Clinical Infectious Diseases

MAJOR ARTICLE

Stool processing methods for Xpert Ultra testing in childhood tuberculosis: A prospective, multi-country accuracy study

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DOI: 10.1093/cid/ciaf289

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Background. Centrifuge-free processing methods support stool Xpert Ultra testing for childhood tuberculosis (TB), but there are limited data on their accuracy, acceptability and usability.

Methods. We conducted a prospective evaluation of stool Xpert Ultra in India, South Africa, and Uganda with three methods: Stool Processing Kit (SPK), Simple One-Step (SOS), and Optimized Sucrose Flotation (OSF). Children <15 years old with presumptive TB had sputum testing with Xpert Ultra and culture. We compared the accuracy of each method to a microbiological reference standard (MRS, TB if Xpert Ultra or culture positive) and a composite reference standard (CRS, TB if Confirmed or Unconfirmed TB). We surveyed laboratory staff to assess acceptability and usability of the methods.

Results. We included 607 children, with a median age of 3.5 years (IQR 1.3-7) and 15.5% HIV positive. Against the MRS, the sensitivities of SPK, SOS and OSF were 36.9% (95% CI 28.6-45.8), 38.6% (95% CI 17.2-51.0), and 31.3% (95% CI 20.2-44.1), respectively. The specificities of SPK, SOS and OSF were 98.2% (95% CI 96.4-99.3), 97.3% (95% CI 93.7-99.1), and 97.1% (95% CI 93.3-99), respectively. The methods were acceptable and usable, but SOS was reported as most feasible to implement in a peripheral facility. Across methods, sensitivity increased among children who were culture-positive (55.0-77.3%) and was low (13-16.7%) against the CRS. Adding stool Xpert Ultra increased sensitivity 0% (OSF) to 11.8% (SPK/SOS) over sputum alone.

Conclusions. Stool processing methods for Xpert Ultra were acceptable, usable, and performed similarly, with highest sensitivity among children with culture-positive TB.

Keywords: child; tuberculosis; diagnostics; stool; Xpert Ultra; centrifuge-free

KEY POINTS: In a multi-country diagnostic accuracy study for childhood pulmonary tuberculosis, three stool processing methods for Xpert Ultra were acceptable, usable and performed similarly. Sensitivity was lower than that of sputum Xpert Ultra, but improved in children with culture-positive disease.

INTRODUCTION

The implementation of low-complexity, automated molecular assays for *Mycobacterium tuberculosis (Mtb)* has improved access to tuberculosis (TB) testing worldwide [1]. However, children are often unable to expectorate sputum, and access to equipment and trained staff to collect induced sputum or gastric aspirates is usually limited to higher-level facilities [2].

Multiple studies have shown that *Mtb* can be detected in the stool of children with pulmonary TB [3-7], in particular with Xpert MTB/RIF and Xpert MTB/RIF Ultra (Xpert Ultra, Cepheid,

Sunnyvale). To support stool Xpert Ultra testing, centrifuge-free stool processing methods have been developed, including the Stool Processing Kit (SPK, FIND, Geneva) [8], Simple One-Step method (SOS, KNCV TB Foundation, The Hague, The Netherlands) [9], and Optimized Sucrose Flotation method (OSF, TB SPEED Consortium) [10]. However, limited comparative data exist on their accuracy, acceptability and usability [11].

Our overall objective was therefore to evaluate and compare the diagnostic accuracy of stool processing methods for Xpert Ultra testing through a prospective, multi-country study. We also assessed their acceptability and usability as reported by laboratory staff.

METHODS

Setting and Participants

We consecutively enrolled children <15 years old from June 2019 to March 2021 in India, South Africa, and Uganda. In India, children were recruited from the All India Institute of Medical Sciences (AIIMS), New Delhi, and KEM Hospital, Pune, with referrals from surrounding outpatient clinics. In South Africa, children were recruited from Red Cross Children's Hospital in Cape Town and Dora Nginza Hospital in Gqeberha. In Kampala, Uganda, children were referred from inpatient and outpatient facilities at Mulago National Referral Hospital and the surrounding area. Children were eligible if they had microbiologically confirmed TB, or at least one symptom of pulmonary TB: unexplained cough for ≥2 weeks, unexplained fever for ≥1 week, unexplained failure to thrive or weight loss, or chest X-ray suggestive of TB. Children on anti-TB treatment for >72 hours were excluded. Caregivers completed a written informed consent, and children aged ≥8 years in Uganda, and ≥7 years in South Africa and India gave assent. Laboratory staff aged ≥18 years who processed the stool samples at each site completed an anonymous survey to assess acceptability and usability.

