# Alternative sputum collection methods for diagnosis of childhood intrathoracic tuberculosis: a systematic literature review

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## ABSTRACT

**Background** Diagnosis of intrathoracic tuberculosis (ITB) is limited in children partly by their difficulty to produce sputum specimen.

**Objective** To systematically review the detection yields of mycobacterial culture and Xpert MTB/RIF from induced sputum (IS), nasopharyngeal aspirate (NPA) and gastric aspirate (GA) in children with presumptive ITB.

**Design** Pubmed, Embase and Biosis databases and grey literature were searched. Randomised controlled trials, cohort, cross-sectional or case control studies using IS, GA and NPA for diagnosis of ITB published between January 1990 and January 2018 were included. Data were extracted on study design, case definition of presumptive ITB, sample collection methods, outcome measures and results.

**Results** 30 studies were selected, including 11 554 children. Detection yields for culture ranged between 1% and 30% for IS, 1% and 45% for GA and 4% and 24% for NPA. For Xpert MTB/RIF, it was between 2% and 17% for IS, 5% and 51% for GA and 3% and 8% for NPA. There was a tendency of better yields with IS when the pretest probability of ITB was low to moderate and with GA when it was high. Sampling a second specimen contributed for 6%–33% of the cumulative yield and combination of different methods significantly increase the detection yields.

**Conclusions** Despite the important study heterogeneity, any of the specimen collection methods offers good potential to confirm childhood ITB. However, their operational challenges were poorly evaluated. In the absence of a sensitive non-sputum based test, only a minority of children with ITB can be confirmed.

### What is already known on this topic?

► Diagnosis of intrathoracic tuberculosis uses sputum specimens that are difficult to collect in children, who are also more likely to present paucibacillary disease.
► Only a small proportion of childhood intrathoracic tuberculosis cases are microbiologically confirmed.
► Xpert can detect two-thirds of children with culture positive samples but is limited by the difficulty to collect specimen, mainly in low-income and middle-income countries.

### What this study adds?

► There is high variability of tuberculosis detection yields from alternative sputum specimen collection methods mainly because studies use different definition of presumptive intrathoracic tuberculosis.
► Combination of different specimen collection methods on the same day is probably as effective as repeated sampling on consecutive days with the same method.
► Operational considerations and training strategy are essential in choosing the appropriate collection method for implementation at low health facility level.

## INTRODUCTION

Childhood intrathoracic tuberculosis (ITB) is challenging to diagnose, because the disease is often paucibacillary and respiratory secretions are difficult to collect.[1] There have been a lot of efforts to improve detection of *Mycobacterium tuberculosis* in biological samples. In children, the Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, USA) allows rapid diagnostic of two-thirds of children with culture positive samples.[2] Unfortunately, due to the absence of diagnostic reference standard of childhood ITB, majority of patients are treated empirically on the basis of clinical or radiological suspicion.[3] Consequently, many diagnosis accuracy studies of childhood ITB only look at the small part of microbiologically confirmed cases.

In high burden low-income and middle-income countries (LMIC), TB in children remains underdiagnosed with only 45% of the estimated number of cases notified to the WHO in 2016[4]. In many of these countries, rising levels of drug resistance makes it essential to achieve the highest possible rates of microbiological confirmation in order to tailor the treatment.

To optimise the performance of microbiological tests on respiratory samples, it is critical to select the best specimen collection method, especially in children who have difficulties in producing sputum spontaneously.[1] The most advanced method, the bronchoalveolar lavage, is not available in many LMIC. Other methods, such as induced sputum (IS), gastric aspirate (GA) or nasopharyngeal aspirate (NPA) are potential good alternatives.

In order to support clinicians and TB programmes in choosing the best method, our study systematically reviewed the value of alternative sputum

collection methods to achieve microbiological confirmation of ITB in children from LMIC. Our main objective was to determine and compare the diagnostic yield of IS, NPA and GA using culture and Xpert in children with presumptive ITB. Our secondary objective was to determine the proportion of additional cases detected when collecting several samples and the value of combining different methods.

