Tuberculosis diagnostic accuracy of stool Xpert MTB/RIF and urine AlereLAM in vulnerable children

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Shareable abstract (@ERSpublications)
Modest sensitivity but high specificity and good feasibility support the use of stool Xpert MTB/RIF in young children at high risk of disseminated tuberculosis; however, urine AlereLAM showed poor specificity and has little applicability https://bit.ly/3oMLNHU

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Abstract

Background Non-sputum-based diagnostic approaches are crucial in children at high risk of disseminated tuberculosis (TB) who cannot expectorate sputum. We evaluated the diagnostic accuracy of stool Xpert MTB/RIF and urine AlereLAM tests in this group of children.

Methods Hospitalised children with presumptive TB and either age <2 years, HIV-positive or with severe malnutrition were enrolled in a diagnostic cohort. At enrolment, we attempted to collect two urine, two stool and two respiratory samples. Urine and stool were tested with AlereLAM and Xpert MTB/RIF, respectively. Respiratory samples were tested with Xpert MTB/RIF and mycobacterial culture. Both a microbiological and a composite clinical reference standard were used.

Results The study analysed 219 children; median age 16.4 months, 72 (32.9%) HIV-positive and 184 (84.4%) severely malnourished. 12 (5.5%) and 58 (28.5%) children had confirmed and unconfirmed TB, respectively. Stool and urine were collected in 219 (100%) and 216 (98.6%) children, respectively. Against the microbiological reference standard, the sensitivity and specificity of stool Xpert MTB/RIF was 50.0% (6/12, 95% CI 21.1–78.9%) and 99.1% (198/200, 95% 96.4–99.9%), while that of urine AlereLAM was 50.0% (6/12, 95% 21.1–78.9%) and 74.6% (147/197, 95% 67.9–80.5%), respectively. Against the composite reference standard, sensitivity was reduced to 11.4% (8/70) for stool and 26.2% (17/68) for urine, with no major difference by age group (<2 and ⩾2 years) or HIV status.

Conclusions The Xpert MTB/RIF assay has excellent specificity on stool, but sensitivity is suboptimal. Urine AlereLAM is compromised by poor sensitivity and specificity in children.

Introduction

Tuberculosis (TB) remains a major infectious disease killer with most cases occurring in resource-limited settings. In 2019, children contributed around 12% of the estimated global TB burden and 16% of deaths among HIV-negative individuals [1]. However, <50% of children estimated to develop TB every year are notified due to a combination of failure to diagnose TB and failure to notify diagnosed cases, making it difficult to assess the true burden of the disease in this population [1]. Nearly a third of all child TB cases live in Africa where many have risk factors for a poor prognosis, such as HIV co-infection and severe malnutrition [2]. In these children, the paucibacillary nature of their disease and difficulty in collection of good quality respiratory specimens are major challenges for the diagnosis of TB.

Consequently, the vast majority of children who are started on TB treatment are started based on clinical presumption. Clinical signs have low sensitivity and low specificity, leading to failure to diagnose children with TB and a tendency to treat children who do not have TB [3]. Children who are more unlikely to have their TB diagnosis confirmed are more likely to have poor outcomes, such as young, HIV-positive or malnourished children [4]. There is an urgent need for a rapid and accurate non-sputum-based TB diagnostic test in this vulnerable group.

The urine lipoarabinomannan (LAM) antigen point-of-care test (Determine TB LAM Ag test, "AlereLAM"; Alere, Waltham, MA, USA) is recommended by the World Health Organization (WHO) to assist the diagnosis of active TB in HIV-positive adults, adolescents and children with TB-suggestive signs or advanced HIV disease, or who are seriously ill or irrespective of TB-suggestive signs if they have CD4 count <200 cells·mm^{-3} [5]. The recommendation for children is mainly based on data obtained in adult studies; the paediatric studies done to date have demonstrated poor sensitivity (50%) and specificity (60%) against mycobacterial culture from respiratory samples [6]. Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) testing from stool provides another non-sputum-based approach for children who are unable to expectorate by retrieving *Mycobacterium tuberculosis* from swallowed respiratory samples. While culture may not be feasible due to contamination and most *M. tuberculosis* dying during passage through the gut, *M. tuberculosis* DNA may still be detected in stool. A pooled sensitivity of 60% and high specificity against a microbiological reference standard from respiratory samples was reported [7]. Neither urine AlereLAM nor stool Xpert MTB/RIF have been evaluated in children at high risk of disseminated TB, which was the purpose of our study.

Methods

Study design and population

This was a prospective diagnostic accuracy study conducted in the Regional Referral Hospital in Mbarara, Uganda. Patients were enrolled between September 2015 and March 2018. After obtaining the parent/guardian's written informed consent (plus assent in children aged >7 years), we enrolled children considered at risk of disseminated TB, defined as aged <2 years, HIV-positive or severely malnourished and with clinical suspicion of TB (supplementary material)

At enrolment, medical history and clinical examinations were performed by the study doctor and followed by sample collection for TB microbiological investigations, digital anterior–posterior and lateral chest radiography, and a tuberculin skin test (TST). All children were reviewed after 1, 2, 8 and 24 weeks. An itemised chest radiography evaluation sheet was used to report radiological findings. TB-suggestive chest radiography was defined by the presence of cavitation, lobar pneumonia, pleural effusion, miliary infiltrates or mediastinal adenopathy. TB treatment decision was made by the site principal investigator who is a paediatrician. If indicated, TB treatment was administered according to national guidelines [8]. A TST using the Mantoux method was done on all children, and defined as positive by an induration of ⩾5 mm in HIV-positive/exposed or malnourished children and ⩾10 mm for all other children [9].

The study received approval from Mbarara University Research Ethics Committee (01/04–15) and the Uganda National Council for Science and Technology (HS 1814).