The study received ethical approval from the Mulago Hospital Research Ethics Committee, the Human Research Ethics Committee of the University of Cape Town, the AIIMS Ethics Committee and the KEM Hospital Research Centre Ethics Committee, and the University of California San Francisco Institutional Review Board.

Reference procedures

Trained staff performed a standard TB evaluation on all children, including a clinical questionnaire (**Supplementary File 1**), physical exam, and chest radiography. Two sputum specimens were collected, including any combination of expectorated or induced sputum, gastric aspirate or nasopharyngeal aspirate. Sputum samples were tested according to standard operating procedures using Xpert Ultra and mycobacterial culture, with Mycobacterial Growth Indicator Tube (MGIT, 37°C for 6 weeks) or Löwenstein-Jensen media (37°C for 8 weeks). Positive Xpert Ultra results include a semi-quantitative level, and trace results were defined as positive per WHO

recommendations [12]. All children had follow-up at 2-3 months to assess any response to TB treatment or resolution of symptoms without treatment.

Stool collection

Study staff asked participants to collect one stool sample in a sterile cup, either directly or transferred from a diaper using a spoon. If the child was unable to produce a sample, a sample could be obtained at home and returned within 3 days.

Stool samples were homogenized when possible and processed fresh, or stored at 2-8°C and tested within 72 hours. Based on available supplies, protocols and training, SPK was introduced first in June 2019, followed by SOS and then OSF in December 2019. When all three methods were performed, we randomized weekly the order of testing and where in the cup the sample was collected.

Stool processing and index testing

Stool samples were processed according to the SPK, SOS, and OSF method before Xpert Ultra testing (See **Supplemental File 2** for a description of the stool processing methods). Of note, they were at different stages of development; SOS was design-locked, while SPK was a prototype design, and OSF had not yet been developed into a kit.

Laboratory staff were blinded to the sputum Xpert Ultra result and clinical treatment decision. The staff were asked to complete a voluntary, anonymous acceptability and usability questionnaire on each method, using a Likert scale ranging from Totally or Partially Disagree to Partially or Totally Agree (**Supplementary File 3**).

TB Classification and Reference Standards

Participants were classified as Confirmed, Unconfirmed or Unlikely TB based on NIH consensus definitions [13]. Confirmed TB was defined as having sputum positive for *Mtb* by Xpert Ultra or culture. Children with Unconfirmed TB did not have microbiological confirmation, but had signs, symptoms and/or radiographic findings consistent with TB, and were started on anti-TB treatment with clinical response at the follow-up visit. Children with Unlikely TB were symptomatic at enrollment, but had negative microbiological testing and symptom resolution without anti-TB treatment at the follow-up visit. A case was unclassifiable if there was insufficient information or follow up to determine TB status. Stool Xpert Ultra results were not used in the TB classification.

We utilized two reference standards. The microbiological reference standard (MRS) classified TB in children with Confirmed TB, and otherwise as not having TB (Unconfirmed or Unlikely TB). The composite reference standard (CRS) includes Unconfirmed TB with Confirmed TB in the definition of TB, and Unlikely TB as negative for TB.

Statistical analysis

We assessed the sensitivity and specificity of stool Xpert Ultra using each method against the MRS and CRS. Secondarily, we calculated the accuracy against sputum Xpert Ultra and culture alone. Children who could not provide a sputum or stool sample were excluded. Participants with non-determinate stool Xpert Ultra results (invalid, error or no result) were excluded; however, we conducted a secondary sensitivity analysis and determined the accuracy if these results were considered negative or positive. For the head-to-head comparison, we included children who had valid results for all three methods, and used McNemar's test with 95% CIs to compare differences in sensitivity and specificity. We also determined the incremental accuracy of performing both stool and sputum Xpert Ultra compared to each test alone against sputum culture. Statistical significance was defined if 95% CIs of the difference did not cross zero.

For the acceptability and usability assessment, we summarized the frequency and proportion of the responses by processing method. Analyses were completed using Stata version 16.1 (StataCorp LLC, College Station, Texas). Findings have been presented in accordance with the Standards for Reporting Diagnostic Accuracy (STARD) guidelines [14].

RESULTS

Participant characteristics

We enrolled 655 children from 1,182 screened across the three countries (**Figure 1**). After excluding 29 children who did not provide a stool sample and 19 children whose TB status was unclassifiable, the final sample size was 607. All children provided a respiratory sample. Most children were from Uganda (n=371, 61.1%) and under 5 years old (n=367, 60.5%, **Table 1**). Half of children (n=308, 50.7%) were underweight, and 89/574 (15.5%) were living with HIV. A total of 147 children (24.2%) had Confirmed TB, with the majority (136/147, 92.5%) being sputum Xpert Ultra positive.

