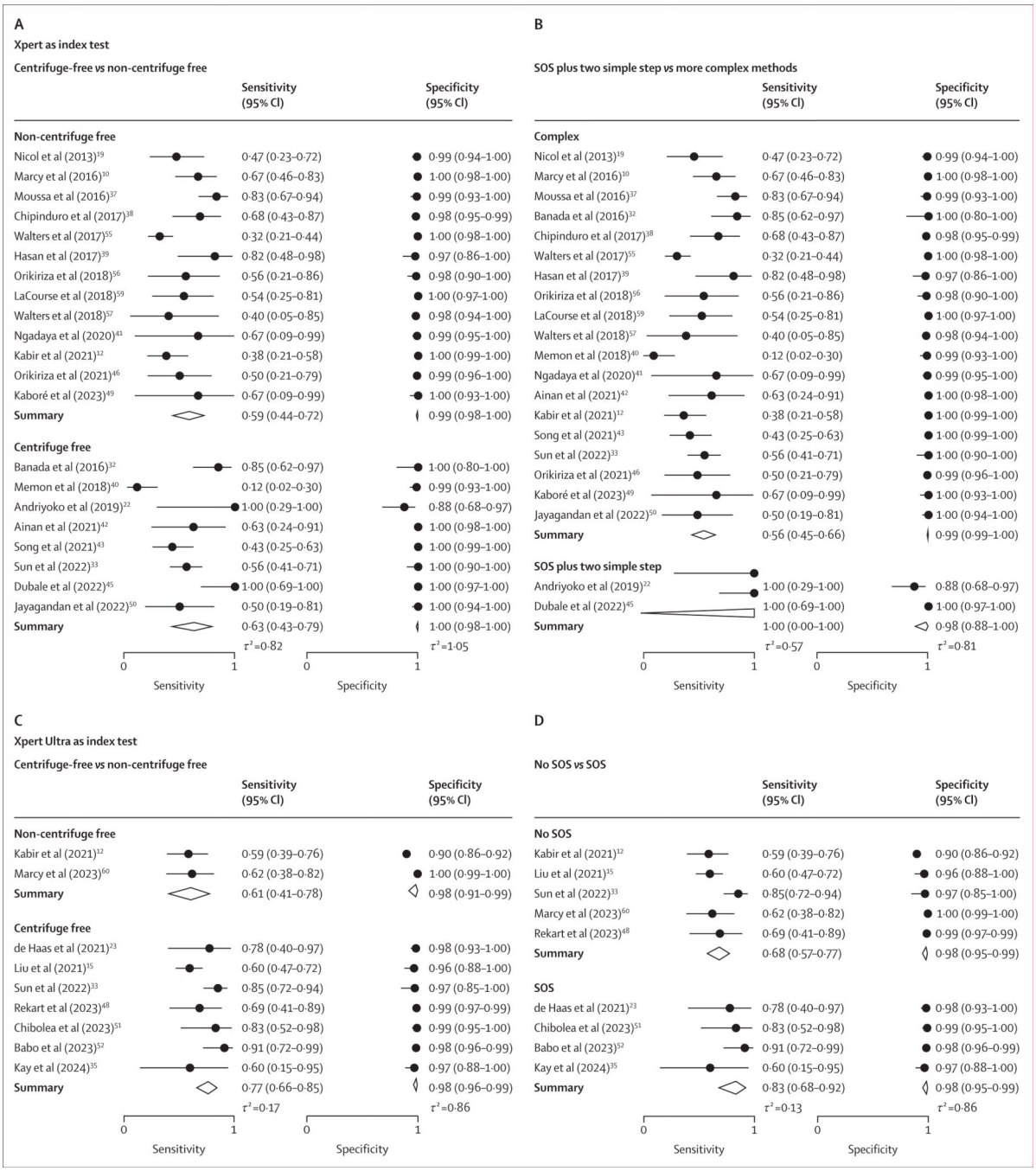


Figure 3: Forest plots of sensitivity and specificity of Xpert and Ultra stratified by stool processing method

Centrifuge-free versus non-centrifuge free (A) and SOS plus two simple step versus more complexity (B) stool processing methods for Xpert as the index test. Centrifuge-free versus non-centrifuge free (C) and no SOS versus SOS (D) stool processing methods for Xpert Ultra as the index test. SOS=simple one step.