Sample collection procedures

On the day of enrolment, collection of one gastric aspirate and one induced sputum or nasopharyngeal aspirate was attempted for microscopy, culture and Xpert MTB/RIF. Induced sputum/nasopharyngeal aspirate (clinician's decision based on child's clinical condition) was performed before the gastric aspirate and after at least 4 h of fasting, as described in the supplementary material. Sodium bicarbonate was added to the gastric aspirate after collection. One stool and one urine sample were collected on both the day of enrolment and the following day. Except for children aged >5 years, fresh urine samples (20–40 mL) were collected in sterile urine containers using a catheter; no urine collection bags were used. In addition, four blood samples were collected among children presenting with fever (>38°C) for bacterial and mycobacterial culture, and all children provided at least 1 mL blood for HIV testing. If indicated, extrapulmonary samples (ascites, cerebrospinal and pleural fluid) were also collected. All samples were collected before the TB treatment decision was made. Finally, where possible, in children who died during the study period consent was sought from their parent/guardians to collect necropsy samples.

Laboratory procedures

Gastric aspirate, induced sputum or nasopharyngeal aspirate, and extrapulmonary and necropsy samples were cultured using commercial Mycobacteria Growth Indicator Tubes (MGITs) (Bactec 960; BD, Franklin Lakes, New Jersey, USA) and in-house Löwenstein–Jensen (LJ) slopes, together with performance of the

Xpert MTB/RIF PCR assay. For all respiratory samples, an equal volume of *N*-acetyl-L-cysteine (NALC) and sodium hydroxide solution was added at 1.25% final concentration, vortexed and allowed to stand at room temperature. Ascites and pleural fluids were processed with 1.25% sodium hydroxide without NALC. After 20 min, the mixture was neutralised with phosphate buffer and centrifuged for 20 min at 3000×*g*. The pellet was resuspended in 2.5 mL PBS, ready for inoculation into LJ and MGIT culture media, and the remnant sample used for Xpert MTB/RIF testing. For LJ, two drops of processed and resuspended pellet were inoculated onto two LJ slopes and incubated for a maximum of 56 days. For MGIT inoculation, PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) was added to limit bacterial overgrowth with OADC (oleic acid, albumin, dextrose and catalase) supplement to enhance the growth of *M. tuberculosis*. MGIT media was inoculated with 500 µL of resuspended pellet and incubated for a maximum of 42 days.

Stool was tested with Xpert MTB/RIF using the sample processing method described by WALTERS *et al.* [10] and tested with Xpert using the manufacturer's instructions for sediments (supplementary material) [11]. Fresh urine samples were tested with AlereLAM within 30 min of collection according to the manufacturer's guidelines [12]. To assess the inter-reader reproducibility, all urine samples collected after January 2017 (protocol amendment) were read by two independent nurses.

Blood samples were tested using a MGIT 9240 instrument (BD) for mycobacterial culture and a Bactec 9240 instrument (BD) for bacterial culture as detailed in the supplementary material. In children with clinical suspicion of *Pneumocystis jirovecii* infection, nasopharyngeal aspirate was also tested with a direct qualitative immunofluorescence microscopy test (Bio-Rad, Hercules, CA, USA). HIV testing and CD4 cell counts followed national guidelines (supplementary material).

Study end-points

The index test "stool Xpert MTB/RIF" result was recorded as *M. tuberculosis* detected, negative or invalid. The index test "urine AlereLAM" result was recorded as 1+, 2+, 3+, 4+ or negative. Two different TB reference standards were used: a microbiological and a composite reference standard. A positive microbiological reference standard was defined by any positive *M. tuberculosis* culture or Xpert MTB/RIF result, excluding stool. A negative microbiological reference standard was defined by one negative culture or Xpert MTB/RIF result from at least two different samples (two respiratory or one respiratory and another sample) without any positive result. For the composite reference standard, at the end of the study, using an algorithmic approach, each case was retrospectively classified as confirmed, unconfirmed or unlikely TB based on the adapted Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease [13]. For the classification, each chest radiograph was read by an independent radiologist who was blinded to the clinical information in addition to the site clinician and a third reader in case of discordant findings. Cases that could not be classified by the algorithm due to incomplete data had their files reviewed by an independent TB paediatrician for final classification; those who did not fit any of the specified criteria were kept as unclassified. Confirmed and unconfirmed TB were grouped together as the positive composite reference standard, while unlikely TB provided the negative reference standard.

Sample size and statistical analysis

Using an expected 50% sensitivity and 80% specificity against the composite reference standard, 10% precision and assuming that 40% of children will be defined as confirmed or unconfirmed TB, the minimum sample size was 241 children. Data were entered using the Voozanoo (Epiconcept, Paris, France) database and analysed with Stata version 13 (StataCorp, College Station, TX, USA). Patient and sample characteristics were reported by age category (<2 and ⩾2 years) and summarised using percentages. Continuous variables were presented as median and interquartile range (IQR). The *M. tuberculosis* detection yields from respiratory samples were calculated for smear microscopy, Xpert MTB/RIF, LJ and MGIT cultures, respectively.

The accuracy of urine AlereLAM and stool Xpert MTB/RIF was calculated against the microbiological and composite reference standards in an intention-to-diagnose approach (a test positive if any of the test results was positive and negative when none was positive). Patients without any stool or urine sample collected were excluded from the accuracy analysis of stool and urine, respectively. Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV), and positive likelihood ratio and negative likelihood ratio were presented with 95% confidence intervals overall, by age groups and by HIV status. Venn diagrams depicted the number of diagnosed children using different tests (Xpert MTB/RIF from respiratory samples and from stool, culture from any sample, and urine AlereLAM). κ analysis assessed the inter-reader analysis of urine AlereLAM [14].