Xpert ultra results

The three methods had a similar proportion of valid results, ranging from 87.4% for OSF to 90.3% for SPK (**Table 1**). As shown in **Table 2**, the proportion of *Mtb* positive results was similar across methods, ranging from 9.3% for OSF to 11.2% for SOS. The cycle threshold (CT) values among those with TB were lower in OSF (median 17.1, IQR 16.6-22.1) compared to SPK and SOS (median 20.6 and 19.8). Stool Xpert Ultra detected rifampin resistance in one child in South Africa using SPK, though this child was not tested with the other two methods.

Table 2 also summarizes the sputum Xpert Ultra results. Sputum was positive in 21.6% of children (131/607), with 64% (48/75) showing trace or very low semi-quantitative results. Only 2 children had non-determinate results.

Accuracy of stool Xpert Ultra with centrifuge-free stool processing methods

Against the MRS, the specificity of all three methods was high (97.1-98.2%), while sensitivity of the SPK, SOS and OSF methods was 36.9%, 38.6% and 31.3%, respectively (**Table 3**). Relative to the MRS, sensitivity was lower with the CRS (range 13.0-16.7% across methods), similar with sputum Xpert Ultra (range 38.3-45%), and higher against sputum culture (range 55-77.3%). Across the methods, sensitivity was significantly higher when the sputum Xpert Ultra semi-quantitative level was Low or higher (95.8-100%), compared to Trace or Very Low (16.7-22.6%, $p < 0.001$ for all methods, **Supplemental Table 1**). If non-determinate results were defined as positive, sensitivity would increase to 38-43.1% and specificity would decrease to 83.9-88.7% (**Supplemental Table 2**). If defined as negative, sensitivity would reduce to 28.1-37.0% and specificity would be overall similar at 97.5-98.4%.

Subgroup analysis is shown in **Supplemental Table 1.** Against the MRS, specificity was higher in Uganda for the SOS and OSF method. By age group, there was a trend towards lower sensitivity in children under 5 years old, and this was statistically significant for SOS (25.9% for children <5 years versus 66.7% for 10-14 years, $p = 0.01$). Sensitivity also tended to be higher in females, and was significantly higher for SPK (45.1% in females vs. 27.1% in males, $p = 0.04$). Of note, the proportion with Confirmed TB was higher among children who were five years and older and who were female (**Supplemental Table 1**).

Head-to-head comparison of the stool processing methods

179 children had valid results from all three approaches (**Figure 1**). Comparing methods, sensitivity and specificity were similar regardless of the reference standard with overlapping 95% CIs (**Table 3**).

Against sputum culture, Xpert Ultra on sputum had higher sensitivity (82.4%, 95% CI 56.6-96.2) than stool Xpert Ultra in all three methods, but it was not statistically significant (**Table 4**). Specificity was significantly lower than stool Xpert Ultra across methods. When sputum and stool Xpert Ultra testing were done concurrently, there was an absolute increase in sensitivity of 17.6-23.5% compared to stool Xpert Ultra alone **(Table 4**), with a reduction in specificity of 11.4-12.1%. The addition of stool Xpert Ultra to sputum Xpert Ultra increased sensitivity 0-11.8%, at a loss of specificity of 2.1-2.9%. The overlap in positive results between sputum culture, sputum Xpert Ultra, and stool Xpert Ultra among those with all three methods are summarized in **Supplemental Figure 1**. Among the 86 Unconfirmed TB cases, SPK and OSF Xpert Ultra detected 3 more cases (3.5%), and OSF Xpert Ultra identified 2 more cases (2.3%).

Acceptability and usability of testing by laboratory staff

The survey was completed by 17 laboratory staff (5 from India, 7 from South Africa, and 5 from Uganda). The majority (12/17, 70.6%) had over three years of TB lab experience, but nearly half (8/17, 47.1%) had no prior experience with stool samples. All participants reported being

comfortable handling stool samples and all agreed that stool testing with Xpert Ultra would be beneficial.

Usability is shown in **Figure 2**. For all three methods, the instructions were clear and posed minimal biosafety concern (>75% agreement). However, SOS was the least time-consuming, and most participants (75%) agreed it could be performed by non-laboratory staff at peripheral facilities with Xpert Ultra but without other laboratory infrastructure (94% agreement). Most respondents believed that all three methods could be performed at a peripheral health center if a microscopy laboratory was present.

DISCUSSION

Stool Xpert Ultra testing has the potential to increase access to molecular TB testing for children, especially in peripheral settings. In this multi-country, prospective evaluation of three stool processing methods for Xpert Ultra testing, we found that stool Xpert Ultra had high specificity, but detected only about a third of microbiologically confirmed cases with similar results across methods. Sensitivity doubled among children with culture-positive TB. Laboratory staff reported that all three methods were acceptable and usable, with SOS being the most practical for use by non-laboratory staff in peripheral facilities. However, ongoing gaps remain in improving TB diagnosis among children who are culture-negative and have paucibacillary disease.

