BMJ Open

Stool specimen for diagnosis of pulmonary tuberculosis in adults: a systematic review

Saima Sultana [1], Sadia Afrin,[2] Mahamudul Hasan [3], Adnan Ansar,[4] KM Saif-Ur-Rahman [2,5,6]

To cite: Sultana S, Afrin S, Hasan M, *et al*. Stool specimen for diagnosis of pulmonary tuberculosis in adults: a systematic review. *BMJ Open* 2023;**13**:e062135. doi:10.1136/bmjopen-2022-062135

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-062135).

Received 18 February 2022
Accepted 18 April 2023

[1]Projahnmo Research Foundation, Dhaka, Bangladesh
[2]Health Systems and Population Studies Division, ICDDRB, Dhaka, Bangladesh
[3]World Health Organization Bangladesh, Dhaka, Bangladesh
[4]School of Nursing and Midwifery, College of Science, Health and Engineering, La Trobe University, Melbourne, Victoria, Australia
[5]Evidence Synthesis Ireland and Cochrane Ireland, University of Galway, Galway, Ireland
[6]College of Medicine, Nursing and Health Sciences, University of Galway, Galway, Ireland

Correspondence to
Dr Saima Sultana;
saima.sultana.jhu@gmail.com

ABSTRACT

Objective To assess the diagnostic accuracy of stool specimens to diagnose pulmonary tuberculosis (PTB) in adults.

Design Systematic review.

Data sources MEDLINE (Ovid), Embase (Ovid), Web of Science and the Cochrane database were searched from inception to 9 March 2023–10 March 2023 using a comprehensive search strategy; reference lists of selected articles and relevant review articles were manually searched.

Eligibility criteria for selecting studies Studies in English reporting diagnostic performance of stool specimens against respiratory specimens using mycobacterial culture or smear microscopy or Xpert assay to diagnose PTB in adults were eligible for this systematic review.

Data extraction and synthesis Two reviewers independently screened the retrieved citations and extracted data. The risk of bias and applicability of results were assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 tool. Narrative data synthesis was performed.

Results A total of 1658 citations were screened, and 28 full-text articles were assessed. Nine studies met the inclusion criteria. The reported sensitivity and specificity of stool culture varied between 21.4% and 63.9%, and 61.5% and 100%, respectively. In stool smear microscopy, sensitivities and specificities ranged from 12.1% to 53.9%, and from 79.5% to 100%, respectively. The reported sensitivities of PCR assays, including Xpert assays, ranged from 69.7% to 100%, with specificities ranging from 69.8% to 100%. Most of the studies had a low risk of bias and a low applicability concern in all domains.

Conclusion This systematic review could not conclude on the diagnostic accuracy of stool specimens for PTB diagnosis in adults. Further studies are required to evaluate the accuracy of stool specimens in adults to enable meta-analyses in updates of this review as well as other systematic reviews.

PROSPERO registration number CRD42021245203.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ A comprehensive, up-to-date search was performed, and rigorous methodologies were employed.
⇒ This review only included articles in English, which might result in us having missed relevant articles published in other languages.
⇒ Significant heterogeneity among the included studies precluded a meta-analysis of study results.

INTRODUCTION

Tuberculosis (TB) is a major infectious disease caused by the bacteria *Mycobacterium tuberculosis* (MTB). Before the COVID-19 pandemic, it was the leading cause of death worldwide due to a single infectious pathogen.[1] In low-income and lower-middle-income countries, it is still ranked among the top 10 causes of mortality.[2] Each year, it affects around 10 million people globally, and kills approximately 1.4 million,[3] with the majority suffering from pulmonary TB (PTB).[4] Accurate diagnosis is one of the major obstacles to global TB control, and WHO has prioritised strengthening diagnostic criteria and tests.[4 5] Early identification of TB is critical to ensure optimal patient care and treatment, including effective TB control.[6] Despite the availability and recent advances in TB diagnostic tests, only 59% of PTB cases reported globally were bacteriologically verified,[1] implying that nearly half of the patients are treated for TB based on clinical symptoms, radiographic findings and contact history with patients with TB. As a result, there is an urgent need to develop an expeditious and accurate TB diagnostic test.

Sputum remains the most commonly used clinical sample and microbiological confirmation of MTB is mostly reliant on sputum samples for PTB diagnosis.[7] Sputum smear microscopy is the most widely available diagnostic test for pulmonary TB in primary healthcare settings,[8 9] while mycobacterial culture is considered the gold standard for bacteriological confirmation with drug susceptibility testing (DST) able to inform appropriate treatment regimen.[10] The nucleic acid amplification tests, such as PCR, real-time PCR

(RT-PCR) and loop-mediated amplification test, are available for TB diagnosis.[11 12] In recent years, Xpert MTB/RIF, using the RT-PCR, has been made widely available at the secondary and tertiary care levels with advantages of higher sensitivity than smear microscopy, the ability to detect rifampicin resistance and to provide rapid bacteriological confirmation within 1–2 days.[11 13 14] Moreover, the WHO 2021 consolidated guidelines on TB has recommended Xpert MTB/RIF as an initial diagnostic test instead of microscopy, culture and DST for both adults and children with signs and symptoms of pulmonary TB.[15]

The diagnostic accuracy of these tests varied by the quality and the amount of MTB in sputum samples.[4] Furthermore, PTB diagnosis is challenging for individuals who are unable to expectorate sputum, which is especially common among young children, HIV patients, the severely ill and the elderly.[16] Sputum induction, gastric or nasopharyngeal aspiration, or fibre-optic bronchoscopy are used as alternative methods to collect respiratory specimens from individuals who are unable to produce expectorated sputum. These procedures are invasive, costly and require qualified technical skills and equipment that might not be readily available, particularly in resource-constrained settings.[17–19] As a result, a non-invasive approach for diagnosing PTB without sputum would benefit these critical patient groups. Stool samples can be used as a substitute for respiratory specimens in the diagnosis of PTB.[20] When sputum is swallowed and MTB passes through the digestive tract, microscopy, culture and PCR testing, including the Xpert assay, can detect MTB in stool specimens.[21 22] Recent systematic reviews on PTB diagnosis in paediatric patients using the Xpert and other PCR-based assays on stool samples have shown high specificity with moderate sensitivity and suggest using stool samples as a rule-in test for PTB diagnosis in children.[23–25] WHO has also recommended stool specimens on Xpert MTB/RIF assay for PTB diagnosis in children in its latest update in 2021.[15]

While a number of studies evaluating the use of stool samples for PTB diagnosis in adults have been published, the utility in the adult population has not yet been assessed through a systematic analysis. Therefore, this systematic review aims to compare the diagnostic accuracy of stool specimens in microscopy, culture and PCR assays against the microbiological reference standard test/s, such as smear microscopy, culture or Xpert assay using respiratory specimen/s to diagnose PTB in adults. This review is intended to serve as a crucial resource for information on the diagnosis of PTB in adults, particularly in patients who provide insufficient sputum or are unable to expectorate sputum.