Table 1:

Characteristics of included studies

	Study setting	Entry criteria or selection of cases	Age for inclusion	Eligible sample size	HIV prevalence, %	Study design	Follow-up ≥2 months	Molecular test in stool	Specimens used for the reference standard	Reference test	Endpoint classification	Bacteriologically confirmed in respiratory samples, n (%)*	Tuberculosis cases, n (%)
Wolf et al (2008)[18]	Peru	Stegen and Toledo score ≥5 points	0–11 years	16	0%	Case–control	NR	IS6110 gene PCR	GA plus NPA	Culture	Culture positive	16 (100%)	16 (100%)
Oberhelman et al (2010)[31]	Peru	Stegen and Toledo ≥5 points cases; no tuberculosis for controls	0–12 years	456	NR	Case–control	NR	IS6110 gene PCR	GA plus NPA	Smear plus L–J	Stegen–Toledo	22 (100%)	218 (100%)
Walters et al (2012)[36]	South Africa	Presumptive tuberculosis	0–13 years	14	8·70%	Cross-sectional	NR	Xpert	GA	Culture	NR	6 (42·9%)	12 (85·7%)
Nicol et al (2013)[19]	South Africa	Presumptive tuberculosis	0–14 years	98	14·80%	Cohort	Yes	Xpert	IS	Xpert and culture	Graham et al (2012)[26]	17 (47·1%)	65 (56·5%)
Marcy et al (2016)[10]	Burkina Faso, Cambodia, Cameroon, and Vietnam	Presumptive tuberculosis plus HIV	0–13 years	267	100%	Cohort	Yes	Xpert	GA or sputum	Xpert and L–J with or without MGIT	Graham et al (2012)[26]	29 (10·7%)	135 (49·6%)
Moussa et al (2016)[37]	Egypt	Presumptive tuberculosis	1–16 years	115	0%	Cross-sectional	NR	Xpert	Sputum and IS	L–J	Graham et al (2012)[26]	36 (31·3%)	97 (84·3%)
Banada et al (2016)[32]	South Africa	Tuberculosis case	0–14 years	38	42·11%	Case–control	No	Xpert	GA or IS	Xpert	Xpert positive	20 (52·6%)	38 (100%)
Walters et al (2017)[55]	South Africa	Presumptive tuberculosis	0–13 years	188	15·40%	Cohort	Yes	Xpert	GA or sputum plus IS	Xpert and MGIT	Graham et al (2015)[27]	35 (18·6%)	128 (68·1%)
Chipinduro et al (2017)[38]	Zimbabwe	Presumptive tuberculosis	5–16 years	218	50·92%	Cross-sectional	NR	Xpert	IS with or without NPA	Xpert and L–J	NR	19 (8·7%)	32 (14·7%)
Hasan et al (2017)[39]	Pakistan	Kenneth-Jones score ≥5	0–15 years	49	Unknown	Cross-sectional	NR	Xpert	Sputum or GA	Xpert and culture	Treatment response	12 (24·5%)	18 (36·7%)
LaCourse et al (2018)[59]	Kenya	New HIV diagnosis	0–12 years	148	100%	Randomised controlled clinical trial	Yes	Xpert	Sputum or GA plus GA	Xpert and MGIT	Graham et al (2015)[27]	11 (7·4%)	63 (42·6%)
Orikiriza et al (2018)[56]	Uganda	Presumptive tuberculosis	1 month to 14 years	71	31%	Cohort	NR	Xpert	Sputum or IS	Smear, Xpert, and	Graham et al (2012)[26]	17 (4·2%)	26 (6·6%)