Stool Xpert Ultra testing showed moderate sensitivity to detect culture-positive TB in children. This is consistent with studies that have shown similar stool and sputum Xpert Ultra accuracy in culture-positive TB [11, 15-17]. However, only 38-45% of children with positive sputum Xpert Ultra results were also positive by stool Xpert Ultra, which was lower than previous studies [11, 18-22]. Stool Xpert performance has been shown to be heterogenous across studies [15, 16], depending on collection and processing methods, age, setting, and co-morbidities. Furthermore, both our study and prior studies found that bacterial burden was associated with stool *Mtb* detection [11, 18]. Our study had a higher proportion of trace or very low positive results on sputum Xpert Ultra compared to previous studies [5, 18], which could increase the likelihood of negative results on stool Xpert Ultra. The majority of our cohort was also under 5 years old, which on subgroup analysis tended to have lower sensitivity and had a lower proportion with Confirmed TB compared to older age groups. In contrast, a companion study in Uganda and Zambia found higher sensitivity of stool Xpert Ultra, but again may have reflected later and more severe disease as these children were mostly hospitalized and less likely to be TB contacts [23].

These findings suggest that while the accuracy of stool Xpert Ultra testing may be higher for culture-positive and more severe TB disease, its sensitivity is limited for children with paucibacillary disease. We found that performing sputum Xpert Ultra in parallel with stool Xpert Ultra, or when stool Xpert Ultra is negative, increased absolute sensitivity by 17.6 to 29.4%. This is supported by other studies that have shown the combination of sputum and stool testing

improved accuracy [18, 24]. The lower specificity of Xpert Ultra on sputum specimens likely reflects detection of culture-negative disease as has been previously reported in children [25, 26]. The addition of stool Xpert Ultra to sputum Xpert Ultra testing had a modest benefit in detection of culture-confirmed and Unconfirmed TB. These findings overall informed a recent WHO Rapid Communication that recommended concurrent stool and respiratory molecular testing for childhood TB [27]. Further work is needed to assess how local TB prevalence and cost-effectiveness impact the benefit of combined testing.

All three centrifuge-free methods had a relatively large proportion of non-determinate results (9.7-12.6%), exceeding the WHO target product profile of <5% [28]. We found that if these individuals had valid results, sensitivity varied by up to 3-7 percentage points, and specificity by 10-14 percentage points. The high proportion of non-determinate results was also observed during the development of these methods (7.8-10%) [10, 29, 30], and in past clinical studies [11, 18]. The reasons may be related to stool consistency, debris in the supernatant and presence of PCR inhibitors. Studies in Ethiopia and Vietnam using SOS found that validity increased as staff gained experience over time, though non-determinate results remained slightly higher than with sputum Xpert Ultra [11, 22]. Further improvements in processing and testing are thus needed to reduce the proportion of non-determinate results.

In the head-to-head comparison, the three methods performed similarly, and laboratory staff found them acceptable and usable. These findings contributed to the WHO guidelines endorsing stool for molecular testing in children [31]. SOS was the least time-consuming, and the most feasible for use in peripheral facilities without laboratory infrastructure. Although SPK and OSF were at earlier stages of development, the findings highlight the preference for methods with fewer supplies and processing steps. SPK development has not continued due to the similar performance and additional supplies required. SOS is being implemented in high TB burden settings, and data suggests increased access to testing and pediatric TB notifications [32], highlighting the importance of non-invasive sample types to increase TB testing for children.

This study is the largest, prospective evaluation comparing stool processing methods for children in multiple high TB-burden settings. We utilized a standardized protocol, and our randomization procedure for stool testing minimized bias in the head-to-head comparison. However, we also acknowledge several limitations. The lack of gold standard TB test for children could bias accuracy estimates; we sought to mitigate this with uniform research-based reference standards and by presenting accuracy across a range of standards. SPK had a larger sample size due to earlier introduction; its diagnostic accuracy estimates may be more reliable compared to SOS and OSF, though the comparative analysis included only participants who underwent all three tests. OSF used less stool than the other methods and may have underestimated its accuracy, although the SOS method has been shown to have similar results at lower stool volumes [30]. Children may have provided a combination of sputum sample types, and we were unable to compare stool Xpert Ultra to specific types such as gastric aspirates. We had few drug-resistant cases and limited HIV positive children with confirmed TB, and further assessment is needed. Sputum Xpert Ultra semi-

quantitative level was not available for all children, limiting stratification by subgroup to assess the association of bacterial load on accuracy. Collecting and testing multiple stool samples may increase accuracy and requires further evaluation. Additionally, we did not include caregivers in our acceptability and usability survey, but a recent study found a preference for stool over respiratory testing [11].