## METHODS
### Search strategy and selection criteria
Randomised controlled trials, cohort, cross-sectional or case–control studies published between January 1990 and f 2018 were included. Reviews, opinion papers and case reports were excluded. Studies from LMIC (World Bank definition) investigating the yield of GA, NPA or IS sampled for culture or Xpert were considered for inclusion. Expectorated samples (ES) were excluded from this review but studies investigating ES could be included if GA, NPA or IS were simultaneously evaluated and separate results were presented. Study participants aged less than 15 years tested for presumptive ITB were included. Presumptive ITB was defined by the presence of at least one symptom suggestive of tuberculosis. Studies that included asymptomatic patients (child contacts or children participating in a vaccination trial or prevalence survey), children investigated for acute pneumonia and children on antituberculosis treatment were excluded.

We conducted systematic search of scientific publications and grey literature in the scientific databases Embase, Medline/Pubmed, BIOSIS and New York Academy of Medicine Grey Literature Report. The key words used were gastric aspirate, induced sputum, nasopharyngeal aspirate, tuberculosis, child and their synonyms. Identification and screening of the records on the basis of their title/abstract was done by a single author (VI). In case no abstract was available, the study was considered as potentially eligible based on the title only. Full-text articles were reviewed independently by two authors (HC and VI) for study eligibility and by a third reviewer (MB) in case of discordance. Reasons for excluding studies were documented.

### Data extraction and analysis
Data on main/secondary outcomes, study, patients and index tests characteristics (age, HIV status, inpatients/outpatients, chest X-ray (CXR) findings) were collected through a structured data collection form separately by HC and VI and discrepancies were discussed with MB. Methodological quality of eligible studies was assessed with the Quadas-2 assessment tool that includes four domains: patient selection, index test, reference standard, flow and timing. Because of the absence of a reference standard of childhood ITB, the items related to reference standard and 2×2 tables were omitted. Signalling questions were added to address the methodological issues of studies comparing index tests between them. It resulted in an 'adapted Quadas-2' (online Supplementary table 1).

After a preliminary review of included studies and before systematic data extraction, we identified nine criteria commonly used to define 'presumptive ITB': symptom complex suggestive of ITB (>1 symptom without specification), cough more than 2 weeks, weight loss or failure to gain weight, fever more than 1 or 2 weeks, painless swelling of superficial nodes, TB contact history, CXR suggestive of ITB or abnormal CXR, positive tuberculin skin test, persistence of symptoms or CXR abnormalities after empirical antibiotic treatment. We defined low pretest probability of ITB by the presence of at least one clinical sign or symptom; moderate probability by the presence of at least

two criteria (including at least one clinical sign or symptom OR presence of the criteria 'symptom complex suggestive of tuberculosis') and high probability by the presence of at least three criteria including at least one clinical sign or symptom OR two criteria including one clinical sign or symptom and persistence of symptoms or CXR abnormalities after empirical antibiotic treatment. If there was no information on the definition of 'presumptive ITB' or if the definition included less than three criteria out of eight criteria (excluding 'symptom complex suggestive of tuberculosis') preventing the classification of high probability, the pretest probability was qualified as 'indeterminate'. We also collected, whenever reported, the number of adverse events per procedure and per patient.

Quantitative data and quality assessments ('adapted Quadas-2') were entered in RevMan V.5.3 to generate descriptive statistics, figures and tables. The yield of a sample collection method for a diagnostic test (Xpert and culture) was defined as the number of patients with a positive test out of the total number of patients tested. Because of the absence of reference standard, sensitivity and specificity could not be calculated. Detection yields with exact binomial 95% CI are presented according to the pretest probability of ITB. Additional yields of second specimen (ie, number of positive patients in the second specimen while being negative on the first) are presented in absolute percentage of study participants.

The study protocol was registered in PROSPERO (International Prospective Register of Systematic Reviews) under the number CRD42014012924.

## RESULTS
### Studies characteristics
Out of 440 records screened, 30 studies (31 articles)[5–35] were included in the review (online Supplementary figure 1).

