1 **Stool processing methods for Xpert Ultra testing in childhood tuberculosis: A**

2 **prospective, multi-country accuracy study**

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48

49   Words

50   Abstract: 241

51   Text: 2,977

52   References: 31

53

54   **KEY POINTS**

55   In a multi-country diagnostic accuracy study for childhood pulmonary tuberculosis, three

56   stool processing methods for Xpert Ultra were acceptable, usable and performed

57   similarly. Sensitivity was lower than that of sputum Xpert Ultra, but improved in children

58   with culture-positive disease.

ABSTRACT

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 respiratory specimen testing with Xpert Ultra and culture. Stool was tested using Xpert Ultra after processing with each method. We compared the accuracy of each method to a microbiological reference standard (MRS) and a composite reference standard (CRS). We surveyed the laboratory staff to assess acceptability and usability of the methods.

Results. We included 607 children, of whom the median age was 3.5 years (IQR 1.3-7), 48% were female, and 15.5% were 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), 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. Laboratory staff reported that the methods were acceptable and usable, but SOS was most feasible to implement in a peripheral facility. Sensitivity increased among children who were culture-positive (55-77.3%) and was low (13-16.7%) against the CRS.

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Conclusions. Stool processing methods for Xpert Ultra were acceptable, usable, and

performed similarly, with highest sensitivity among children with culture-positive TB.

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

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 [3-6], 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) [7], Simple One-Step method (SOS, KNCV TB Foundation, Hague, Netherlands) [8], and Optimized Sucrose Flotation method (OSF, TB SPEED Consortium) [9]. However, limited comparative data exist on their accuracy, acceptability and usability [10].

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.

106 **METHODS**

107 *Setting and Participants*

108 We consecutively enrolled children under 15 years old from June 2019 to March 2021 in

109 India, South Africa, and Uganda. In India, children were recruited from two tertiary care

110 centers: the All India Institute of Medical Sciences (AIIMS), New Delhi, and KEM

111 Hospital, Pune, with referrals from surrounding outpatient clinics. In South Africa,

112 children were recruited from two tertiary hospitals, Red Cross Children's Hospital in

113 Cape Town and Dora Nginza Hospital in Gqeberha. In Kampala, Uganda, children were

114 referred from Mulago National Referral Hospital inpatient and outpatient facilities, and

115 hospitals and clinics from the surrounding area. Enrollment was paused for periods

116 during the COVID-19 pandemic, and each site followed local regulations of when they

117 could resume. Children were eligible if they had microbiologically confirmed TB, or at

118 least one symptom of pulmonary TB: unexplained cough for ≥2 weeks, unexplained

119 fever for ≥1 week, unexplained failure to thrive or weight loss, or chest X-ray suggestive

120 of TB. Children already on anti-TB treatment for >72 hours were excluded. Caregivers

121 completed a written informed consent, and children aged ≥8 years in Uganda, and ≥7

122 years in South Africa and India gave assent according to local ethical guidelines.

123 Laboratory staff aged ≥18 years who processed the stool samples at each site

124 completed an anonymous survey to assess acceptability and usability.

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126 The study received ethical approval from the Mulago Hospital Research Ethics

127 Committee, the Human Research Ethics Committee of the University of Cape Town, the

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128   AIIMS Ethics Committee and the KEM Hospital Research Centre Ethics Committee, and

129   the University of California San Francisco Institutional Review Board.

130

131   *Reference Procedures*

132   Trained staff performed a standard TB evaluation on all children, including a clinical

133   questionnaire, physical exam, and chest radiography. Two sputum specimens were

134   collected, including expectorated or induced sputum, gastric aspirate or nasopharyngeal

135   aspirate, and tested using Xpert Ultra and liquid or solid mycobacterial culture according

136   to standard operating procedures. Sputum Xpert Ultra trace results were defined as

137   positive [11].

138

139   *Stool Collection*

140   Study staff asked participants to collect one stool sample in a sterile cup at enrollment,

141   either directly or transferred from a diaper using a spoon. If the child was unable to

142   produce a sample, the caregiver was provided with a cup and was asked to return the

143   sample as soon as obtained within 3 days.

144   Stool samples were homogenized when possible and processed fresh, or stored

145   at 2-8°C and tested within 72 hours. Based on available supplies, protocols and training,

146   SPK was introduced first in June 2019, followed by SOS and then OSF in December

147   2019. When all three methods were tested, we randomized the order of testing and

148   where each sample was collected in the cup each week.