CONCLUSIONS

Three stool processing methods achieved similar accuracy with Xpert Ultra, and performed best among children who are *Mtb* culture-positive. All methods were acceptable, but SOS was the most feasible to be implemented in peripheral facilities without a laboratory. Centrifuge-free stool processing methods may increase access to Xpert Ultra for children, but sensitivity is lower than sputum Xpert Ultra in children with culture-negative disease. Additional implementation and economic evaluations are needed to assess the benefits of stool testing when sputum Xpert Ultra testing in children is feasible. Stool testing alone may not eliminate the diagnostic gap for pediatric TB, but could play an important role as part of a comprehensive package of TB diagnostic approaches and highlights the need to develop more sensitive assays on easily accessible samples to detect TB in children.

NOTES

The work was supported by the National Institutes of Health, through the National Institute of Allergy and Infectious Diseases [U01AI152087 to AC and CMD; and R01AI131617 to DA], and the National Heart, Lung, and Blood Institute [K23HL153581 to DJ]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. FIND provided funding to all the sites and the SPK supplies. Cepheid provided in-kind support of Xpert Ultra cartridges and GeneXpert machines, but had no role in the study design, analysis, interpretation and decision to publish.

For potential conflicts of interest, AM, AT, CMD, MPN, MR, DA, PB and FIND were involved in the development of SPK, PD and ET for SOS, and MB and ML for OSF. DA holds patents related to tuberculosis detection and drug treatment, and receives royalty payments for one or more of these patents that have been licensed by Rutgers University to Cepheid. The other authors declare no conflicts of interest.

ACKNOWLEDGEMENTS: We thank the study participants, their caregivers, and clinical and research staff at each site. We also thank FIND staff Flavio Ambrogiani, Aradhana Chauhan, Loghanathan Prabakaran, Sunita Singh, and Rita Szekely for their support. We greatly appreciate Cepheid for donating Xpert Ultra cartridges and loaning GeneXpert machines. De-identified data used for the analysis is available on request.

For author contributions: conceptualization was by DJ, PN, MPN, HJZ, RL, AB, AC, MR, EW, CMD, methodology by DJ, PN, MPN, MB, ML, PD, DA, PB, and CMD, investigation by DJ, MPN, RC, PW, HJZ, LW, RL, UBS, AB, AT, AM, AC, and EW, analysis by DJ, RC, and LW, writing original draft by DJ, review and edits by all authors, and resources by DJ, AC, MR, EW, and CMD.