METHODS

This systematic review was conducted following the methodology of Cochrane Systematic Reviews of Diagnostic Test Accuracy[26] and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy criteria.[27] The published protocol describes the methodology in detail, including the formulation of a search strategy, the screening data extraction, the appraisal of included articles for quality assessment and data synthesis.[16] This systematic review is registered in the International Prospective Register of Systematic Reviews (CRD42021245203).

Search strategy and selection criteria

Search terms, keywords and synonyms such as "Mycobacterium tuberculosis" or "MTB," "pulmonary tuberculosis" or "PTB," "tuberculosis," or "TB," "adults" or "elderly," "stool" or "faeces" or "faecal," and "diagnosis" or "diagnostic" and different combinations of the keywords were used to develop a comprehensive search strategy. Four databases: MEDLINE (Ovid), Embase (Ovid), Web of Science and Cochrane database were searched with no date restrictions. The initial search was conducted in April 2021 and it was updated on 9 March 2023–10 March 2023 (final searches are available in online supplemental file 1). In addition, to prevent missing relevant studies, reference lists of identified studies and relevant reviews were also searched.

Studies were selected in compliance with the following inclusion criteria:

I. Examined stool specimens in adults (18 years and older) with presumptive/active PTB using microscopy or culture or PCR assays (index test).
II. PTB diagnosis was accompanied by bacteriological confirmation of MTB in the respiratory samples using culture and/or microscopy, and/or Xpert assay (reference test).
III. Study design: retrospective and prospective cross-sectional and cohort studies, randomised controlled trials, and case–control studies that evaluated stool samples for PTB diagnosis.
IV. Studies that evaluated diagnostic accuracy and/or supplied enough data to generate diagnostic accuracy metrics (true positive, false positive, true negative, false negative).
V. Studies that also used banked/stored sputum and stool specimens for PTB diagnosis.
VI. Studies including both adults and children, and disaggregated adult data were available.

Studies were excluded if (1) study participants were below 18 years; (2) stool samples were not tested for PTB diagnosis; (3) case reports, reviews, conference proceedings, and abstracts, editorials, and commentaries and (4) articles in languages other than English.

Study screening and selection

Search results were imported into Covidence,[28] and duplicate articles were removed. Two review authors (SS and KMS-U-R/SA) independently screened the titles and abstracts of all included articles as per the predefined eligibility criteria, followed by the full-text review of all selected articles (SS and KMS-U-R/SA). Any discrepancies

were resolved through discussion, or a third reviewer (MH) was consulted, where necessary.

Data extraction and assessment of study quality

Data extractions were independently carried out by two review authors (SS and MH) using a predefined data collection template. Any discrepant judgements were discussed and resolved by a third reviewer (KMS-U-R). Attempts were taken to communicate with the study corresponding author/s to obtain additional data.

Two reviewers (KMS-U-R and MH) independently assessed the risk of bias and applicability of the included studies using the Quality Assessment of Diagnostic Accuracy Studies tool.[29] Non-consensus between the reviewers was resolved through consultation with the third reviewer (SS).

Data synthesis

A descriptive synthesis was performed following the review objective and outcome measures. Meta-analyses could not be performed due to heterogeneity in included studies and insufficient data. Exact binomial 95% CIs were computed for all estimates of sensitivity and specificity.

Patient and public involvement

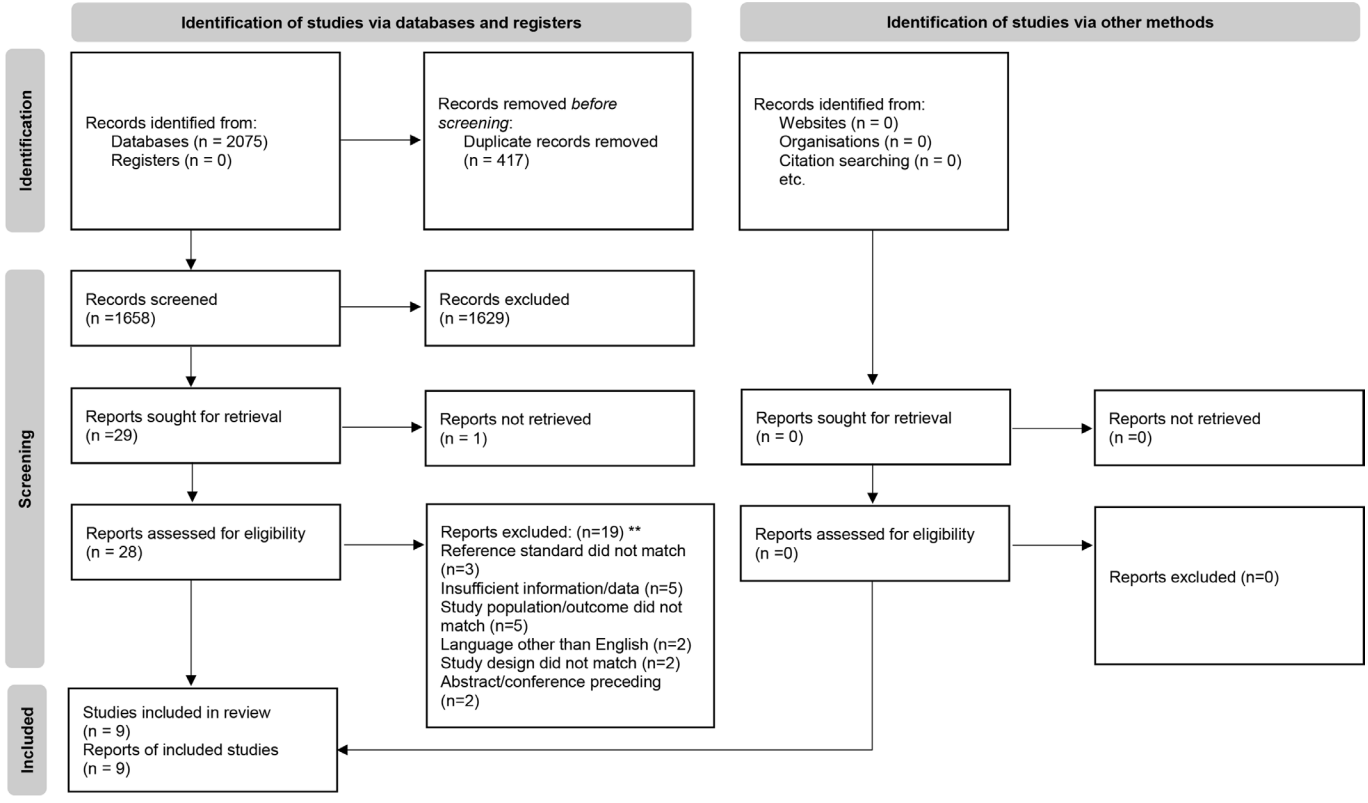
None.