149

150   *Stool processing and index testing*

8

151 Stool samples were processed using the following methods before Xpert Ultra testing

152 (**Supplemental Figure 1**):

153   1. SPK. The kit (42T for FIND) included a squeeze bottle with mixing beads, a stool

154   processing buffer (SPB) and a filter cap. Five milliliters (mL) of the SPB and 5 mL

155   of Xpert Ultra Sample Reagent (SR) were pipetted into the squeeze bottle. One

156   gram of stool was added to the squeeze bottle, shaken vigorously, and incubated

157   at room temperature for 30 minutes. The mixture was then squeezed through the

158   filter cap into the Xpert Ultra cartridge.

159   2. SOS: 0.8-1 gram of solid stool was added to an 8 mL SR bottle. If the stool was

160   soft or liquid, then respectively 2 or 4 mL of SR were replaced with the same

161   volume of stool. Shaking and incubation for 10 minutes were performed twice,

162   and then two mL of supernatant were pipetted into the Xpert Ultra cartridge.

163   3. OSF: 0.5 gram of stool was added to 10 mL of 50% Sheather's solution, and

164   emulsified using wooden sticks and manually shaking. After 30 minutes of

165   incubation to allow sedimentation, 0.5 mL of supernatant were mixed with 1.8 mL

166   of SR and incubated for 15 minutes before being added to the Xpert Ultra

167   cartridge.

168 Of note, the three methods were at different stages of development. SOS was design-

169 locked, while SPK was a prototype design, and OSF had not yet been developed into a

170 kit.

171   Laboratory staff were blinded to the sputum Xpert Ultra result and clinical

172 treatment decision. Within three months of the start of enrollment, the staff were asked

173 to complete a voluntary, anonymous acceptability and usability questionnaire on each

174   method, using a Likert scale ranging from Totally or Partially Disagree to Partially or

175   Totally Agree.

176

177   *TB Classification*

178   Participants were classified as Confirmed, Unconfirmed or Unlikely TB based on NIH

179   consensus definitions [12]. Confirmed TB was defined as having sputum positive for

180   *Mtb* by Xpert Ultra or culture. Children with Unconfirmed TB did not have microbiological

181   confirmation, but had signs, symptoms and/or radiographic findings consistent with TB

182   and had clinical response to anti-TB treatment. Children with Unlikely TB had symptom

183   resolution without anti-TB treatment. A case was unclassifiable if there was insufficient

184   information or follow up to determine TB status. Stool Xpert Ultra results were not used

185   in the TB classification.

186

187   *Statistical Analysis*

188   Our primary analysis assessed the sensitivity and specificity of stool Xpert Ultra using

189   each method, against a microbiological reference standard (MRS) that classified TB in

190   children with Confirmed TB, and otherwise as not having TB (Unconfirmed or Unlikely

191   TB). Children who could not provide a sputum or stool sample were excluded.

192   Participants with non-determinate stool Xpert Ultra results (invalid, error or no result)

193   were excluded; however, we conducted a sensitivity analysis using an intention-to-

194   diagnose approach that considered their results as either negative or positive. For the

195   head-to-head comparison, we included children who had valid results for all three

196   methods, and used McNemar's test with 95% CIs to compare differences in sensitivity

10

197 and specificity. Statistical significance was defined if 95% CIs of the difference did not

198 cross zero.

199   Secondarily, we assessed accuracy using three additional reference standards:

200 1) a composite reference standard (CRS) that included Unconfirmed TB in the definition

201 of TB; 2) sputum Xpert Ultra result alone; and 3) sputum culture results alone. In

202 addition, we determined the incremental accuracy of using both stool and sputum Xpert

203 Ultra compared to each test alone against sputum culture.

204   For the acceptability and usability assessment, we summarized the frequency

205 and proportion of the responses by processing method. Analyses were completed using

206 Stata version 16.1 (StataCorp LLC, College Station, Texas). Findings have been

207 presented in accordance with the Standards for Reporting Diagnostic Accuracy

208 (STARD) guidelines [13].

209

210 **RESULTS**

211 *Participant Characteristics*

212 We enrolled 655 children from 1,182 screened across the three countries (**Figure 1**).

213 After excluding 29 children who did not provide a stool sample and 19 children whose

214 TB status was unclassifiable, the final sample size was 607. Most children were from

215 Uganda (n=371, 61.1%) and under 5 years old (n=367, 60.5%, **Table 1**). Of the 574

216 children, 89 (15.5%) were living with HIV and 308 (50.7%) were underweight. A total of

217 147 children (24.2%) had Confirmed TB, with the majority (136/147, 92.5%) being

218 sputum Xpert Ultra positive.