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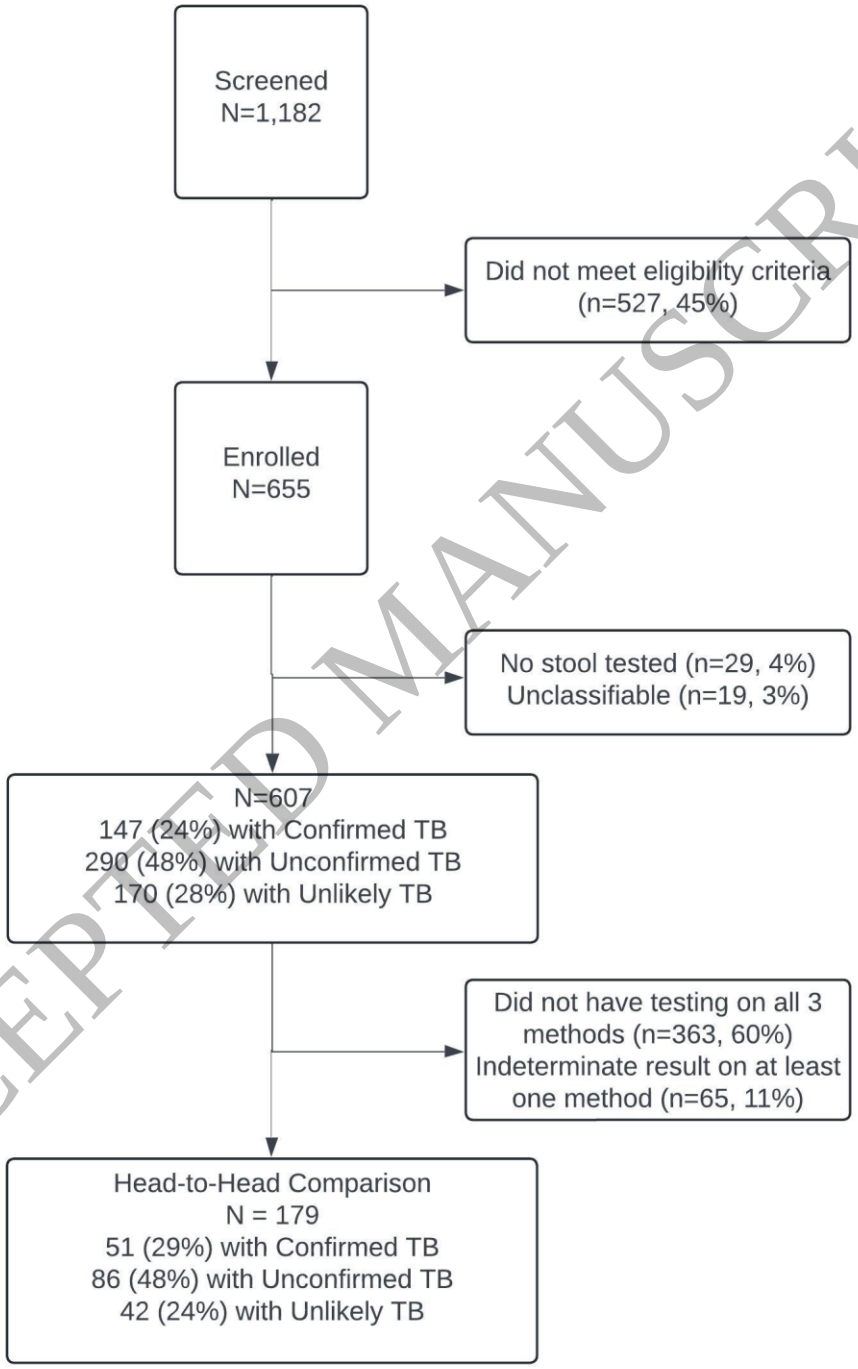
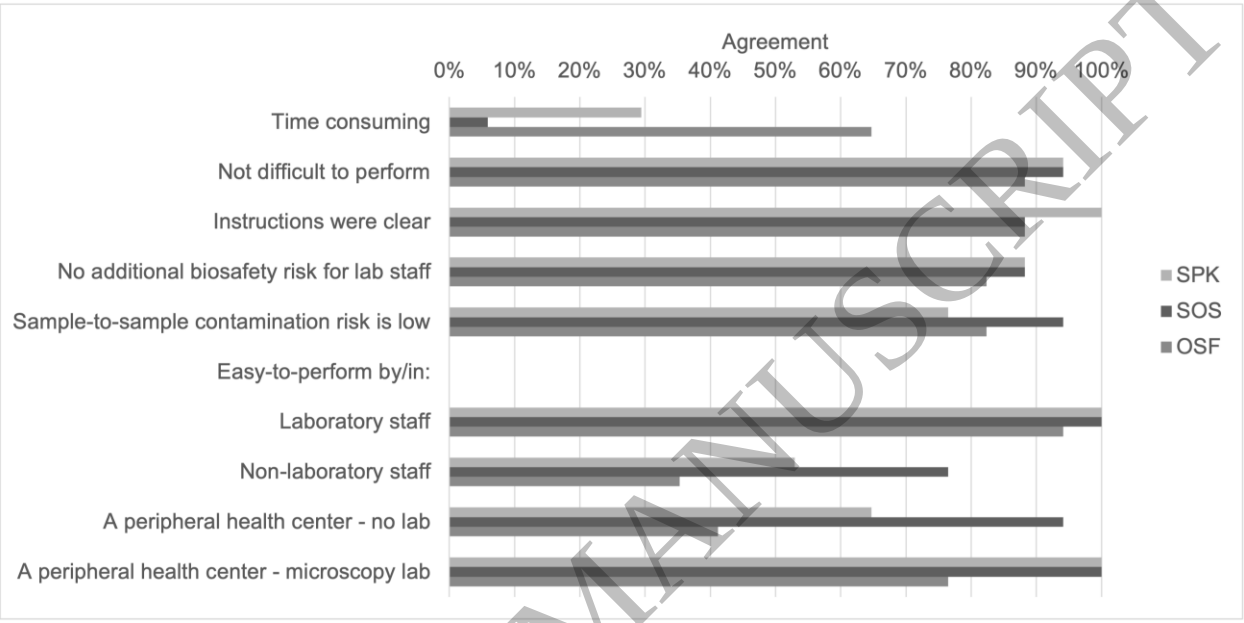
FIGURE LEGENDS

Figure 1. Flow of Participants



Alt Text: A flow chart that shows the number of children who were screened, enrolled, and then included in the head-to-head comparison of stool processing methods for Xpert Ultra. Number and reasons for exclusion are also indicated.