RESULTS

A total of 2075 citations were identified through the database search. After duplicates were removed, 1658 unique citations remained. In total, 1629 citations were excluded during the title and abstract screening, and 28 full-text articles were reviewed. Of these, nine articles were eligible for inclusion (figure 1).

Characteristics of the included studies

The included studies were conducted in 10 countries, most of them from Asian countries,[30–34] 3 studies from Africa[35–37] and 1 study from Europe.[38] Of these, four studies were conducted in five high-TB-burden countries—Bangladesh,[34] China,[32] Ethiopia,[35] and Thailand and Vietnam,[33] including one study in a high TB/HIV burden country.[37] Most studies (7 out of 9, 78%) were carried out prospectively.[32–38] The number of microbiologically positive (PTB patients) and negative (non-TB healthy individuals) cases in the included studies ranged from 65 to 187, with a total of 998 participants. Two studies recruited only persons living with the HIV[33 35] whereas three studies did not report on the HIV status of the participants,[32 34 38] and the rest of the studies had a mixed population. Seven studies used expectorated sputum as the specimen type for the reference standard and one study used transtracheal aspirate sputum specimens.[32] Reference standard tests were variable across the



**multiple reasons applicable

Figure 1 PRISMA flow diagram. **multiple reasons applicable. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

studies, six studies[32–37] had culture as the reference standard while other studies reported a combination of reference standards (eg, culture and/or smear microscopy and PCR). All studies reported different types of culture media for the reference standard. Six studies performed all three diagnostic tests (culture, microscopy and PCR) on the stool specimen,[30 31 34 35 37 38] one study used only culture[33] and the rest two performed PCR tests.[32 36] Among the studies that performed PCR tests, three studies performed Xpert MTB/RIF assay,[30 32 34] including one study used Xpert MTB/RIF ultra (Xpert ultra)[37] and the remaining studies used other PCR assays such as IS6110, quantitative PCR, Region of Difference 9-based PCR, Transcription Reverse-transcription Concerted reaction and TB molecular bacterial load assay. Online supplemental table 1 summarises the key characteristics of the included studies. A substantial variation was also observed in stool processing methods across the studies (online supplemental file 2).

Diagnostic accuracy results

The sensitivity of stool specimen culture ranged from 21.4% to 63.9% in the 7 studies, among them, 2 studies using 2 culture media reported the diagnostic accuracy results separately.[30 37] The specificities of stool culture were varied between 61.5% and 100%. The reported sensitivities of stool smear microscopy were relatively low, ranging from 12.1% to 53.9% in the 6 studies. By contrast, the specificity was uniformly high across most of the studies, ranging from 79.5% to 100%. The sensitivity and specificity of the Xpert MTB/RIF assay ranged from 85.7% to 90.6% and 93.9% to 100%, respectively, while the reported sensitivity and specificity of Xpert ultra were 83.6% and 87.2%. For other PCR assays, the sensitivity and specificity varied from 69.7% to 100% and 69.8% to 97.3%, respectively, in the 5 studies. The sensitivity and specificity of the included studies are presented in online supplemental table 2 and figure 2.

Methodological quality of included studies

The risk of bias was high in three studies for the patient selection domain for using a case–control study design,[31 34] and for an unclear sampling strategy,[30] the remainder had a low risk of bias. For the index test and the flow and timing domain, all studies were judged to have a low risk of bias. The risk of bias was unclear in one of the studies[31] for the reference standard domain, as it was uncertain whether the reference standard results were interpreted without knowledge of the index test results. There were low applicability concerns for patient selection, index test and reference standard domains across all the studies, except one study[31] was judged to have unclear applicability concerns for reference standard domains. The summary of the risk of bias assessment of the included studies is presented in figure 3.

DISCUSSION

TB is a major global health problem, claiming millions of lives each year and contributes significantly to the disease burden across the world. Early and correct diagnosis is critical to prevent TB morbidity and mortality. However, despite significant progress in technologies and methods in TB diagnosis, rapid and early diagnosis remains a key challenge for PTB diagnosis. Sputum is the most widely used clinical specimen for PTB testing, yet it possesses some challenges in the acquisition of a sputum sample and the performance of the diagnostic test/s often depends on the quality of the sputum specimen.[4] This systematic review aimed to evaluate the diagnostic performance of stool specimens in PTB diagnosis in adults.