219

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 Xpert Ultra results with sputum samples. Sputum samples were 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

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, compared to the MRS (**Table 3**). Sensitivity was lower with the CRS (range 13.0-16.7%), similar compared to sputum Xpert Ultra (range 38.3-45%) and higher compared to 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**). In the intention-to-diagnose analysis, sensitivity ranged from 33.3-43.1% for SPK, 37.0-41.1% for SOS, and 28.1-38.0% for OSF. Specificity ranged from 88.7-98.4% for SPK, 84.0-97.6% for SOS and 83.9-97.5% for OSF (**Supplemental Table 2**).

12

243   Subgroup analysis is shown in **Supplemental Table 1.** Against the MRS,

244   specificity was higher in Uganda for the SOS and OSF method. By age group, there

245   was a trend towards lower sensitivity in children under 5 years old, and this was

246   statistically significant for SOS (25.9% for children <5 years versus 66.7% for 10-14

247   years, p = 0.01). Sensitivity also tended to be higher in females, and was significantly

248   higher for SPK (45.1% in females vs. 27.1% in males, p = 0.04).

249

250   *Head-to-head comparison of the stool processing methods*

251   179 children had valid results from all three approaches (**Figure 1**). Comparing

252   methods, sensitivity and specificity were similar regardless of the reference standard

253   with overlapping 95% CIs (**Table 3**).

254

255   Against sputum culture, Xpert Ultra on sputum had higher sensitivity (82.4%, 95% CI

256   56.6-96.2) than stool Xpert Ultra in all three methods, but it was not statistically

257   significant (**Table 4**). Specificity was significantly lower than stool Xpert Ultra across

258   methods. When respiratory and stool Xpert Ultra testing were done concurrently, the

259   sensitivity ranged from 82.4% (OSF) to 94.1% (SPK and SOS) against sputum culture.

260   This represented an absolute increase of 17.6-23.5% compared to stool Xpert Ultra

261   alone **(Table 4)**, with a reduction in specificity of 11.4-12.1%. The addition of stool Xpert

262   Ultra to sputum Xpert Ultra increased sensitivity by 0-11.8%, at a loss of specificity by

263   2.1-2.9%.

264

265   *Acceptability and usability of testing by laboratory staff*

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266   The survey was completed by 17 laboratory staff (5 from India, 7 from South Africa, and

267   5 from Uganda). The majority (12/17, 70.6%) had over three years of TB lab

268   experience, but nearly half (8/17, 47.1%) had no prior experience with stool samples. All

269   participants reported being comfortable handling stool samples and all agreed that stool

270   testing with Xpert Ultra would be beneficial.

271   Usability is shown in **Figure 2**. For all three methods, the instructions were clear

272   and posed minimal biosafety concern (>75% agreement). However, SOS was the least

273   time-consuming, and most participants (75%) agreed it could be performed by non-

274   laboratory staff at peripheral facilities with Xpert Ultra but without other laboratory

275   infrastructure (94% agreement). Most respondents believed that all three methods could

276   be performed at a peripheral health center if a microscopy laboratory was present.

277

278   **DISCUSSION**

279   Stool Xpert Ultra testing has the potential to increase access to molecular TB testing for

280   children, especially in peripheral settings. In this multi-country, prospective evaluation of

281   three stool processing methods for Xpert Ultra testing, we found that stool Xpert Ultra

282   had high specificity, but detected only about a third of microbiologically confirmed cases

283   with similar results across methods. Sensitivity doubled among children with culture-

284   positive TB. Laboratory staff reported that all three methods were acceptable and

285   usable, with SOS being the most practical for use by non-laboratory staff in peripheral

286   facilities. However, ongoing gaps remain in improving TB diagnosis among children who

287   are culture-negative and have paucibacillary disease.

288

289   Stool Xpert Ultra testing showed moderate sensitivity to detect culture-positive

290   TB in children. This is consistent with studies that have shown similar stool and sputum

291   Xpert Ultra accuracy in culture-positive TB [10, 14-16]. However, only 38-45% of

292   children with positive sputum Xpert Ultra results were also positive by stool Xpert Ultra,

293   which was lower than previous studies [10, 17-21]. One reason for this difference is that

294   stool xpert performance has been shown to be heterogenous [14, 15], depending on

295   collection and processing methods, age, setting, and co-morbidities. This is supported

296   by the subgroup analysis where we noted differences by country, age group, and sex.