Figure 2. Usability of stool-based Xpert Ultra testing with centrifuge-free processing methods



Alt Text: Bar chart that shows the percent agreement of laboratory staff on questions related to the usability of stool Xpert Ultra, stratified by stool processing method.

Table 1. Sample Characteristics

Characteristic[a]	Median (IQR) or n (%)
Age	3.5 (1.3-7)
<5 years old	367 (60.5)
5-9 years	164 (27.0)
10-14 years	76 (12.5)
Female	292 (48.1)
HIV positive (n=574)	89 (15.5)
Median CD4 (n=72)	715 (261-1228)
Underweight	308 (50.7)
History of TB (n=604)	19 (3.2)

History of known TB contact (n=602)	314 (52.2)
Country	
India	58 (9.6)
South Africa	178 (29.3)
Uganda	371 (61.1)
Reported Cough	563 (92.8)
Reported Fever	462 (76.1)
Failure to thrive	122 (20.1)
Xpert Ultra positive result on sputum	136 (22.4)
Culture on sputum positive for *M. tuberculosis*	63 (10.4)
TB Classification	
Confirmed TB	147 (24.2)
Unconfirmed TB	290 (47.8)
Unlikely TB	170 (28.0)
Stool collection method (n=596)	
Diaper	159 (26.4)
Cup	428 (71.1)
Other	15 (2.5)
Stool processing method with valid result[b]	
SPK (n=585)	528 (90.3)
SOS (n=285)	253 (88.8)
OSF (n=270)	236 (87.4)

a. N= 607 unless indicated
b. Introduction of SPK testing began first, followed by SOS and OSF, with respective totals noted

Table 2. Summary of stool and sputum Xpert Ultra Results[1]

	SPK (N=585)	SOS (N=285)	OSF (N=270)[2]	Sputum Sample (N=607)[3,4]
MTB Positive, n (%)	55 (9.4)	32 (11.2)	25 (9.3)	131 (21.6)
Median CT value	20.6 (17.1-24.3)	19.8 (16.7-25.4)	17.1 (16.6-22.1)	-
Trace	10 (18.2)	7 (21.9)	4 (16.7)	36 (48)
Very Low	14 (25.5)	8 (25)	3 (12.5)	12 (16)
Low	19 (34.6)	11 (34.4)	12 (50)	7 (9.3)
Medium	11 (20)	5 (15.6)	4 (16.7)	11 (14.7)
High	1 (1.8)	1 (3.1)	1 (4.2)	9 (12)
RIF resistant	1 (1.8)	0	0	12 (16)
MTB Negative, n (%)	473 (80.9)	221 (77.5)	211 (78.2)	474 (78.1)
Non-determinate results, n (%)	57 (9.7)	32 (11.3)	34 (12.6)	2 (0.3)
Invalid	41 (7.0)	25 (8.8)	13 (4.8)	1 (0.2)
Error	14 (2.4)	7 (2.5)	20 (7.4)	1 (0.2)
No Result	2 (0.3)	0	1 (0.4)	0

MTB: *M. tuberculosis*; RIF: Rifampin; SPK: Stool Processing Kit; SOS: Simple-One-Step; OSF: Optimized Sucrose Flotation

1. Summary of all testing, not limited to children who had all three stool processing methods, and is indicated by totals
2. One missing OSF semi-quantitative result

DOI: 10.1093/cid/ciaf289

3. Median CT values not available for respiratory Xpert Ultra as results from South Africa came from a referral laboratory
4. Data on semi-quantitative level and rifampin resistance not available in South Africa, or if the child had sputum Xpert Ultra testing prior to enrollment (n=75)

Table 3. Diagnostic accuracy of stool Xpert Ultra, by centrifuge-free processing method

Reference Standard	Sensitivity, n/N, % (95% CI)			Specificity, n/N, % (95% CI)		
	SPK	SOS	OSF	SPK	SOS	OSF
All valid results[1]						
MRS	48/130, 36.9% (28.6-45.8)	27/70, 38.6% (27.2-51)	20/64, 31.3% (20.2-44.1)	391/398, 98.2% (96.4-99.3)	178/183, 97.3% (93.7-99.1)	167/172, 97.1% (93.3-99)
CRS	53/381, 13.9% (10.6-17.8)	31/186, 16.7% (11.6-22.8)	23/177, 13.0% (8.4-18.9)	145/147, 98.6% (95.2-99.8)	66/67, 98.5% (92-100)	57/59, 96.6% (88.3-99.6)
Sputum Xpert Ultra	46/120, 38.3% (29.6-47.6)	25/63, 39.7% (27.6-52.8)	21/60, 35.0% (23.1-48.4)	398/407, 97.8% (95.8-99)	181/188, 96.3% (92.5-98.5)	171/175, 97.7% (94.3-99.4)
Sputum culture	36/58, 62.1% (48.4-74.5)	17/22, 77.3% (54.6-92.2)	11/20, 55.0% (31.5-76.9)	408/421, 96.9% (94.8-98.3)	188/199, 94.5% (90.3-97.2)	181/92, 94.3% (90-97.1)
Head-to-Head Comparison (N=179)[2]						
MRS	19/51, 37.3% (24.1-51.9)	21/51, 41.2% (27.6-55.8)	17/51, 33.3% (20.8-47.9)	124/128, 96.9% (92.2-99.1)	124/128, 96.9% (92.2-99.1)	125/128, 97.7% (93.3-99.5)