The literature search and screening process identified nine articles that met the inclusion criteria. This systematic review identified substantial variation in the included studies in terms of study subjects, use of reference standards and index test/s, including stool processing methods. Hence, we could not conclude on the diagnostic accuracy of stool specimens for PTB diagnosis from this systematic review. A considerable variation was observed in diagnostic accuracies, particularly for sensitivities and all with wide CIs. These variations in accuracy estimates might be owing to differences in the included studies as mentioned earlier. However, specificities were found to be higher among the included studies compared with sensitivity estimates. Furthermore, in contrast to observed between-study variations of stool culture and smear microscopy, PCR assays including Xpert MTB/RIF assays demonstrated higher sensitivity (69.7%–100%). Previous systematic reviews and meta-analyses evaluating the performance of stool specimens in PTB diagnosis in children using Xpert MTB/RIF, including in-house molecular tests showed a pooled sensitivity of 67%,[24] 50%[23] and 57%.[25] A Cochrane review also reported a sensitivity of 61.5% and a specificity of 98.5% in paediatric stool specimens for Xpert MTB/RIF, while the sputum Xpert MTB/RIF showed slightly higher sensitivity and specificity at 64.6% and 99%, respectively.[39] It is noteworthy to mention that there has been a lack of standardised protocols for stool specimen collection and processing that also influence the diagnostic yields, particularly for molecular assays,[24 25] hence requiring further investigations to evaluate and compare the performances of these different methods. Furthermore, the observed low sensitivities in stool culture can be explained by the fact that the decontamination procedure prior to culture might lead to the destruction of the *MTB bacilli* and result in a reduction of viable bacillary loads in culture. A previous study also suggested an enhanced decontamination technique including increasing stool sample volume to achieve a greater diagnostic yield of the stool culture for PTB diagnosis.[40] Three out of nine included studies were considered to have a high risk of bias in the patient selection domain due to using a case–control design or an unclear sampling method. Of note, all of the included studies had relatively smaller sample sizes, meaning they are not sufficiently powered to provide statistically significant diagnostic accuracy estimates. Although, sample size estimation in diagnostic test studies depends on a number

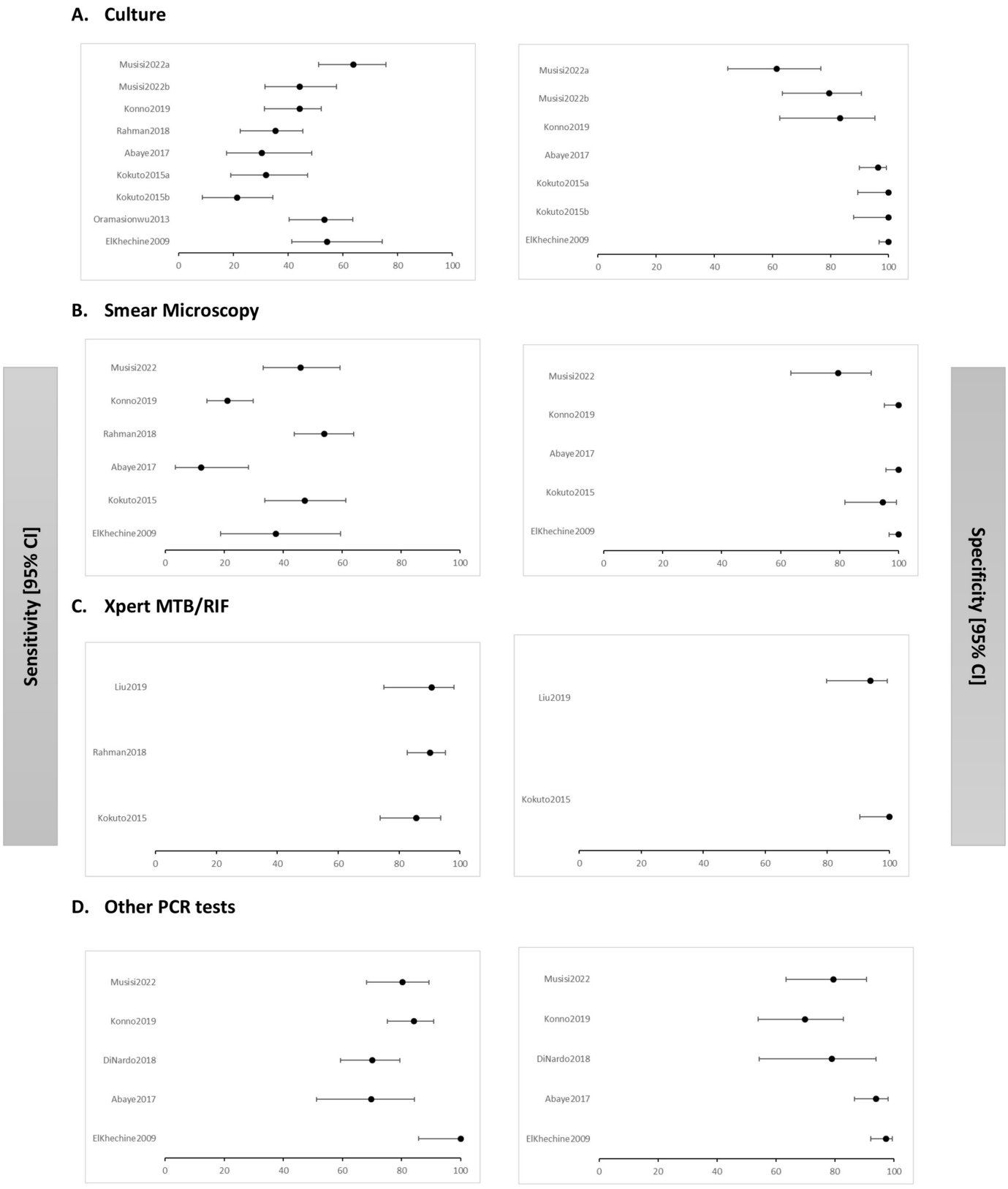


Figure 2 Forest plots showing sensitivity and specificity of the stool specimen in different tests.

of prespecified parameters, a minimum of 300 subjects is required to evaluate both the sensitivity and specificity of the diagnostic test.[41] Thus, future studies should consider appropriate study design including a larger sample size to provide robust evidence using stool samples.