297   Furthermore, both our study and prior studies found that bacterial burden was

298   associated with stool *Mtb* detection [10, 17]. Our study had a higher proportion of trace

299   or very low positive results on sputum Xpert Ultra compared to previous studies [5, 17],

300   which could increase the likelihood of negative results on stool Xpert Ultra. Moreover, a

301   companion stool Xpert Ultra study in Uganda and Zambia found higher sensitivity, but

302   these children were mostly hospitalized and less likely to be TB contacts (28% versus

303   52% in our study), and so may have reflected later and more severe disease [22].

304

305   These findings suggest that while the accuracy of stool Xpert Ultra testing may

306   be higher for culture-positive and more severe TB disease, its sensitivity is limited for

307   children with paucibacillary disease [6]. This has important implications, because stool-

308   based testing is particularly helpful in peripheral settings where children may present

309   earlier, and in children under five who are more likely to have paucibacillary disease.

310   We found that performing sputum Xpert Ultra in parallel with stool Xpert Ultra, or when

311   stool Xpert Ultra is negative, increased absolute sensitivity by 17.6 to 29.4%. This is

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312 supported by other studies that have shown the combination of sputum and stool testing

313 improved accuracy [17, 23]. Although specificity was lower for Xpert Ultra on sputum

314 specimens using a culture-based reference standard, this likely reflects detection of

315 culture-negative disease that has been previously reported in children [24, 25]. The

316 addition of stool Xpert Ultra to sputum Xpert Ultra testing had a modest benefit. These

317 findings informed a recent WHO Rapid Communication that recommended concurrent

318 stool and respiratory molecular testing for childhood TB [26]. Further work is needed to

319 assess how local TB prevalence and cost-effectiveness inform the benefit of combined

320 testing.

321 All three centrifuge-free methods had a relatively large proportion of non-

322 determinate results (9.7-12.6%), exceeding the WHO target product profile of <5% [27].

323 This was also observed during the development of these methods (7.8-10%) [9, 28, 29],

324 and in past clinical studies [10, 17]. The reasons may be related to stool consistency,

325 debris in the supernatant and presence of PCR inhibitors. Studies in Ethiopia and

326 Vietnam using SOS found that validity increased as staff gained experience over time,

327 though non-determinate results remained slightly higher than with sputum Xpert Ultra

328 [10, 21]. In the intention-to-diagnose analyses, we found that including non-determinate

329 results varied sensitivity by 3-7%, and specificity by 10-14%. Further improvements in

330 processing and testing are needed to reduce the proportion of non-determinate results.

331 In the head-to-head comparison, the three methods performed similarly, and

332 laboratory staff found them acceptable and usable. These findings contributed to the

333 WHO guidelines endorsing stool for molecular testing in children [30]. SOS was the

334 least time-consuming, and the most feasible for use in peripheral facilities without

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335 laboratory infrastructure. Although SPK and OSF were at earlier stages of development,

336 the findings highlight the preference for methods that require fewer supplies and

337 processing steps. SPK development has not continued due to the similar performance

338 and additional supplies required. SOS is being implemented in high TB burden settings,

339 and data suggests increased access to testing and pediatric TB notifications [31],

340 further highlighting the importance of non-invasive sample types to increase access to

341 TB testing for children.

342

343 This study is the largest, prospective evaluation comparing stool processing

344 methods for children in multiple high TB-burden settings. We utilized a standardized

345 protocol and reference standards across sites, and our randomization procedure for

346 stool testing minimized bias in the head-to-head comparison. However, we also

347 acknowledge several limitations. SPK had a larger sample size due to earlier

348 introduction; its diagnostic accuracy estimates may be more reliable compared to SOS

349 and OSF, though the comparative analysis included only participants who underwent all

350 three tests. We had few drug-resistant cases and further assessment is needed.

351 Additionally, we did not include caregivers in our acceptability and usability survey, but a

352 recent study found that most caregivers preferred stool over respiratory testing [10].