Results

Patient characteristics

Of 238 inpatients, 19 were excluded for not meeting the defined study entry criteria (figure 1). Of the 219 children included in the analysis, 157 (71.7%) were aged <2 years, 31 (14.1%) were aged ⩾2 years and HIV-positive, and 31 (14.1%) were aged ⩾2 years and HIV-negative with severe malnutrition. Overall, 107 (48.9%) were female, the median (IQR) age was 16.4 (9.7–29.2) months and 72 (32.9%) were HIV-positive. Cough >2 weeks (183 (83.6%)) and reduced playfulness (205 (93.6%)) were the most common reported TB symptoms, and 20/202 (9.9%) children had a TB-suggestive chest radiograph, as interpreted by the site clinician. After the second independent reading and the review of radiographs with discordant findings by a third reader, 31/200 (15.5%) had a chest radiograph interpreted as TB-suggestive. The majority of children (184 (84.0%)) had a diagnosis of severe malnutrition using either weight for height z-score <−3SD, presence of bilateral oedema or mid-upper arm circumference measure <115 mm (table 1).

Microbiological results

Overall, 213 children (97.3%) had at least one respiratory sample and 211 (96.3%) had two samples collected. Respiratory samples could not be collected in six children who died within 24 h after enrolment (table 2). All children were able to provide at least one stool sample. 72 children (32.9%) died during follow-up; 23 (31.9%) had necropsy samples collected. There were 16/424 (3.8%) respiratory samples positive by either smear microscopy, Xpert MTB/RIF or culture. The detection yields by microscopy, LJ culture, MGIT culture and Xpert MTB/RIF assay were 2.4% (10/424), 2.7% (11/403), 2.9% (11/375) and 2.8% (12/423), respectively, after excluding bacterial contamination, non-tuberculous mycobacteria (NTM) growth and invalid Xpert MTB/RIF results (table 3). At the patient level, 8/213 (3.8%) children had at least one respiratory sample positive with Xpert MTB/RIF: 3.2% (5/154) in the group aged <2 years *versus* 5.1% (3/59) for those aged ⩾2 years (p=0.688). 20 children had extrapulmonary samples investigated for TB: blood (n=15), cerebrospinal fluid (n=2), ascites (n=2) and pleural fluid (n=1). All samples were negative. Bacterial blood culture was performed in 67 children and nine (13.4%) of them had bacterial growth: n=3 *Staphylococcus aureus*, n=3 *Salmonella species*, n=2 *Klebsiella pneumonia* and n=1 *Haemophilus influenzae*. Of the 23 patients with necropsy samples, four (17.4%) had *M. tuberculosis*-positive culture results, including two who were not previously detected from respiratory samples.

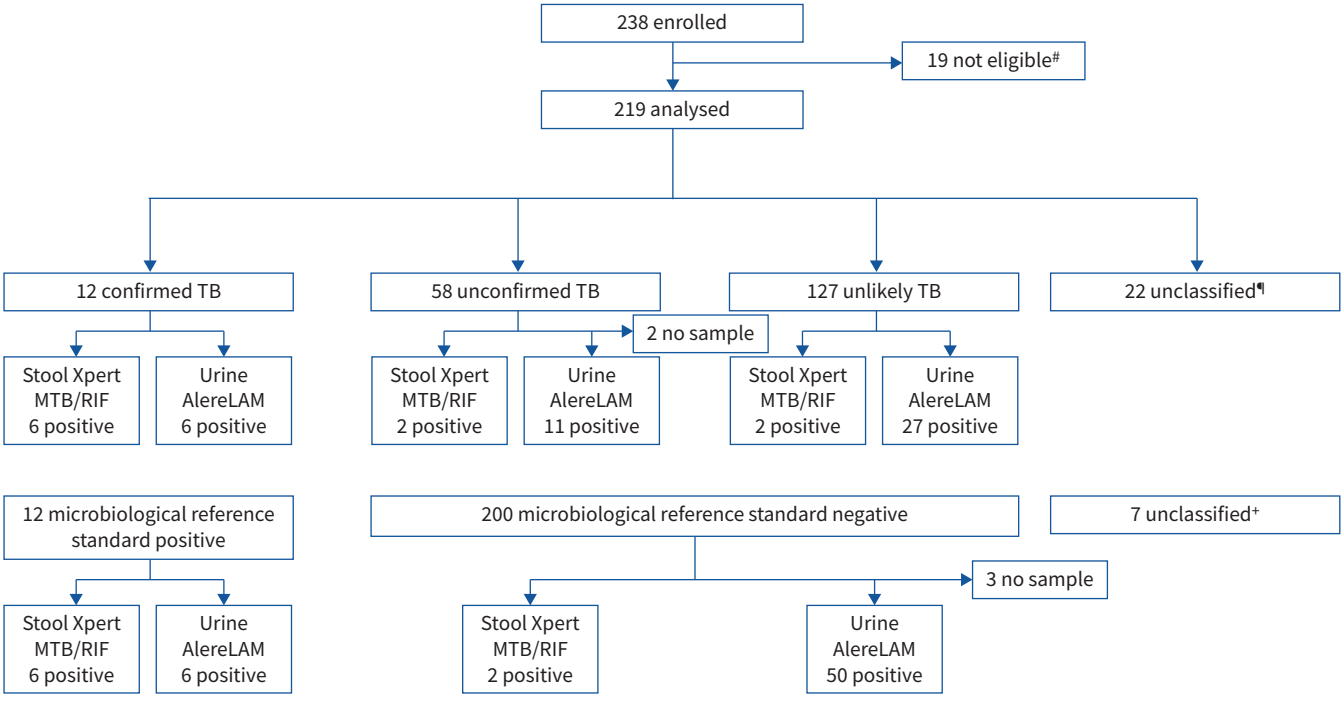

FIGURE 1 Study profile. #: nine children did not have risk factor criteria of disseminated TB (<2 years, HIV-positive or severe malnutrition) and 10 children had only one tuberculosis (TB)-suggestive criteria; ¶: in 22 children it was impossible to apply the case definition due to lack of data, the main reason (20/22) being early death of the children; +: six children had no respiratory sample collected because they died within 24 h after enrolment and one had an unclassified microbiological reference standard because the child had only one negative sample.