Further research using standardised procedures is needed to provide accuracy estimates of stool specimens in adult PTB diagnosis through meta-analysis which will be crucial evidence on the TB diagnostic landscape for early detection and initiating treatment, particularly for

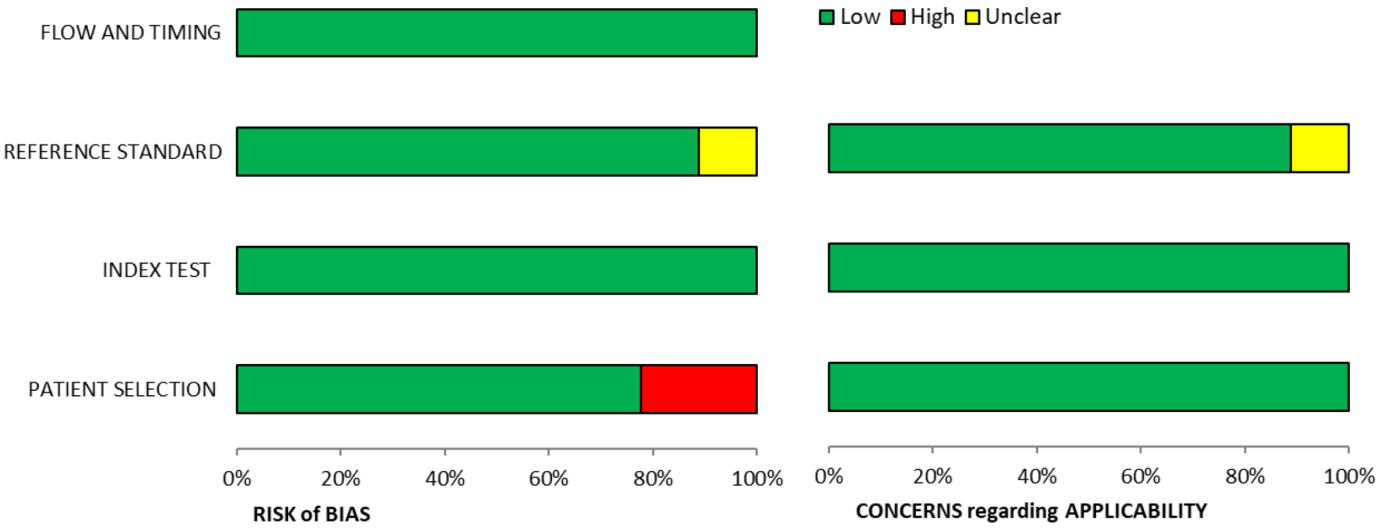


Figure 3 Summary of risk of bias assessment and applicability using QUADAS-2. QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies-2.

those who are unable to provide sputum specimens. More studies using Xpert MTB/RIF would be beneficial as WHO's current guideline recommends using Xpert MTB/RIF over smear microscopy or culture for individuals with signs and symptoms of PTB.[15]

Our systematic review has several limitations. First, the heterogeneous nature of the included studies and paucity of data prohibited us from performing a meta-analysis and concluding on the diagnostic accuracy of stool specimens for PTB diagnosis in adults. Second, we only included articles published in the English language which might result in missing relevant articles written in other languages. Nonetheless, to the best of our knowledge, this is the first systematic review looking into the diagnostic accuracy of stool specimens in adults for PTB diagnosis using several diagnostic tests, that is, culture, microscopy and PCR assays, including Xpert MTB/RIF. An important strength of this review was to use a comprehensive search strategy using several search databases to identify all relevant studies. Screening, study selection, quality assessment and data extraction were independently undertaken by two reviewers, with assessments compared and disagreements resolved by discussion or consultation with a third reviewer.

CONCLUSION

This systematic review could not conclude on the diagnostic accuracy of stool specimens for PTB diagnosis in adults. Further studies are required to evaluate the accuracy of stool specimens in adults to enable meta-analyses in updates of this review as well as other systematic reviews. Further diagnostic accuracy studies following standardised protocols and procedures are warranted to generate more evidence on the diagnostic accuracy of stool specimens to diagnose PTB in adults and to perform meta-analyses in future reviews.

Acknowledgements The authors (KMSUR, SA) would like to acknowledge the contribution of the current donors providing unrestricted support to icddr,b that include: the Governments of Bangladesh, Canada, Sweden and the UK. We gratefully acknowledge these donors for their support and commitment to icddr,b's research efforts.

Contributors SS conceptualised and designed this systematic review. SS and KS-U-R led the search strategy. SS, KS-U-R and MH obtained and apprised data. SS and SA developed the first draft, with revisions from AA. All authors reviewed the manuscript and provided intellectual inputs and approved the final version. SS is the guarantor of this review.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information.

ORCID iDs
Saima Sultana http://orcid.org/0000-0001-6129-918X
Mahamudul Hasan http://orcid.org/0000-0002-2683-9803
KM Saif-Ur-Rahman http://orcid.org/0000-0001-8702-7094