353

354 **CONCLUSIONS**

355 Three stool processing methods achieved similar accuracy with Xpert Ultra, and

356 performed best among children who are *Mtb* culture-positive. All methods were

357 acceptable, but SOS was the most feasible to be implemented in peripheral facilities

358   without a laboratory. Centrifuge-free stool processing methods may increase access to

359   Xpert Ultra for children in those settings, but sensitivity is lower than sputum Xpert Ultra

360   in children with culture-negative disease. Additional implementation and economic

361   evaluations are needed to assess the benefits of stool testing when sputum Xpert Ultra

362   testing in children is feasible. Stool testing alone may not eliminate the diagnostic gap

363   for pediatric TB, but could play an important role as part of a comprehensive package of

364   TB diagnostic approaches and highlights the need to develop more sensitive assays on

365   easily accessible samples to detect TB in children.

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389 For potential conflicts of interest, MPN, DA, PB and FIND were involved in the

390 development of SPK, PD and ET for SOS, and MB and ML for OSF. DA holds patents

391 related to tuberculosis detection and drug treatment, and receives royalty payments for

392 one or more of these patents that have been licensed by Rutgers University to Cepheid.

393 The other authors declare no conflicts of interest.

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Figure 1. Flow of Participants
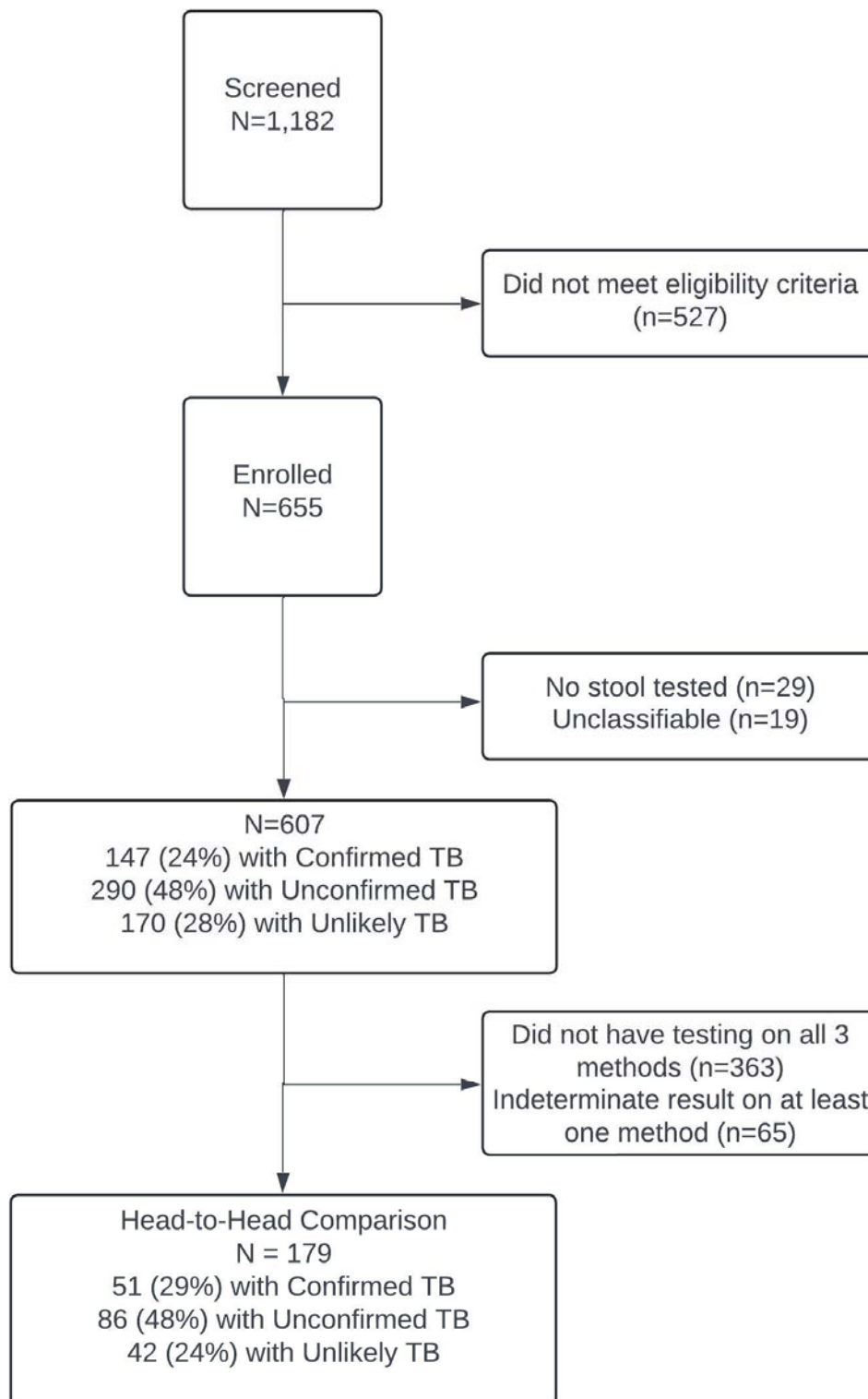
Figure 2. Usability of stool-based Xpert Ultra testing with centrifuge-free processing methods
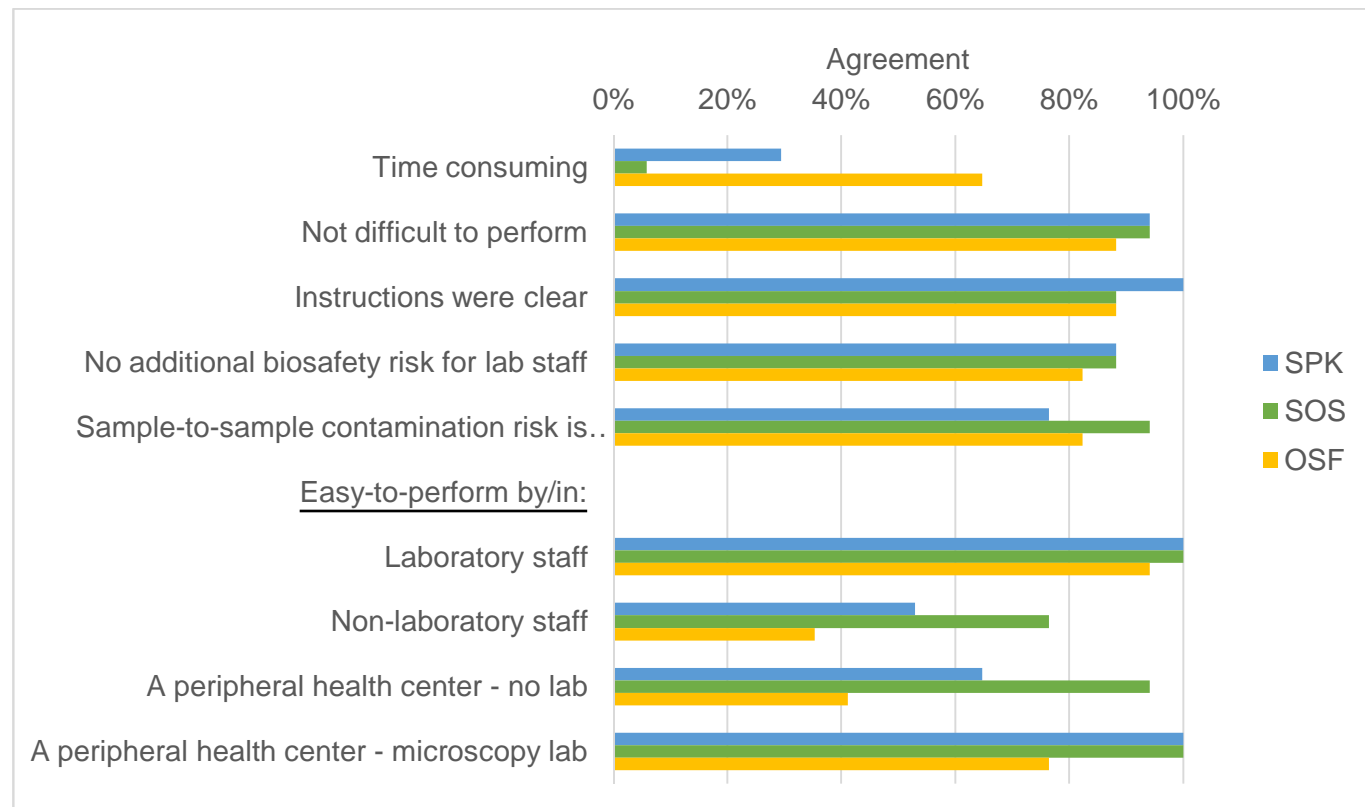
Table 1. Sample Characteristics

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
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)	181/1881, 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

	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, 94.1% (71.3-99.9)	115/140, 82.1% (74.8-88.1)	16/17, 94.1% (71.3-99.9)	115/140, 82.1% (74.8-88.1)	14/17, 82.4% (56.6-96.2)	116/140, 82.9% (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)

processing method

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