The total number of study participants was 11 554. The greater number of patients were from India (n=3098, 27%)[11 12 14–16 33] and South Africa (n=3312, 29%).[10 18–20 22 27 29 32 35] There was great heterogeneity of definitions of presumptive ITB and patient characteristics across studies (table 1). Most studies were done in tertiary care centres and only two[20 32] in primary care centres. Half of the studies included inpatients and outpatients. Pretest probability of ITB was estimated as 'moderate' in half of the studies and could not be assessed in four studies[15 23 25 28] (online Supplementary table 2). In 23 (77%) studies, majority of children were aged <5 years. When reported, proportion of HIV patients across studies varied from 8%[20] to 70%.[8] Culture, Xpert and both were used in 29, 13 and 12 studies, respectively. Solid culture media was used in nine studies, liquid media in 11 and both media in seven studies.

### Specimen collection procedures
GA was performed early morning, after an overnight or 4–8 hours fast. Only two studies[29 30] reported the minimum volume of gastric liquid aspirated: 10 mL for the first and 20 mL for the second. Four studies[16 25 29 33] reported the use of a normal saline wash to collect gastric liquid and in two studies, the use of sterile water in case of aspiration of less than 3 mL of gastric fluid.[7 27] NPA was preceded by instillation of normal saline in three studies[7 19 20] but not in two studies.[5 8] IS procedure was well described and standardised in 19/20 studies (online Supplementary table 3). Main differences were the type of nebulizer used (jet vs ultrasonic), the concentration of the hypertonic saline (3 vs 5%) and use of physiotherapy to help induction. Physiotherapy was performed in nine studies. In 16 studies,