REFERENCES

1 WHO. *Global tuberculosis report 2021*. Geneva: WHO, 2021.
2 WHO. The top 10 causes of death: World Health organization. 2020. Available: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death
3 WHO. *Global tuberculosis report 2020*. Geneva: World Health Organization, 2020.
4 Datta S, Shah L, Gilman RH, *et al*. Comparison of sputum collection methods for tuberculosis diagnosis: a systematic review and pairwise and network meta-analysis. *Lancet Glob Health* 2017;5:e760–71.
5 WHO. *Global tuberculosis report 2014*. Geneva: World Health Organization, 2014.
6 Agrawal M, Bajaj A, Bhatia V, *et al*. Comparative study of GeneXpert with Zn stain and culture in samples of suspected pulmonary tuberculosis. *J Clin Diagn Res* 2016;10:DC09–12.
7 Pai M, Schito M. Tuberculosis diagnostics in 2015: landscape, priorities, needs, and prospects. *J Infect Dis* 2015;211 Suppl 2(Suppl 2):S21–8.
8 WHO. *Systematic screening for active tuberculosis: principles and recommendations*. Geneva, Switzerland: World Health Organization, 2013.
9 WHO. *Approaches to improve sputum smear microscopy for tuberculosis diagnosis: expert group meeting report*. Geneva: World Health Organization, 2009.
10 WHO. *Implementing tuberculosis diagnostics: A policy framework*. Geneva: World Health Organization, 2015.
11 Pai M, Nicol MP, Boehme CC. Tuberculosis diagnostics: state of the art and future directions. *Microbiol Spectr* 2016;4.
12 Namuganga AR, Chegou NN, Mayanja-Kizza H. Past and present approaches to diagnosis of active pulmonary tuberculosis. *Front Med (Lausanne)* 2021;8:709793.
13 Horne DJ, Kohli M, Zifodya JS, *et al*. Xpert MTB/RIF and Xpert MTB/RIF ultra for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2019;6:CD009593.
14 WHO. *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system: policy statement*. Geneva: World Health Organization, 2011.
15 WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization, 2021.
16 Sultana S, Ansar A, Saif-Ur-Rahman KM. Stool specimen for diagnosis of pulmonary tuberculosis in adults: protocol for a systematic review and meta-analysis. *BMJ Open* 2021;11:e052212.
17 Tan H-K, Fan S-J, Xu Y-C, *et al*. The clinical diagnostic value of Xpert MTB/RIF for the detection of Mycobacterium tuberculosis in gastric aspirates. *Biosci Rep* 2020;40.
18 Luo W, Lin Y, Li Z, *et al*. Comparison of sputum induction and bronchoscopy in diagnosis of sputum smear-negative pulmonary tuberculosis: a systemic review and meta-analysis. *BMC Pulm Med* 2020;20.
19 Ruiz Jiménez M, Guillén Martín S, Prieto Tato LM, *et al*. Induced sputum versus gastric lavage for the diagnosis of pulmonary tuberculosis in children. *BMC Infect Dis* 2013;13:222.
20 Datta S, Gonzales-Huerta LE, Evans CA. The potential for testing stool to reduce tuberculosis missed diagnoses and misdiagnoses. *Am J Trop Med Hyg* 2018;99:243–5.
21 Walters E, Demers A-M, van der Zalm MM, *et al*. Stool culture for diagnosis of pulmonary tuberculosis in children. *J Clin Microbiol* 2017;55:3355–65.
22 Donald PR, Schaaf HS, Gie RP, *et al*. Stool microscopy and culture to assist the diagnosis of pulmonary tuberculosis in childhood. *J Trop Pediatr* 1996;42:311–2.
23 Gebre M, Cameron LH, Tadesse G, *et al*. Variable diagnostic performance of stool Xpert in pediatric tuberculosis: a systematic review and meta-analysis. *Open Forum Infect Dis* 2021;8:ofaa627.
24 MacLean E, Sulis G, Denkinger CM, *et al*. Diagnostic accuracy of stool Xpert MTB/RIF for detection of pulmonary tuberculosis in children: a systematic review and meta-analysis. *J Clin Microbiol* 2019;57:e02057-18.
25 Mesman AW, Rodriguez C, Ager E, *et al*. Diagnostic accuracy of molecular detection of Mycobacterium tuberculosis in pediatric stool samples: a systematic review and meta-analysis. *Tuberculosis (Edinb)* 2019;119:101878.
26 Cochrane Collaboration. *Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy*.
27 Salameh J-P, Bossuyt PM, McGrath TA, *et al*. Preferred reporting items for systematic review and meta-analysis of diagnostic test accuracy studies (PRISMA-DTA): explanation, elaboration, and checklist. *BMJ* 2020;370:m2632.
28 Covidence systematic review software. Melbourne, Australia: Veritas Health Innovation, 2017.
29 Whiting PF, Rutjes AWS, Westwood ME, *et al*. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155:529–36.
30 Kokuto H, Sasaki Y, Yoshimatsu S, *et al*. Detection of Mycobacterium tuberculosis (MTB) in fecal specimens from adults diagnosed with pulmonary tuberculosis using the Xpert MTB/rifampicin test. *Open Forum Infect Dis* 2015;2:ofv074.
31 Konno A, Narumoto O, Matsui H, *et al*. The benefit of stool Mycobacterial examination to diagnose pulmonary tuberculosis for adult and elderly patients. Journal of clinical tuberculosis and other Mycobacterial diseases 2019;16:100106.
32 Liu R, Liang Q, Shang Y, *et al*. Genexpert of stool versus gastric lavage fluid for the diagnosis of pulmonary tuberculosis in severely ill adults. *Infection* 2019;47:611–6.
33 Oramasionwu GE, Heilig CM, Udomsantisuk N, *et al*. The utility of stool cultures for diagnosing tuberculosis in people living with the human immunodeficiency virus. *Int J Tuberc Lung Dis* 2013;17:1023–8.
34 Rahman SMM, Maliha UT, Ahmed S, *et al*. Evaluation of Xpert MTB/RIF assay for detection of Mycobacterium tuberculosis in stool samples of adults with pulmonary tuberculosis. *PLoS One* 2018;13:e0203063.
35 Abaye GE, Abebe T, Worku A, *et al*. Detection of Mycobacterium tuberculosis from the stool of HIV sero-positive individuals suspected of pulmonary tuberculosis. *PLoS One* 2017;12:e0177529.
36 DiNardo AR, Kay AW, Maphalala G, *et al*. Diagnostic and treatment monitoring potential of a stool-based quantitative polymerase chain reaction assay for pulmonary tuberculosis. *Am J Trop Med Hyg* 2018;99:310–6.
37 Musisi E, Sessolo A, Kaswabuli S, *et al*. High Mycobacterium tuberculosis bacillary loads detected by tuberculosis molecular bacterial load assay in patient stool: a potential alternative for nonsputum diagnosis and treatment response monitoring of tuberculosis. *Microbiol Spectr* 2022;10:e02100-21.
38 El Khéchine A, Henry M, Raoult D, *et al*. Detection of Mycobacterium tuberculosis complex organisms in the stools of patients with pulmonary tuberculosis. *Microbiology (Reading)* 2009;155(Pt 7):2384–9.
39 Kay AW, González Fernández L, Takwoingi Y, *et al*. Xpert MTB/RIF and Xpert MTB/RIF ultra assays for active tuberculosis and rifampicin resistance in children. *Cochrane Database Syst Rev* 2020;8:CD013359.
40 Oberhelman RA, Soto-Castellares G, Caviedes L, *et al*. Improved recovery of Mycobacterium tuberculosis from children using the microscopic observation drug susceptibility method. *Pediatrics* 2006;118:e100–6.
41 Bujang MA, Adnan TH. Requirements for minimum sample size for sensitivity and specificity analysis. *J Clin Diagn Res* 2016;10:YE01–6.