TABLE 1 Demographics and clinical characteristics of enrolled children according to age groups

	Overall (N=219)	<2 years (N=157)	⩾2 years (N=62)	p-value
Female	107 (48.9)	74 (47.1)	33 (53.2)	0.455
Age, months	16.4 (9.7–29.2)	13.0 (7.9–17.1)	50.7 (36.4–73.0)	
HIV-positive	72 (32.9)	41 (26.1)	31 (50.0)	0.002
CD4 cells, mm^{-3} (N=40)	262.5 (17–868)	587.0 (69–1000)	162.0 (13–840)	
BCG vaccination	204 (93.1)	145 (92.4)	59 (95.2)	0.695
BCG scar	172 (84.3)	127 (87.8)	45 (76.3)	0.202
TB contact history				
With confirmed TB case	8 (3.7)	6 (3.8)	2 (3.2)	0.595
With non-confirmed TB case	10 (4.6)	8 (5.1)	2 (3.2)	0.425
Received antibiotics before enrolment	191 (87.2)	136 (86.6)	55 (88.7)	0.823
Reported TB-suggestive symptoms				
Cough >2 weeks	183 (83.6)	132 (84.1)	51 (82.3)	0.744
Fever >7 days	147 (67.1)	104 (66.2)	43 (69.3)	0.659
Night sweats >2 weeks	51 (23.3)	35 (22.3)	16 (25.8)	0.579
Fatigue/reduced playfulness >2 weeks	205 (93.6)	145 (92.4)	60 (96.8)	0.229
Failure to gain weight >3 months	100 (45.7)	73 (46.5)	27 (43.5)	0.693
Nutritional status				
Weight for height <–2SD	162 (74.0)	124 (79.0)	38 (61.3)	0.007
Weight for height <–3SD	135 (83.3)	102 (82.2)	34 (89.5)	
MUAC[#] <115 mm	106/169 (62.7)	93/130 (71.5)	13/39 (33.3)	<0.001
Bilateral pedal oedema	38 (17.4)	15 (9.6)	23 (37.1)	<0.001
Body temperature >38°C	76 (34.7)	53 (33.8)	23 (37.1)	0.640
Peripheral oxygen saturation <90%	13 (5.9)	12 (7.6)	1 (1.6%)	0.089
Peripheral lymphadenopathy	37 (16.9)	26 (16.6)	11 (17.7)	0.834
Positive TST[¶]	7/195 (3.6)	5/145 (3.4)	2/50 (4.0)	0.574
Chest radiography findings[+]				0.713
Normal	89/202 (44.1)	64/149 (42.9)	25/53 (47.2)	
Suggestive of TB[§]	20/202 (9.9)	14/149 (9.4)	6/53 (11.3)	
Abnormal not suggestive of TB	93/202 (46.0)	71/149 (47.6)	22/53 (41.5)	

Data are presented as n (%), median (interquartile range) or n/N (%), unless otherwise stated. BCG: bacille Calmette–Guérin; TB: tuberculosis; MUAC: mid-upper arm circumference measure; TST: tuberculin skin test. [#]: measured in all children 6 months to 5 years of age; [¶]: defined by an induration of ⩾5 mm in HIV-positive/exposed or malnourished children and ⩾10 mm for all other children; [+]: primary clinician's interpretation; [§]: cavitation, pleural effusion, miliary, mediastinal adenopathy and lobar pneumonia.

TABLE 2 Sample characteristics per patient and age category

	Overall (N=219)	<2 years (N=157)	⩾2 years (N=62)	p-value
Respiratory samples				
Two samples	211 (96.3)	154 (98.1)	57 (91.9)	0.029
NPA+GA	187 (88.6)	151 (98.0)	36 (63.3)	<0.001
Sputum+IS/sputum	13 (6.2)	0 (0)	13 (22.8)	<0.001
Sputum/IS+GA	8 (3.8)	1 (0.6)	7 (12.3)	<0.001
GA+GA	3 (1.4)	2 (1.3)	1 (1.7)	0.613
One sample only	2 (0.9)	0 (0.0)	2 (3.2)	
Sputum	2 (0.9)	0 (0.0)	2 (3.2)	
Stool samples				
Two samples	205 (93.6)	151 (96.2)	54 (87.1)	0.027
One sample only	14 (6.4)	6 (3.8)	8 (12.9)	0.027
Urine samples				
Two samples	204 (93.1)	149 (94.9)	55 (88.7)	0.135
One sample only	12 (5.5)	6 (3.8)	6 (9.7)	0.103

Data are presented as n (%), unless otherwise stated. NPA: nasopharyngeal aspirate; GA: gastric aspirate; IS: induced sputum.

TABLE 3 Mycobacteriological results from various respiratory samples using different detection methods

	All samples	Sputum	Induced sputum	Gastric aspirate	Nasopharyngeal aspirate
Microscopy	N=424	N=26	N=10	N=201	N=187
Negative	414 (97.6)	26 (100)	10 (100)	196 (97.5)	182 (97.3)
Scanty	4 (0.9)	0	0	1 (0.5)	3 (1.6)
1+	3 (0.7)	0	0	2 (1.0)	1 (0.5)
2+	2 (0.5)	0	0	2 (1.0)	0
3+	1 (0.2)	0	0	0	1 (0.5)
LJ culture	N=424	N=26	N=11	N=197	N=190
Negative	392 (92.4)	24 (92.3)	10 (90.9)	179 (90.9)	179 (94.2)
MTBc-positive	11 (2.6)	2 (7.7)	0	5 (2.5)	4 (2.1)
Contaminated	21 (4.9)	0	1 (9.1)	13 (6.6)	7 (3.7)
MGIT culture	N=424	N=26	N=11	N=197	N=190
Negative	364 (85.8)	22 (84.6)	9 (81.8)	164 (83.2)	169 (88.9)
MTBc	11 (2.6)	1 (3.8)	0	6 (3.0)	4 (2.1)
NTM	2 (0.5)	0	0	2 (1.0)	0
Contaminated	47 (11.1)	3 (11.5)	2 (18.8)	25 (12.7)	17 (8.9)
Xpert MTB/RIF	N=424	N=26	N=10	N=201	N=187
Negative	411 (96.9)	26 (100)	10 (100)	192 (95.5)	183 (97.9)
MTBc	12 (2.8)	0	0	8 (3.9)	4 (2.1)
RIF-sensitive	12 (100)	0	0	8 (100)	4 (100)
Invalid	1 (0.2)	0	0	1 (0.5)	0