**Table 1** Characteristics of included studies

| | N patient | Sample collection method | Facility | In/outpatient | TB incidence/100 000pop | Pretest probability | Age (months), mean (SD) or median (IQR) | % CXR findings suggestive | TST pos, % | Contact history, % | HIV pos, % | Malnourished % | Culture media | Xpert MTB-RIF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Al-Aghbari et al[5] (Yemen) | 203 | 1NPA+3GA+3IS/ES | Tertiary | IO | 49 | Low | 60 (?-?) | U | U | 51 | U | U | S | N |
| Bates et al[6] (Zambia) | 865 | 1GA+1ES | Tertiary | IO | 427 | Low | 24 (12–74) | U | U | U | 28 | 23 | L | Y |
| Iriso et al[17] (Uganda) | 101 | 1IS | Tertiary | IO | 179 | High | 26 (17) | 100 | 47 | 44 | 49 | 38 | S | N |
| Joel et al[28] (Botswana) | 1394 | 1IS | Tertiary | IO | 408 | Indeterminate | 46 (16–101) | U | U | 30 | 47 | U | S | N |
| Kabir et al[24] (Bangladesh) | 91 | 1GA | Tertiary | I | 221 | High | 18 (9–36) | U | U | U | U | 85.2 | SL | N |
| Lopez et al[25] (Mozambique) | 775 | 1GA+1IS | Tertiary | O | 551 | Indeterminate | U | U | U | U | U | U | SL | N |
| Lu et al[26] (China) | 127 | 1GA | Tertiary | I | 64 | High | U | U | U | U | 0 | U | S | Y |
| Maciel et al[30] (Brazil) | 104 | 3GA | Tertiary | U | 46 | Moderate | U | U | U | U | 0 | U | U | N |
| Marcy et al[31]c | 281 | 3ES/2–3GA+1NPA | Tertiary | U | U | Low | 7.2 (4,1–7,2)b | U | U | U | 100 | 68.4 | SL | Y |
| Moore et al[32] (South Africa) | 269 | 2IS | Primary | O | 1003 | Moderate | 38 (19–61) | 56 | 46 | 68 | 0? | U | L | N |
| Mukherjee et al[33] and Singh et al[14] (India)a | 403 | 2IS+2GA | Tertiary | O | 176 | High | 97 (44–168) | 100 | 92 | 37 | U | U | L | Y |
| Nansumba et al[34] (Uganda) | 126 | 2IS | Tertiary | IO | 179 | Moderate | 97 (44–168) | U | U | U | 33 | U | SL | N |
| Nicol et al[35] (South Africa) | 452 | 2IS | Tertiary | I | 1003 | Moderate | 19 (11–46) | 51 | 66 | U | 24 | 34 | L | Y |
| Oberhelman et al[7] (Peru) | 216 | 2GA+2NPA | Tertiary | IO | 95 | Moderate | 40 (?) | U | 91 | 84 | U | U | SL | N |
| Owens et al[8] (Uganda) | 94 | 1IS+1NPA | Tertiary | IO | 179 | High | 48 (4–44) | 72 | 54 | 34 | 70 | 23 | S | N |
| Palme et al[9] (Ethiopia) | 296 | 2GA+3ES | Tertiary | IO | 247 | High | U | 98 | U | U | 18 | 84 | S | N |
| Planting et al[10] (South Africa) | 690 | 2IS | Tertiary | I | 1003 | Moderate | 27 (13–64) | U | U | U | 24 | 25 | L | Y |
| Qureshi et al[11] (India) | 60 | 3GA+3IS | Tertiary | I | 176 | Moderate | 48 (?) | U | U | 84 | U | U | SL | N |
| Raizada et al[12] (India) | 2541 | 1GA | Mixed | OI | 176 | Low | U | U | U | U | U | U | None | Y |
| Sabi et al[21] (Tanzania) | 192 | 1IS | Tertiary | I | 287 | Low | 23 (14–53) | U | 2.1 | | 29 | 49.5 | S | Y |
| Schaaf et al[16] (South Africa) | 235 | 2GA | Tertiary | IO | 1003 | High | 30 (?) | U | 35 | 42 | 0 | 36 | L | N |
| Sekadde et al[13] (Uganda) | 250 | 1IS | Tertiary | IO | 179 | Moderate | 36 (16–75) | 79 | 34 | 34 | 42 | 27 | SL | Y |
| Shata et al[23] (Malawi) | 29 | 1IS | Tertiary | IO | 163 | Indeterminate | U | 93 | 44 | U | U | U | S | N |
| Singhal[15] (India) | 44 | 2IS+2GA | Tertiary | U | 176 | Indeterminate | U | U | U | U | U | U | U | N |
| Somu et al[16] (India) | 50 | 2GA | Tertiary | O | 176 | High | 61 (36) | 100 | 74 | U | U | 66 | S | N |
| Thomas et al[18] (South Africa) | 118 | 3GA+1IS/ES | Secondary | IO | 1003 | Low | 56 (37) | 71 | U | 53 | 65 | 44 | L | Y |
| Walters et al[27] | 379 | 2GA/ES+2IS/ES | Tertiary | IO | 1003 | Low | 15.9 (9,2–29.3) | 28.3 | 28 | 56.5 | 13.7 | 44.3 | L | Y |
| Zar et al[29] (South Africa) | 250 | 3GA+3IS | Tertiary | I | 1003 | Moderate | 13 (6–24) | U | U | U | 56 | U | L | N |
| Zar et al[19] (South Africa) | 535 | 2IS+2NPA | Tertiary | I | 1003 | Moderate | 19 (11–38) | 62 | 36 | U | 22 | 13 | L | Y |
| Zar et al[20] 2 (South Africa) | 384 | 2IS+2NPA | Primary | O | 1003 | Moderate | 38 (21–57) | 67 | 67 | U | 8 | 13 | L | Y |

aMukherjee et al[33] and Singh et al[14] (two publications with complementary results from the same cohort).
bIn years.
cBurkina Faso, Cambodia, Cameroon, Vietnam.
CXR, chest X-ray; ES, expectorated sputum; GA, gastric aspirate; I, inpatient; IS, induced sputum; L, liquid; NPA, nasopharyngeal aspirate; O, outpatient; pop, population; pos, positive; S, solid; TST, tuberculin skin test; U, unknown