	Study setting	Entry criteria or selection of cases	Age for inclusion	Eligible sample size	HIV prevalence, %	Study design	Follow-up ≥2 months	Molecular test in stool	Specimens used for the reference standard	Reference test	Endpoint classification	Bacteriologically confirmed in respiratory samples, n (%)*	Tuberculosis cases, n (%)
										L–J plus MGIT			
Walters et al (2018)[57]	South Africa	Presumptive tuberculosis	0–13 years	148	16·20%	Cohort	Yes	Xpert	Sputum or IS plus GA	Xpert and MGIT	Graham et al (2015)[27]	3 (2·0%)	63 (42·6%)
DiNardo et al (2018)[16]	Eswatini	Tuberculosis case	No limit	38	54·05%	Case–control	Yes	qPCR	GA plus IS	Xpert and Culture	Graham et al (2012)[26]	10 (26·3%)	38 (100%)
Memon et al (2018)[40]	India	Probable tuberculosis	6months to 15 years	100	3%	Cross-sectional	NR	Xpert	GA and IS	MGIT	Graham et al (2012)[26]	26 (26·0%)	100 (100%)
Hanrahan et al (2019)[58]	South Africa	Presumptive tuberculosis	2 months to 10 years	119	17·65%	Cohort	Yes	Xpert	Sputum or NPA plus IS, plus NPA with or without GA	Smear, Xpert, and MGIT	Graham et al (2015)[27]	4 (3·4%)	104 (87·4%)
Andriyoko et al (2019)[22]	Indonesia	Presumptive tuberculosis screening workout	0–14 years	29	NR	Cross-sectional	NR	Xpert	GA in <5 year olds; IS in >5 year olds	Xpert	Bacteriological confirmation	3 (10·3%)	NR
Mesman et al (2019)[17]	Peru	Presumptive tuberculosis local	0–14 years	259	<0·1%	Case–control	NR	Tru-Tip	Sputum plus IS or GA	L–J and liquid culture	NR	22 (20·8%)	106 (100%)
Ngadaya et al (2020)[41]	Tanzania	Presumptive tuberculosis	1 year to no limit	108	NR	Cross-sectional	NR	Xpert	Sputum	Xpert and culture	Bacteriological confirmation	3 (2·8%)	NR
Ainan et al (2021)[42]	Tanzania	Presumptive tuberculosis or positive contact	1 month to 14 years	225	6·2%	Cross-sectional	NR	Xpert	IS or GA	Xpert and L–J	Bacteriological confirmation	8 (3·5%)	50 (22·2%)
Kabir et al (2021)[12]	Bangladesh	Presumptive tuberculosis	0–14 years	447	NR	Cross-sectional	NR	Xpert and Ultra	IS	Xpert, Ultra, and culture	Bacteriological confirmation	29 (6·5%)	68 (15·2%)
Song et al (2021)[43]	Kenya	Presumptive tuberculosis	0–5 years	294	24·33%	Cross-sectional	NR	Xpert	IS or GA plus NPA plus ST	Xpert and MGIT	Bacteriological confirmation	31 (10·5%)	NR
Osório et al (2021)[44]	Mozambique	SAM	0–5 years	45	22%	Cross-sectional	NR	Xpert	NPA plus Stool	Xpert and culture	Graham et al (2015)[27]	4 (8·9%)	17 (37·8%)
de Haas et al (2021)[23]	Ethiopia	Presumptive tuberculosis	0–15 years	123	NR	Cross-sectional	NR	Ultra	GA	Ultra, L–J, and MGIT	NR	9 (7·3%)	NR

	Study setting	Entry criteria or selection of cases	Age for inclusion	Eligible sample size	HIV prevalence, %	Study design	Follow-up ≥2 months	Molecular test in stool	Specimens used for the reference standard	Reference test	Endpoint classification	Bacteriologically confirmed in respiratory samples, n (%)*	Tuberculosis cases, n (%)
Liu et al (2021)[15]	China	Presumptive tuberculosis	0–15 years	126	0%	Cohort	Yes	Ultra	IS, NPA, or GA	Xpert and MGIT	Graham et al (2015)[27]	43 (34·1%)	88 (69·9%)
Dubale et al (2022)[45]	Ethiopia	Presumptive tuberculosis	0–15 years	152	NR	Cross-sectional	Yes	Xpert	GA or sputum	Xpert and L–J	Graham et al (2015)[27]	10 (6·6%)	20 (13·2%)
Orikiriza et al (2021)[46]	Uganda	Presumptive tuberculosis and risk of dissemination	NR	219	32·90%	Cross-sectional	Yes	Xpert	1 GA plus one IS or NPA	Smear, Xpert, L–J, and MGIT	Graham et al (2015)[27]	12 (5·5%)	70 (32·0%)
Marcy et al (2023)[60]	Cambodia, Cameroon, Côte d'Ivoire, Mozambique, Uganda, and Zambia	Severe pneumonia	2 months to 4 years	902	5·10%	Cluster randomised trial	Yes	Ultra	NPA, GA, or PL	Ultra	Clinical diagnosis	26 (2·2%)	88 (7·5%)
Sun et al (2022)[33]	China	Tuberculosis case	NR	175	NR	Case–control	NR	Xpert and Ultra	GA	Xpert and culture	Graham et al (2015)[27]	48 (34·0%)	141 (100%)
Kesarwani et al (2022)[34]	India	Tuberculosis case	1 year to no limit	30	NR	Case–control	NR	IS6110 gene PCR	GA	Smear and culture	Entry criteria	11 (36·7%)	30 (100%)
Agarwal et al (2022)[47]	India	Presumptive tuberculosis	6 months to 12 years	75	0%	Cross-sectional	NR	Xpert	GA	MGIT	National guidelines	11 (14·7%)	28 (37·3%)
Rekart et al (2023)[48]	Tajikistan	Presumptive tuberculosis	0–14 years	688	1·30%	Cross-sectional	Yes	Ultra	IS	Smear, Ultra, and culture (L–J and MGIT)	Graham et al (2012)[26]	18 (2·8%)	206 (29·9%)
Kaboré et al (2023)[49]	Burkina Faso	Presumptive tuberculosis	No limit	51	NR	Cross-sectional	No	Xpert	GA or sputum	L–J and Xpert	NA	26 (44·6%)	NR
Jayagandan et al (2022)[50]	India	Presumptive tuberculosis	0–10 years	75	NR	Cross-sectional	NR	Xpert and multiplex PCR	GA or IS	MGIT and Xpert	NA	10 (13·3%)	NR
Chibolea et al (2023)[51]	Zambia	Presumptive tuberculosis	0–5 years	114	23%	Cross-sectional	NR	Ultra	GA	MGIT and Ultra	Clinician's criteria	12 (10·5%)	NR
Babo et al (2023)[52]	Ethiopia	Presumptive tuberculosis	0–14 years	368	1·1%	Cross-sectional	NR	Ultra	GA or sputum	Ultra	National guidelines	23 (6·2%)	36 (9·6%)
Singhal et al (2024)[53]	India	Presumptive tuberculosis	0–18 years	100	NR	Cross-sectional	NR	Xpert	GA	Xpert and MGIT	Graham et al (2012)[26]	57 (57%)	76 (76·0%)