Supplementary table 1: Characteristics of the included studies

Study	Country	Study design	No of participants*	Age in years (median/mean)	HIV status (% HIV +ve)	Specimen type for reference standard	Reference standard	Index test/s	Type of culture media used for reference/index test
Kokuto 2015 (30)	Japan	Retrospective case control	93	Median: 59.6	0%	Sputum	Culture or molecular diagnostics (TRC)	Culture, Microscopy, Xpert MTB/RIF	MGIT, 2% Ogawa
Konno 2019 (31)	Japan	Retrospective investigation	187	Mean: 74	3.7%	Sputum	Culture, or microscopy	Culture, Microscopy, TRC Rapid	MGIT, 2% Ogawa
Liu 2019 (32)	China	Cross sectional	65	Median: 42	Not reported	Transtracheal aspirate sputum	Culture	Xpert MTB/RIF	MGIT
Oramasionwu 2013 (33)	Cambodia, Thailand, Viet Nam	Cross sectional	94**	Median: 29	100%	Sputum	Culture	Culture	Lowenstein Jensen or BACTEC MGIT 960 or BACTEC 9050/9120
Rahman 2018 (34)	Bangladesh	Case control	102	Mean: 33.4 (PTB)	Not reported	Sputum	Culture	Culture, Microscopy, Xpert MTB/RIF	Lowenstein Jensen
Abaye 2017 (35)	Ethiopia	Cross sectional	117	Mean: 34.5	100%	Sputum	Culture	Culture, Microscopy, Region of Difference (RD)9–PCR	Lowenstein Jensen
DiNardo 2018 (36)	Swaziland	Cross sectional	106	Median: 32	68% (PTB)	Sputum (expectorate /induced)	Culture	Quantitative PCR (qPCR)	Middlebrook 7H9
Musisi 2022 (37)	Uganda	Cross sectional	100	Median: 34	35%	Sputum	Culture	Culture, Microscopy, Xpert ultra, TB-MBLA	MGIT, Lowenstein Jensen
ElKhechine 2009 (38)	France	Cross sectional	134	Mean: 37 (PTB)	Not reported	Sputum	Culture or microscopy and real-time PCR	Culture, Microscopy, IS6110 PCR	BACTEC, Lowenstein Jensen

*Implied only bacteriologically confirmed positive PTB or negative TB cases; ** excluded cases with positive culture on both sputum and non-stool extrapulmonary sample

Supplementary table 2: Diagnostic accuracy results

Test type	Study	Sensitivity [95% CI]	Specificity [95% CI]
Culture	Kokuto 2015a (30)	21.4 [11.6-34.4]	100 [89.4-100]
	Kokuto 2015b (30)	31.9 [19.1-47.1]	100 [88-100]
	Konno 2019 (31)	44.2 [36.4-52.1]	83.3 [62.6-95.3]
	Oramasionwu 2013 (33)	53.2 [42.6-63.6]	*
	Rahman 2018 (34)	35.3 [26.1-45.4]	**
	Abaye 2017 (35)	30.3 [15.6- 48.7]	96.4 [89.9-99.3]
	Musisi 2022a (37)	63.9 [50.6-75.8]	61.5% [44.6-76.6]
	Musisi 2022b (37)	44.3 [31.5-57.6]	79.5 [63.5-90.7]
	ElKhechine 2009 (38)	54.2 [32.8-74.4]	100 [96.7-100]
AFB Microscopy	Kokuto 2015 (30)	47.3 [33.7-61.2]	94.6[81.8-99.3]
	Konno 2019 (31)	21.1 [14-29.7]	100 [95.1-100]
	Rahman 2018 (34)	53.9 [43.8-63.8]	*
	Abaye 2017 (35)	12.1 [3.4-28.2]	100 [95.7-100]
	Musisi 2022 (37)	45.9 [33.1-59.2]	79.5 [63.5-90.7]
	ElKhechine 2009 (38)	37.5 [18.8-59.4]	100 [96.7-100]
Xpert MTB/RIF	Kokuto 2015 (30)	85.7 [73.8-93.6]	100 [90.5-100]
	Liu 2019 (32)	90.6 [75-98]	93.9 [79.8-99.3]
	Rahman 2018 (34)	90.2 [82.7-95.2]	*
Xpert Ultra	Musisi 2022 (37)	83.6 [71.9-91.8]	87.2 [72.6-95.7]
Other PCR assay	Konno 2019 (31)	84.2 [75.3-90.9]	69.8 [53.9-82.8]
	Abaye 2017 (35)	69.7 [51.3-84.4]	94 [86.7-98]
	DiNardo 2018 (36)	70.1 [59.4-79.5]	78.9 [54.4-93.9]
	Musisi 2022 (37)	80.3 [68.2-89.4]	79.5 [63.5-90.7]
	ElKhechine 2009 (38)	100 [85.8-100]	97.3 [92.2-99.4]

*not reported; **non-TB healthy controls were not microbiologically confirmed. Therefore, we did not include specificity results

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily (1946 to March 09, 2022)

#	Searches	Results
1	exp Mycobacterium tuberculosis/	56893
2	(Mycobacterium tuberculosis or MTB or mycobacterium tuberculosis complex).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	76134
3	exp Tuberculosis/	204979
4	exp Tuberculosis, Pulmonary/	77546
5	(TB or PTB or pulmonary tuberculosis or lung tuberculosis).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	106486
6	1 or 2 or 3 or 4 or 5	276944
7	exp Adult/	7902127
8	exp Aged/	3439008
9	exp "Aged, 80 and over"/	1012459
10	exp Middle Aged/	4710681