**Table 2** Comparison of culture or Xpert MTB/RIF detection yield or sensitivity between different sputum collection methods in same patients

| Studies | N | Pretest probability | Methods | Test | Difference in yields (%) (95% CI) | Difference in sensitivity (%) (95% CI) | P values |
|---|---|---|---|---|---|---|---|
| Zar et al[29]* | 250 | Moderate | 2IS vs 2GA | Culture | +5.3%, 15% (11 to 20) vs 8% (5 to 12) | | 0.018† |
| Qureshi et al[11] | 60 | Moderate | 3IS vs 3GA | Culture | +5%, 13% (3 to 18) vs 8% (6 to 25) | | |
| Mukherjee et al[33]* | 403 | High | 2IS vs 2GA | Culture | −15%, 18% (14 to 22) vs 33% (28 to 37) | | <0.001† |
| Singh et al[14]* | 130 | High | 2IS vs 2GA | Xpert | −10%, 22% (15 to 30) vs 32% (24 to 40) | +2.5%, 70% (56.2 to 80.9) vs 67.5% (56.5 to 76.9) | 0.770 |
| Owens[8]* | 94 | High | 1IS vs 1NPA | Culture | −2%, 22% (14 to 32) vs 24% (15 to 34) | | >0.5† |
| Zar et al[19]* | 396 | Moderate | 2IS vs 2NPA | Culture | +3%, 15% (12 to 19) vs 12% (9 to 16) | | <0.01 |
| Zar et al* | 396 | Moderate | 2IS vs 2NPA | Xpert | +1%, 9% (6 to 12) vs 8% (5 to 12) | +6.3%, 71.4% (60.0 to 82.9) vs 65.1% (53.0 to 77.2) | 0.440 |
| Zar et al[20]* | 389 | Moderate | 2IS vs 2NPA | Xpert | +1%, 4% (2 to 7) vs 3% (1 to 5) | +18%, 57% (39 to 75) vs 39% (24 to 58) | 0.180 |
| Oberhelman et al[7]* | 216 | Moderate | 2GA vs 2NPA | Culture | +5%, 10% (6 to 15) vs 6% (3 to 10) | | <0.05† |
| Al-Aghbari[5] | 203 | Low | 1GA vs 1NPA | Culture | +2%, 9% (5 to 14) vs 7% (4 to 11) | | |

*Paired samples.
†Exact McNemar's test.
GA, gastric aspirate; IS, induced sputum; NPA, nasopharyngeal aspirate.

bronchodilators were administered before induction and in eight studies samples were collected after nasopharyngeal suction. In Moore et al,[32] Planting et al[10] and Sabi et al,[21] 60%, 21% and 1.6% of samples, respectively, were expectorated spontaneously after IS.

### Detection yields

Yield of Xpert and culture according to specimen collection method and pretest probability of ITB are reported in online Supplementary figures 1 and 2 and online Supplementary tables 4 and 5. The detection yield (first sample) for culture ranged between 1% and 30% for IS, 1% and 45% for GA and 4% and 24% for NPA across studies. For Xpert, it was between 2% and 17% for IS, 5% and 51% for GA and 3% and 8% for NPA. In Lu et al,[26] Xpert detected much more cases than culture (51.2 vs 19.7%) from the same GA sample.

Using 10% threshold of detection yield in patients with low to moderate pretest probability, the proportion of studies reporting yield of culture and Xpert >10% tended to be higher for IS (7/12, 58.3% and 2/8, 25.0%) than for GA (1/7, 14.3% and 0/3) or NPA (1/4, 25.0% and 0/3). When the pretest probability was high, using a threshold of detection yield of 20%, the proportion of studies with culture detection yield >20% was similar betwenn GA and IS (4/6, 66.7% vs 2/3, 66.7%) and tended to be higher for GA with Xpert (2/2 vs 0/1). Yield of culture performed on NPA was available from one study[8] with high probability of ITB (24%). In primary care setting, IS had a lower detection yield than in tertiary setting.[20 32]

Nine studies[5 7 8 11 14 19 20 29 33] presented the detection yields of different specimen collection methods in same patients (table 2). Four studies[11 14 29 33] compared IS and GA, three using culture[11 29 33] and one using Xpert.[14] Detection yield was higher for GA in two studies with high pretest probability and for IS in two studies with moderate pretest probability of ITB. Of three studies comparing detections yields between IS and NPA, two favoured IS with a very low difference in yields ranging between 1% and 3%, all in patients with moderate probability of ITB.