	Study setting	Entry criteria or selection of cases	Age for inclusion	Eligible sample size	HIV prevalence, %	Study design	Follow-up ≥2 months	Molecular test in stool	Specimens used for the reference standard	Reference test	Endpoint classification	Bacteriologically confirmed in respiratory samples, n (%)*	Tuberculosis cases, n (%)
Torane et al (2023)[54]	India	Confirmed tuberculosis	0–15 years	50	NR	Cross-sectional	NR	Xpert	GA or sputum	Xpert	NA	50 (100%)	50 (100%)
Kay et al (2024)[35]	Eswatini, Mozambique, and Tanzania	Tuberculosis case	10 years or older	97	NR	Case–control	Yes	Ultra	Sputum or IS	Ultra and MGIT	Clinician's criteria	5 (13·2%)	38 (100%)

GA=gastric aspirate. IS=induced sputum. L–J=Löwenstein–Jensen. MGIT=Mycobacteria growth indicator tube. NPA=nasopharyngeal aspirate. NR=not reported. PL=pleural liquid. qPCR=quantitative PCR. SAM=severe acute malnutrition. ST=string test.

*For cross-sectional studies the percentage of bacteriologically confirmed cases is expressed over the whole sample, for case–control studies it was represented from the number of tuberculosis cases.

Table 2:

Additionality of stool Xpert Ultra for *Mycobacterium tuberculosis* bacteriological confirmation

	Final number of participants	Cohort type	Respiratory sample type	Bacterially confirmed respiratory sample, n (%)	Bacterially confirmed including stool, n (%)	Positive in stool and not clinically diagnosed	Additionality in absolute %	Relative additionality, ratio
Kabir et al (2021)[12]	447	Presumptive tuberculosis	Induced sputum	29 (6·5%)	66 (14·8%)	0	8·3%	2·3
de Haas et al (2021)[23]	123	Presumptive tuberculosis	Gastric aspirate	9 (7·3%)	11 (8·9%)	0	1·6%	1·2
Liu et al (2021)[15]	126	Presumptive tuberculosis *	Gastric aspirate	56 (44·4%)	60 (47·6%)	2	3·2%	1·1
Sun et al (2022)[33]	141	Tuberculosis case definition †	Gastric aspirate	81 (57·4%)	100 (70·9%)	1	13·5%	1·2
Marcy et al (2023)[60]	902	Pneumonia	Nasopharyngeal aspirate, gastric aspirate, or pleural liquid	19 (2·1%)	22 (2·4%)	0	0·3%	1·2
Rekart et al (2023)[48]	688	Presumptive tuberculosis	Gastric aspirate	11 (1·6%)	25 (3·6%)	0	2·0%	2·3
Kay et al (2024)[35]	38	Tuberculosis case definition ‡	Induced sputum or sputum	5 (13·2%)	7 (18·4%)	0	5·3%	1·4
Total	2465	..	..	210 (8·5%)	291 (11·8%)	..	3·3%	1·4

* Presumptive tuberculosis with abnormal chest x-ray.

† Definite and probable tuberculosis.

‡ Started on treatment.