Data are presented as n (%), unless otherwise stated. LJ: Löwenstein–Jensen; MTBc: *Mycobacterium tuberculosis* complex; MGIT: Mycobacteria Growth Indicator Tube; NTM: non-tuberculous mycobacteria; RIF: rifampicin.

Out of 424 stool samples tested by Xpert MTB/RIF, 409 (96.5%) had a negative result and 15 (3.5%) had *M. tuberculosis* detected. None detected rifampicin resistance. Of 219 children with at least one stool sample, nine (4.1%) had *M. tuberculosis* detected. Out of 420 urine samples tested with AlereLAM, 89 (21.2%) had a positive result. Of the 89 positive results, 64 (71.9%), 10 (11.2%), eight (9.0%) and

TABLE 4 Microbiological reference standard and consensus case definition

	Overall	<2 years	⩾2 years
Microbiological reference standard	N=212	N=154	N=58
Positive reference standard (any positive)	12 (5.7)	8 (5.2)	4 (6.9)
Culture-positive (LJ or MGIT)	8	5	3
Culture-negative and Xpert-positive	2	1	1
Culture- and/or Xpert-negative and necropsy-positive[#]	2	2	0
Consensus case definition	N=197[¶]	N=146	N=51
Confirmed TB	12 (6.1)	8 (5.5)	4 (7.8)
Unconfirmed TB	58 (29.4)	37 (25.3)	21 (41.2)
TST-positive and ⩾1 TB-suggestive criteria[+]	3	2	1
TST-negative or not done and ⩾2 suggestive criteria	55	35	20
⩾2 TB-suggestive criteria	8	5	3
⩾1 TB-suggestive symptom+suggestive chest radiography	21	15	6
⩾1 TB-suggestive symptom+TB treatment response	15	8	7
⩾1 TB-suggestive symptom+TB exposure	11	7	4
Unlikely TB	127 (64.5)	101 (69.2)	26 (51.0)

Data are presented as n (%) or n, unless otherwise stated. LJ: Löwenstein–Jensen; MGIT: Mycobacteria Growth Indicator Tube; TB: tuberculosis; TST: tuberculin skin test. [#]: an 8-month-old advanced HIV-positive child with negative culture and Xpert MTB/RIF results from gastric aspirate and nasopharyngeal aspirate clinically diagnosed and started on TB treatment but died after 1 week of treatment, and a HIV-exposed 5-month-old child with clinical suspicion of pneumocystosis, negative culture and Xpert results from gastric aspirate, nasopharyngeal aspirate and stool and negative LAM results who died 2 weeks after starting both high-dose cotrimoxazole and TB therapy; [¶]: in 22 children it was impossible to apply any case definition due to lack of data, the main reason (20/22) being early death of the children; [+]: TB-suggestive symptoms or chest radiography suggestive of TB or positive clinical response to TB treatment.

TABLE 5 Diagnostic accuracy of stool Xpert MTB/RIF against different reference standards

	Sensitivity, n/N, % (95% CI)	Specificity, n/N, % (95% CI)	PPV, n/N, % (95% CI)	NPV, n/N, % (95% CI)	LR+, % (95% CI)	LR−, % (95% CI)
Confirmed TB (microbiological reference)						
Overall	6/12, 5.0 (21.1–78.9)	198/200, 99.0 (96.4–99.9)	6/8, 75.0 (34.9–96.8)	198/204, 97.1 (93.7–98.9)	50.0 (11.2–222.0)	0.5 (0.3–0.9)
First stool only	5/12, 41.7 (15.2–72.3)	198/200, 99.0 (96.4–99.9)	5/7, 71.4 (29.0–96.3)	198/205, 96.6 (93.1–98.6)	41.7 (9.0–193.0)	0.6 (0.4–0.9)
Two stools	6/11, 54.5 (23.4–83.2)	191/193, 99.0 (96.3–99.9)	6/8, 75.0 (34.9–96.8)	191/196, 97.4 (94.2–99.2)	48.2 (10.9–214.1)	0.5 (0.3–0.9)
<2 years[#]	4/8, 50.0 (15.7–84.3)	146/146, 100 (97.5–100)	4/4, 100 (39.8–100)	146/150, 97.3 (93.3–99.3)	NA	0.5 (0.25–1.0)
⩾2 years[#]	2/4, 50.0 (6.8–93.2)	52/54, 96.3 (87.2–99.5)	2/4, 50.0 (6.8–93.2)	52/54, 96.3 (87.2–99.5)	13.5 (2.5–72.2)	0.5 (0.2–1.4)
HIV-positive[#]	1/4, 25.0 (0.6–80.1)	65/65, 100 (94.5–100)	1/1, 100 (2.5–100)	65/68, 94.6 (87.6–99.1)	NA	0.8 (0.4–1.3)
HIV-negative[#]	5/8, 62.5 (24.5–91.5)	128/130, 98.5 (94.5–99.8)	5/7, 71.4 (29.0–96.3)	128/131, 97.7 (93.4–99.5)	40.6 (9.3–177.8)	0.4 (0.2–0.9)
Confirmed and unconfirmed TB (consensus case definition)						
Overall	8/70, 11.4 (5.1–21.3)	127/127, 100 (97.1–100)	8/8, 100 (63.1–100)	127/189, 67.2 (60.0–73.8)	NA	0.9 (0.8–1.0)
First stool only	7/70, 10.0 (4.1–19.5)	127/127, 100 (97.1–100)	7/7, 100 (59.0–100)	127/190, 66.8 (59.7–73.5)	NA	0.9 (0.8–1.0)
Two stool samples	8/68, 11.8 (0.5–21.9)	124/124, 100 (97.1–100)	8/8, 100 (63.0–100)	124/184, 67.4 (60.1–74.1)	NA	0.9 (0.8–1.0)
<2 years[#]	4/45, 8.9 (2.5–21.2)	101/101, 100 (96.4–100)	4/4, 100 (39.8–100)	101/142, 71.1 (62.9–78.4)	NA	0.9 (0.8–1.0)
⩾2 years[#]	4/25, 16.0 (4.5–36.1)	26/26, 100 (86.8–100)	4/4, 100 (39.8–100)	26/47, 55.3 (40.1–69.8)	NA	0.8 (0.7–1.0)
HIV-positive[#]	1/21, 4.8 (1.2–23.8)	39/39, 100 (91.1–100)	1/1, 100 (2.5–100)	39/59, 66.1 (52.6–77.9)	NA	0.9 (0.9–1.0)
HIV-negative[#]	7/49, 14.3 (5.9–27.2)	82/82, 100 (95.6–100)	7/7, 100 (59.0–100)	82/124, 66.1 (57.1–74.4)	NA	0.9 (0.8–1.0)

PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio; TB: tuberculosis; NA: not applicable. [#]: all samples used for these analyses.

seven (7.9%) were of grade 1+, 2+, 3+ and 4+, respectively. Of 216 children with at least one urine sample, 59 (26.3%) had a positive urine AlereLAM result.

Diagnostic accuracy of stool Xpert MTB/RIF and urine LAM

12 (5.5%) children had a positive microbiological reference standard and 58 (26.5%) were classified as unconfirmed TB (table 4). Using a microbiological reference standard, the sensitivity, specificity, PPV and NPV of stool Xpert MTB/RIF was 50.0% (6/12), 99.0% (198/200), 75.0% (6/8) and 97.1% (198/204), respectively. The sensitivity remained the same in the two age groups and was reduced among HIV-positive children (1/4 (25%)). Against the composite reference standard, the sensitivity and NPV of stool Xpert MTB/RIF was reduced to 11.4% (8/70) and 67.2% (127/189), respectively, with an increase of specificity (127/127) and PPV (8/8) to 100% (table 5).

Using the microbiological reference standard, the sensitivity, specificity, PPV and NPV of AlereLAM (all samples included) was 50.0% (6/12), 74.6% (147/197), 10.7% (6/56) and 96.1% (147/153), respectively. Specificity increased to 86.9% (166/191) when using two urine samples. The sensitivity remained the same regardless of age group and HIV status with a specificity <80%. Against a composite reference standard, the sensitivity was reduced to 25.0% (17/68) without any increase of specificity (73.2% (93/127)). The sensitivity did not change much according to HIV status (table 6).

Among the 275 AlereLAM tests (from 141 children) read by two independent nurses, the positive and negative agreements were 26/33 (78.8%) and 242/249 (97.2%) with κ=0.867 (0.771–0.964). Of the 50

TABLE 6 Diagnostic accuracy of urine AlereLAM overall against different reference standards

	Sensitivity, n/N, % (95% CI)	Specificity, n/N, % (95% CI)	PPV, n/N, % (95% CI)	NPV n/N, % (95% CI)	LR+, % (95% CI)	LR−, % (95% CI)
Confirmed TB (microbiological reference)						
Overall	6/12, 50.0 (21.1–78.9)	147/197, 74.6 (67.9–80.5)	6/56, 10.7 (4.0–21.9)	147/153, 96.1 (91.7–98.5)	2.0 (1.1–3.6)	0.7 (0.4–1.2)
First urine only	5/12, 41.7 (15.2–72.3)	159/197, 80.7 (74.5–86.0)	5/43, 11.6 (3.9–25.1)	159/166, 95.8 (91.5–98.3)	2.2 (1.0–4.5)	0.7 (0.4–1.2)
Two urine samples	5/12, 41.7 (15.2–72.3)	166/191, 86.9 (81.2–91.3)	5/30, 16.7 (5.6–34.7)	166/173, 95.9 (91.8–98.4)	3.2 (1.5–6.8)	0.7 (0.4–1.1)
<2 years[#]	4/8, 50.0 (15.7–84.3)	105/144, 72.9 (64.9–79.8)	4/43, 9.3 (2.6–22.1)	105/109, 96.3 (90.9–99.0)	1.8 (0.9–3.9)	0.7 (0.3–1.4)
⩾2 years[#]	2/4, 50.0 (6.8–93.2)	42/53, 79.2 (65.9–89.1)	2/13, 15.4 (1.9–45.4)	42/44, 95.4 (84.5–99.4)	2.4 (0.8–7.3)	0.6 (0.2–1.7)
HIV-positive[#]	2/4, 50.0 (6.8–93.2)	50/63, 79.4 (67.3–88.5)	2/15, 13.3 (1.7–40.5)	50/52, 96.1 (86.8–99.5)	2.4 (0.8–7.2)	0.6 (0.2–1.7)
HIV-negative[#]	4/8, 50.0 (15.7–84.3)	93/129, 72.1 (63.5–79.6)	4/40, 10.0 (2.8–23.7)	93/97, 95.9 (89.8–98.9)	1.8 (0.8–3.8)	0.7 (0.3–1.4)
Confirmed and unconfirmed TB (consensus case definition)						
Overall	17/68, 25.0 (15.3–37.0)	93/127, 73.2 (64.6–80.7)	17/51, 33.3 (20.8–47.9)	93/144, 64.6 (56.2–72.4)	0.4 (0.2–0.5)	1.0 (0.9–1.2)
First urine only	13/68, 19.1 (10.6–30.5)	101/127, 79.5 (71.5–86.2)	13/39, 33.3 (19.1–50.2)	101/156, 64.7 (56.7–72.2)	0.9 (0.5–1.7)	1.0 (0.9–1.2)
Two urine samples	10/67, 14.9 (7.4–25.7)	106/124, 85.5 (78.0–91.2)	10/28, 35.7 (18.6–55.9)	106/163, 65.0 (57.2–73.3)	1.0 (0.5–2.1)	1.0 (0.9–1.1)
<2 years[#]	12/43, 27.9 (15.3–43.7)	75/101, 74.3 (64.6–82.4)	12/38, 31.6 (17.5–48.6)	75/106, 70.7 (61.1–79.2)	1.1 (0.6–1.9)	1.0 (0.8–1.2)
⩾2 years[#]	5/25, 20.0 (6.8–40.7)	18/26, 69.2 (48.2–85.7)	5/13, 38.5 (13.9–68.4)	18/38, 47.4 (31.0–64.2)	0.6 (0.2–1.7)	1.2 (0.9–1.6)
HIV-positive[#]	5/20, 25.0 (8.7–49.1)	30/39, 76.9 (60.7–88.9)	5/14, 35.7 (12.8–64.9)	30/45, 66.7 (51.0–80.0)	1.1 (0.4–2.8)	1.0 (0.7–1.3)
HIV-negative[#]	12/48, 25.0 (13.6–39.6)	58/82, 70.7 (59.6–80.3)	12/36, 33.3 (18.6–51.0)	58/94, 61.7 (51.1–71.5)	0.8 (0.5–1.5)	1.1 (0.9–1.3)