However, the difference was significant in the Zar et al's[19] study with 396 patients. In the study[8] among children with high pretest probability, NPA had a slight better culture (+2%) detection yield but the difference was not significant. Only two studies[5 7] compared GA with NPA, with a trend for better culture detection yield for GA.

Sampling a second specimen contributed for 6%–10% of the cumulative yield in children with low pretest probability and for 6%–33% in children with moderate or high probability of ITB, regardless of the specimen collection method and microbiological test used (figures 1 and 2).

Seven studies assessed the detection yield on combining different sample collection methods (table 3). The contribution of one method alone ranged between 66% and 100% for GA, 49% and 95% for IS and 55% and 87.5% for NPA. In three studies, combining 2–3 methods on the first day detected between 74% and 100% of cases detected over 2–3 consecutive days.

### Tolerability

Only seven studies[10 18 21 28 29 32 34] reported tolerability data and only for the IS. The proportion of patients with adverse events ranged between 0.4% and 27.9% for nose bleeding, 0%–1.1% for wheezing, 0%–1.6% for transient hypoxia and 0.5%–4.0% for vomiting. In one study,[10] 14 (2%) children required bronchodilator after the procedure due to wheezing that was reversible in all children after bronchodilator. In this study, median oxygen saturation was 98% (IQR 97–98) before IS (online Supplementary table 6)

### DISCUSSION

Our systematic review confirms that culture and Xpert yields for detection of ITB are low in children with presumptive ITB and shows conflicting data on comparison of detection yields between GA, IS and NPA. As expected, the detection yields are correlated to the pretest probability of ITB that is related to the
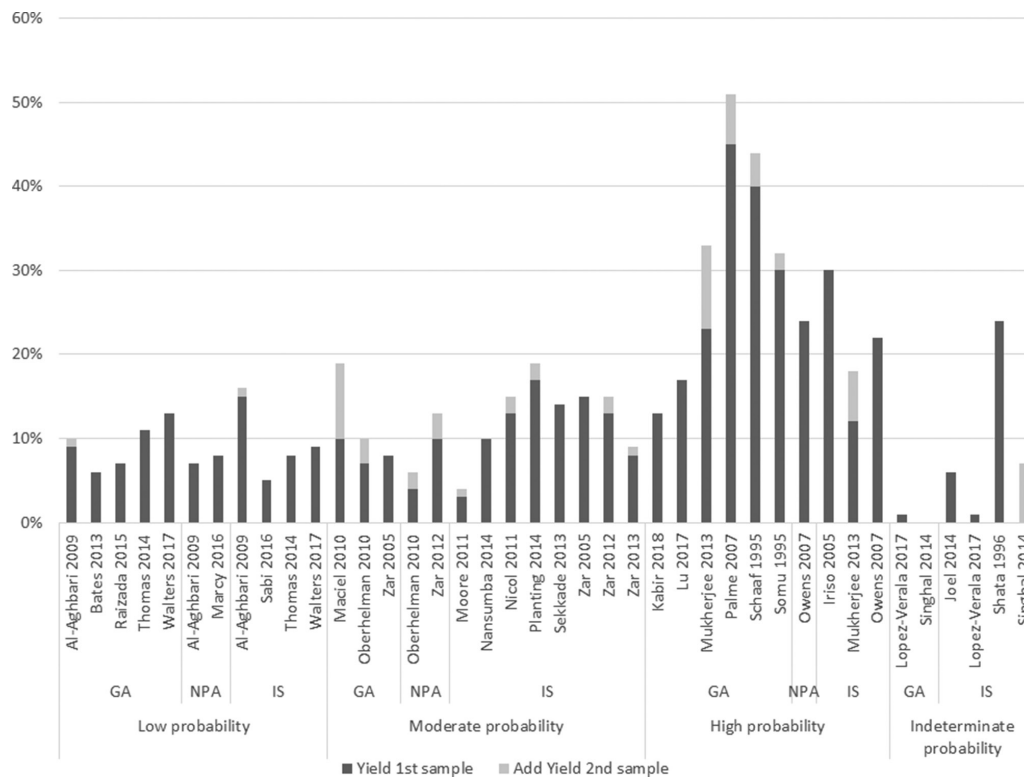
**Figure 1** Yield of culture according to specimen collection method and pretest probability of intrathoracic tuberculosis. GA, gastric aspirate; IS, induced sputum; NPA, nasopharyngeal aspirate.