CRS	22/137, 16.1% (10.3-23.3)	24/137, 17.5% (11.6-24.9)	19/137, 13.9% (8.6-20.8)	41/42, 97.6% (87.4-99.9)	41/42, 97.6% (87.4-99.9)	41/42, 97.6% (87.4-99.9)
Sputum Xpert Ultra	17/44, 38.6% (24.4-54.5)	19/44, 43.2% (28.3-59)	17/44, 38.6% (24.4-54.5)	129/135, 95.6% (90.6-98.4)	129/135, 95.6% (90.6-98.4)	132/135, 97.8% (93.6-99.5)
Sputum culture (n=157)	12/17, 70.6% (44-89.7)	13/17, 76.5% (50.1-93.2)	9/17, 52.9% (27.8-77)	132/140, 94.3% (89.1-97.5)	131/140, 93.6% (88.1-97)	132/140, 94.3% (89.1-97.5)

CI: Confidence interval; CRS: Composite Reference Standard; MRS: Microbiological Reference Standard; SPK: Stool processing kit; SOS: Simple-One-Step; OSF: Optimized Sucrose Flotation

1. Sensitivity and specificity calculated for each method based on total number of valid results, and not limited to only children who completed all three methods. Denominator indicated for each method by reference standard.
2. Accuracy among children who had valid results by all three methods, N=179 except as noted for culture (157 with valid culture results).

Table 4. Comparison and incremental accuracy of Xpert Ultra with a sputum and stool specimen, by stool processing method

	SPK		SOS		OSF	
Comparison of sputum Xpert Ultra versus stool Xpert Ultra[1,2] n/N, % (95% CI)	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
Xpert Ultra Sputum	14/17, 82.4% (56.6-96.2)	119/140, 85% (78-90.5)	14/17, 82.4% (56.6-96.2)	119/140, 85% (78-90.5)	14/17, 82.4% (56.6-96.2)	119/140, 85% (78-90.5)
Xpert Ultra Stool	12/17, 70.6% (44-89.7)	132/140, 94.3% (89.1-97.5)	13/17, 76.5% (50.1-93.2)	131/140, 93.6% (88.1-97)	9/17, 52.9% (27.8-77)	132/140, 94.3% (89.1-97.5)
Difference % (95% CI)	11.8% (-21.8 to 45.3)	-9.3% (-16.2 to -2.3)	5.9% (-25.6 to 37.4)	-8.6% (-15.4 to 1.8)	29.4% (1.9 to 57)	-9.2% (-15.9 to -2.7)
Incremental accuracy of sputum Xpert Ultra and stool Xpert Ultra[1,2] n/N, % (95% CI)	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
Xpert Ultra Sputum + Stool	16/17,	115/140, 82.1%	16/17, 94.1% (71.3-99.9)	115/140, 82.1%	14/17, 82.4% (56.6-96.2)	116/140, 82.9%

	94.1% (71.3-99.9)	(74.8-88.1)		(74.8-88.1)		(75.6-88.7)
Incremental change vs. Xpert Ultra Stool alone, Difference % (95% CI)	23.5% (-2.5 to 50)	-12.1% (-18.3 to -6)	17.6% (-5.4 to 41.7)	-11.4% (-17.4 to -5.4)	29.4% (1.9 to 57)	-11.4% (-17.4 to -5.4)
Incremental change vs. Xpert Ultra Sputum alone, Difference % (95% CI)	11.8% (-9.4 to 33)	-2.9% (-6.3 to 0.6)	11.8% (-9.4 to 33)	-2.9% (-6.3 to 0.6)	0 (-5.9 to 5.9)	-2.1% (-5.2 to 1)

CI: Confidence interval;

1. Among children who had valid Xpert Ultra results for sputum and with all three stool processing method
2. Sputum culture-based reference standard

DOI: 10.1093/cid/ciaf289