11	(aged or elder* or adult*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	8946855
12	7 or 8 or 9 or 10 or 11	8946860
13	exp Feces/	107456
14	(stool or f?eces or f?ecal).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	210628
15	((stool or f?eces or f?ecal) adj3 (analysis or sample* or specimen*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	48531
16	13 or 14 or 15	216070
17	exp Diagnosis/	9296766
18	exp "Diagnostic Techniques and Procedures"/	7828075
19	exp "Sensitivity and Specificity"/	644623
20	diagnos*.mp.	5839263

21	(diagnos* adj3 (accuracy or performance)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	93653
22	17 or 18 or 19 or 20 or 21	11692041
23	6 and 12 and 16 and 22	254

Database: Embase Classic+Embase (1947 to March 9, 2022)

#	Searches	Results
1	exp Mycobacterium tuberculosis/	85907
2	(Mycobacterium tuberculosis or MTB or mycobacterium tuberculosis complex).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	108425
3	exp Tuberculosis/	296667
4	exp Tuberculosis, Pulmonary/	81940
5	(TB or PTB or pulmonary tuberculosis or lung tuberculosis).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	191949

6	1 or 2 or 3 or 4 or 5	392093
7	exp Adult/	11110912
8	exp Aged/	3729387
9	exp "Aged, 80 and over"/	207051
10	exp Middle Aged/	2180061
11	(aged or elder* or adult*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	12258291
12	7 or 8 or 9 or 10 or 11	12258331
13	exp Feces/	95973
14	(stool or f?eces or f?ecal).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	311211
15	((stool or f?eces or f?ecal) adj3 (analysis or sample* or specimen*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	95040
16	13 or 14 or 15	311211
17	exp Diagnosis/	8393516
18	exp "Diagnostic Techniques and Procedures"/	8393516

19	exp "Sensitivity and Specificity"/	474829
20	diagnos*.mp.	7898663
21	(diagnos* adj3 (accuracy or performance)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	7898663
22	17 or 18 or 19 or 20 or 21	23174442
23	6 and 12 and 16 and 22	954

Database: Web of Science (Search date: March 10, 2023)

#	Searches	Results
1	TS=(Mycobacterium tuberculosis OR MTB OR mycobacterium tuberculosis complex)	85791
2	TS=(TB or PTB or pulmonary tuberculosis or lung tuberculosis)	139341
3	#2 OR #1	190871
4	TS=(aged or elder* or adult*)	5671293
5	TS=(stool or f$eces or f$ecal)	207449
6	TS=((stool or f$eces or f$ecal) NEAR/3 (analysis or sample* or specimen*))	53111
7	#6 OR #5	207449
8	TS=diagnos*	3279053
9	TS=(diagnos* NEAR/3 (accuracy or performance))	

		121227
10	#9 OR #8	3279053
11	#10 AND #7 AND #4 AND #3	144

Cochrane database (Search date: March 10, 2023)

#	Searches	Results
1	(Mycobacterium tuberculosis OR MTB OR mycobacterium tuberculosis complex):ti,ab,kw	1525
2	(TB OR PTB OR pulmonary tuberculosis OR lung tuberculosis):ti,ab	6546
3	(aged OR elder* OR adult*):ti,ab,kw	1151590
4	(stool OR f*eces OR f*ecal):ti,ab,kw	23025
5	(stool OR f*eces OR f*ecal NEXT analysis OR sample* OR specimen*):ti,ab,kw	194603
6	(diagnos*):ti,ab,kw	287053
7	(diagnostic NEXT accuracy OR performance):ti,ab,kw	129788
8	#6 OR #7	390185
9	#1 OR #2	7020
10	#4 OR #5	198512
11	#3 AND #8 AND #9 AND #10	389

Supplementary file 2: Stool sample collection, storage and processing in the included studies

Study	Timing of stool collection relative to start anti-TB treatment	Stool sample collected per participant	Stool volume	Stool storage prior to processing	Stool processing method			
					Pretreatment, decontamination and neutralization	Homogenization process	Centrifugation	Filtration
Kokuto 2015 (30)	< 7 days	1	2 cm^3	Not reported	distilled water, 3% NACL-NaOH, PBS	vortex mixing	Yes	Not reported
Konno 2019 (31)	Not reported	1	100 – 200 µl	Not reported	semi alkaline proteinase, NALC-NaOH, PBS	Not reported	Yes	Not reported
Liu 2019 (32)	Prior to treatment		1gm	Not reported	PBS, sample reagent	vortex mixing	Not reported	Not reported
Oramasionwu 2013 (33)	Prior to treatment	1	1 gm	Not reported	sterile water, 1% NACL-NaOH, PBS	emulsifying with glass beads	Yes	Yes, sterile gauze
Rahman 2018 (34)	Not reported	1	2 gm	-20° C	sterile normal saline, NALC-NaOH-Na-citrate, PBS	vortex mixing	Yes, twice	Not reported

Study	Timing of stool collection relative to start anti-TB treatment	Stool sample collected per participant	Stool volume	Stool storage prior to processing	Stool processing method			
					Pretreatment, decontamination and neutralization	Homogenization process	Centrifugation	Filtration
Abaye 2017 (35)	Prior to treatment	1	1 gm	4° C	NALC-NaOH, Tris buffer, 1% chlorhexidine digluconate, PBS	Emulsifying with glass beads and vortex mixing	Yes	Yes
DiNardo 2018 (36)	Median of 5 days (0-14)	1	50 mg	-80° C	MP Fast DNA soil kit	Bead-beating	Not reported	Not reported
Musisi 2022 (37)	Not reported	1	12 gm	-20°C	OM-S, PBS	Not reported	Yes	Not reported
ElKhechine 2009 (38)	Prior to treatment	Not reported	Not reported	Not reported	Tris buffer, PBS, 1% chlorhexidine digluconate	Vortex mixing	Yes	Yes, filtration vial kit

NALC-NaOH: N-acetyl-L-cysteine-sodium hydroxide; PBS: Phosphate buffer solution; NALC-NaOH-Na-citrate: N-acetyl-L-cysteine (NALC)-NaOH-Na-citrate solution (0.5% NALC, 4% NaOH, and 2.94% Na-citrate); OM-S: OMNIgene-sputum reagent