definition of presumptive TB. In children with low pretest probability, yields are low and IS tends to detect more cases than GA and NPA. In children with high pretest probability, detection yields tend to be better with GA. These trends are more obvious in sufficiently powered studies comparing head to head IS and GA, like in the Zar et al[29] and Mukherjee et al[33] studies. Although
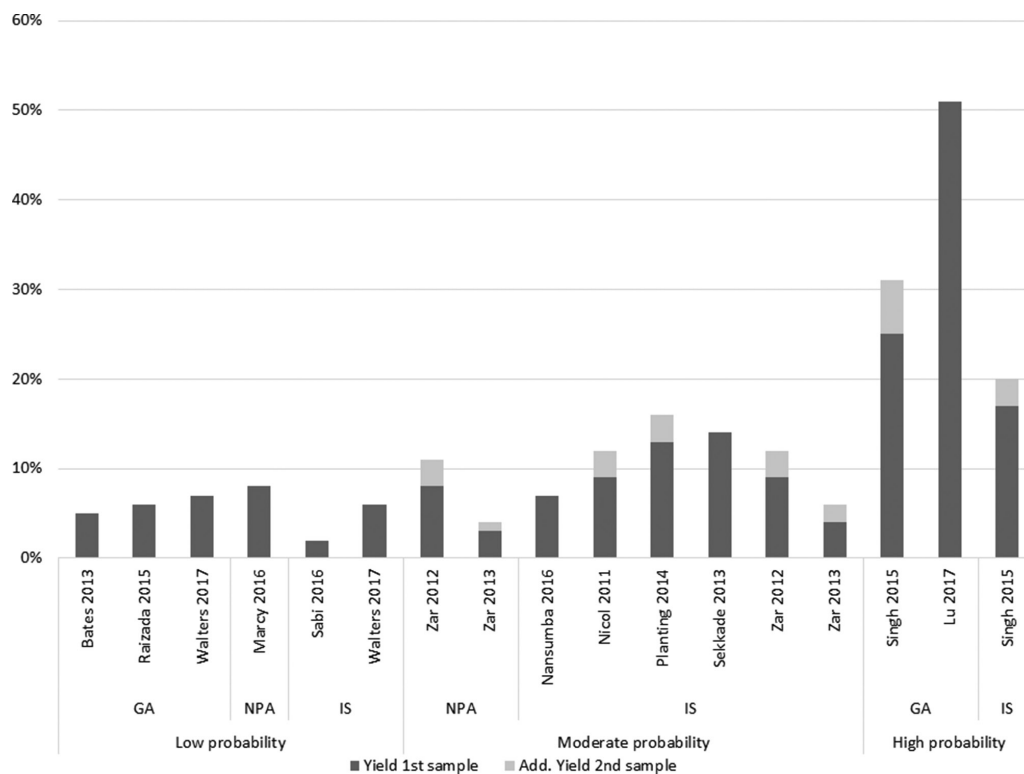


**Figure 2** Yield of Xpert MTB/RIF according to specimen collection method and pretest probability of intrathoracic tuberculosis. GA, gastric aspirate; IS, induced sputum; NPA, nasopharyngeal aspirate.