PPV: positive predictive value; NPV: negative predictive value; LR likelihood ratio; TB: tuberculosis. [#]: all samples used for these analyses.

children with false-positive AlereLAM results, 39 (78.0%) had low grade (1+) results and 25 (50.0%) had only one positive result out of two tests performed; of the six "true-positive" cases, five (83.3%) had higher grade positive results (>1+) confirmed in both urine specimens. Bacterial pathogens were isolated in the urine of 16/50 (32.0%) children with a false-positive AlereLAM result; only 1/6 (16.7%) with a "true-positive" result (p=0.402).

Among the 15 children with a final diagnosis of confirmed TB by any sample (including stool) tested with either culture or Xpert MTB/RIF, Xpert MTB/RIF from stool, from respiratory samples and from both sample types detected nine (60.0%), eight (53.3%) and 11 (73.3%) of them, respectively. The combination of urine AlereLAM and Xpert MTB/RIF from stool identified 11/15 confirmed cases (73.3%) but only 7/59 (11.9%) children with a positive AlereLAM result were microbiologically confirmed (figure 2).

Discussion

In this prospective cohort of children at risk of developing disseminated TB, non-sputum-based diagnostic methods showed modest diagnostic accuracy. Stool Xpert MTB/RIF detected half of the confirmed cases with high specificity. The sensitivity was slightly lower than the pooled sensitivity (67%, 95% CI 52–79%) reported in a recent meta-analysis and did not increase among HIV-positive children as suggested by the review [7]. Variable sensitivity across studies could be explained by different risk cohorts and the use of different stool processing methods [7, 15–18]. Our results are in the range of sensitivity (32–89%) and specificity (95–99%) of studies using additional PBS and centrifugation [15–17, 19–21]. The modest sensitivity is balanced by excellent feasibility (almost 100% collection in our study). Further optimisation of this method is expected in the near future with improved specimen processing and using the more