**Table 3** Yield of culture and Xpert MTB-RIF with combination of different specimen collection methods and contribution of each method

| Study | Pretest probability | Specimen collection strategy (test) | Yield of combinations, n/N (%) | Yield of combinations on first day, n (%) | Contribution per method* | | |
|---|---|---|---|---|---|---|---|
| | | | | | GA, n (%) | NPA, n (%) | IS, n (%) |
| Al-Aghbari et al[5] | Low | 1NPA+3GA+3IS/ES (culture) | 29/213 (13.6) | 29 (100) | 19 (66) | 14 (48) | 27 (93) |
| Mukherjee et al[33] | High | 2GA+2IS (culture) | 147/403 (36.5) | 109 (74) | 131 (89) | | 72 (49) |
| Singh et al[14] | High | 2GA+2IS (Xpert) | 49/130 (38) | 40 (81) | 41 (84) | | 29 (59) |
| Oberhelman et al[7] | Moderate | 2GA+2NPA (culture) | 22/210 (10.5) | NA | 22 (100) | 12 (55) | |
| Owens et al[8] | High | 1NPA+1IS (culture) | 24/88 (27.3) | 24 (100) | | 21 (87.5) | 19 (79) |
| Zar et al[29] | Moderate | 3GA+3IS (culture) | 58/250 (23.2) | NA | 38 (66) | | 51 (88) |
| Zar et al[37] | Moderate | 2NPA+2IS (culture) | 63/396 (15.9) | NA | | 48 (76) | 60 (95) |
| Zar et al[19] | Moderate | 2NPA+2IS (Xpert) | 51/396 (12.9) | NA | | 41 (80) | 45 (88) |

*Number of children with a positive test per specimen collection method divided by the total number of children with a positive test by combination of methods.
ES, expectorated sputum; GA, gastric aspirate; IS, induced sputum; NA, not applicable; NPA, nasopharyngeal aspirate.

the performance of the IS and GA might be affected differently by the pretest probability of ITB, other factors could explain the variation like the difference in age, HIV prevalence, level of health facility and experience of the study personnel with different methods. The review shows the benefit of collecting two specimens instead of one. This often requires repeating the collection over two consecutive days, which can be challenging for outpatients and for settings with limited bed capacity. However, we also report that collecting two different specimens on the first day could be a good alternative.

This review has several limitations. (1) The absence of reference standard to diagnose ITB in children precludes attempt to compare accuracy of the methods. (2) Only few studies had appropriate design to compare yields of sputum collection methods in paired samples.[14 19 20 29 33] (3) We did not include string test in our review due to the few number of studies and its poor applicability to young children who cannot swallow the device.[31 34] (4) Due to study heterogeneity, particularly in the definition of 'clinical suspicion of ITB', we could not perform a meta-analysis.(5) Most studies were conducted in tertiary care centres limiting the generalisability of the results to lower centres. The generalisability of the results is also limited by the fact that two-thirds of the study participants were from South-Africa or India. (6) Very few data was available on feasibility aspects of the methods, which is crucial for their use in primary care centres of LMIC.

Both IS and GA are recommended by WHO for diagnosis of ITB in children unable to produce sputum.[36] However, in most LMIC, these methods are poorly implemented, especially at secondary or primary healthcare settings due to operational challenges.[37] IS requires adequate training of health workers, equipment and hygiene precautions and GA requires admission of the child. Improving diagnostic capacity at secondary and primary healthcare centres is crucial to increase case detection. NPA might be an easier and better accepted method compared with IS or GA, but its implementation would require optimisation of equipment (aspirator) and material (mucus extractor). Recently, a great interest emerged from the use of stool to detect TB bacilli that are swallowed for the diagnosis of childhood TB. Despite variable sensitivity of Xpert on stools (32%–85%) between studies, and laboratory challenges to find the best sample processing method to remove the PCR inhibiting factors common in stool, this specimen could be a good alternative to sputum in children.[31 38–42] Authors have shown that Xpert on the combination of stool and NPA could achieve similar detection yields as 2SI or 2GA, offering a good opportunity for decentralisation of the microbiological diagnosis of childhood TB.[31 43] The

use of Xpert Ultra cartridge that has a level of detection close to the one of culture with the upcoming GeneXpert Omni Point of Care platform combined to more child friendly specimens collection methods like NPA and stools could significantly improve the microbiological diagnostic capacity of childhood ITB in LMIC but the logistic implications in programmatic conditions will need to be assessed.[44]

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