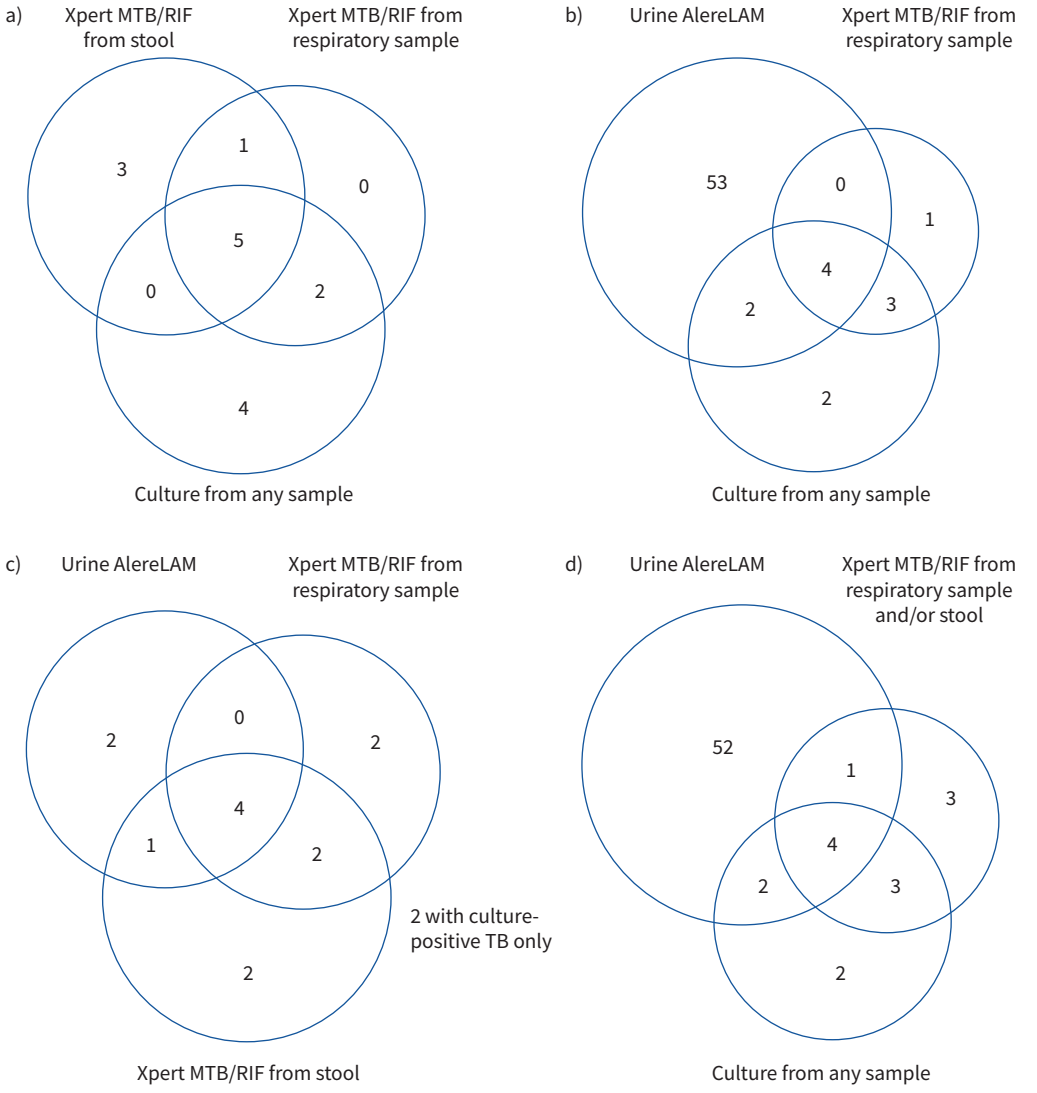

FIGURE 2 Venn diagrams showing intersection of positive results from AlereLAM, Xpert TB/RIF and mycobacterial culture. a) Between Xpert MTB/RIF from stool, Xpert from respiratory samples and mycobacterial culture from any sample. b) Between urine AlereLAM, Xpert MTB/RIF from respiratory samples and mycobacterial culture from any sample. c) Between urine AlereLAM, Xpert MTB/RIF from stool and Xpert MTB/RIF from respiratory samples among patients with confirmed tuberculosis (TB) by mycobacterial culture or Xpert MTB/RIF results from any sample. d) Between urine AlereLAM, Xpert MTB/RIF from respiratory sample and/or stool, and culture from any sample.

sensitive Xpert Ultra cartridge [22–25]. A recent study has shown an important increase in sensitivity with Xpert Ultra (58.6%) compared with Xpert MTB/RIF (37.9%) from stool in children, but with some loss in specificity (from 100% to 88.1%), mainly due to the difficulties in interpreting the trace positive results [26].

For urine AlereLAM, we could not detect increased sensitivity when selecting children at increased risk of disseminated TB compared with what was previously reported among children with presumptive TB (28–48%) or HIV-positive (43–50%) in Africa [6, 17–27]. However, a recent study in India reported higher sensitivity (73%) with the same assay among children with both clinical presumption of TB and recent TB contact history [28]. Like previous evaluations in children (60–97%), we reported a low specificity (74.1%) [6, 17, 29, 30]. The specificity increased to 86.9% with two samples collected, but two urine LAM tests would unlikely be used in routine practice in low-resource settings. The low specificity is usually attributed to bacterial contamination, especially with the use of urine bags for specimen collection in young children, and corynebacterial organisms found on the skin have LAM-like lipoglycans [31–36].

Although we used catheterisation during sampling in our study, we still encountered a high proportion (20%) of bacterial contamination. Unfortunately, we did not have the capacity to identify bacteria that also include LAM-like lipoglycans.

The specificity of urine AlereLAM may also be weakened by the lack of a proper reference standard if based on respiratory samples only. In our study, necropsy samples, when available, contributed to 2/12 (16.7%) microbiological reference standard positive results. The majority of the false-positive AlereLAM results were of low grade, which could indicate a low bacillary load and may support the hypothesis that these samples could not be detected by the microbiological reference standard. In order to improve upon this, a composite reference was used with very limited benefit on the specificity. One of the limitations of this composite reference standard was that 10% of cases could not be properly classified due to early death (20/22 (90.9%)). Although a high proportion of children had been on antibiotics before inclusion, it is unlikely that they were exposed to antibiotics with antimycobacterial effects such as fluoroquinolones.

The use of the urine LAM-based assay in children would require optimisation to improve both the sensitivity and specificity of the assay, which should include enhanced sample collection to prevent bacterial contamination in young children. Emerging data using the new Fujifilm SILVAMP TB LAM ("FujiLAM"; Fujifilm, Tokyo, Japan) suggest increased sensitivity compared with AlereLAM (65% *versus* 31%) but with minimal improvements in specificity [35, 37–39]. Further prospective studies to show the value of FujiLAM in children are needed.

The major study limitation was the low number of microbiologically confirmed TB cases, which affected the precision of the primary end-point. Despite intensive sampling and testing, we only confirmed TB in 6.8% (15/219) of children, including three detected on stool only. This very low prevalence was unexpected but may be explained by the low TB exposure reported; only 3.7% of children had a recent TB contact history with a bacteriologically confirmed case and only 3.6% had a positive TST result. The low mycobacterial culture yield might have been affected by rigorous attempts to limit bacterial contamination, potentially at the expense of *M. tuberculosis* recovery rate in paucibacillary samples. Another limitation was the absence of fine-needle aspiration biopsy in case of peripheral lymphadenitis.

In conclusion, the study provided additional evidence for the use of the Xpert MTB/RIF assay from stool for the diagnosis of TB in highly vulnerable children. Considering the difficulties in obtaining other respiratory samples from this group, our findings support the WHO recommendation that stool is an important respiratory sample to also consider for childhood TB diagnosis [40]. Further evaluation using the more sensitive Xpert Ultra cartridge is needed. The urine AlereLAM assay had poor diagnostic accuracy and little diagnostic value in this high-risk group. Additional evidence using the new FujiLAM assay is warranted to assess potentially improved diagnostic accuracy.

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