Title

A Prospective Evaluation of Xpert MTB/RIF Ultra for Childhood Pulmonary Tuberculosis in Uganda

Journal

Journal of the Pediatric Infectious Diseases Society, 10(5)

ISSN

2048-7193

Authors

Jaganath, Devan
Wambi, Peter
Reza, Tania F
et al.

Publication Date

2021-05-28

DOI

10.1093/jpids/piaa159

Peer reviewed

Journal of the Pediatric Infectious Diseases Society

ORIGINAL ARTICLE

A Prospective Evaluation of Xpert MTB/RIF Ultra for Childhood Pulmonary Tuberculosis in Uganda

Devan Jaganath,[1,2,3,a] Peter Wambi,[4,a] Tania F. Reza,[2,3] Jascent Nakafeero,[4] Ernest O. Aben,[4] Emma Kiconco,[4] Gertrude Nannyonga,[4] Moses Nsereko,[4] Moorine P. Sekadde,[5] Mary Mudiope,[6] Midori Kato-Maeda,[2,3] Jeffrey Starke,[7] Alfred Andama,[8] Swomitra Mohanty,[9] Eric Wobudeya,[4] and Adithya Cattamanchi,[2,3,10]

[1]Division of Pediatric Infectious Diseases, University of California–San Francisco, San Francisco, California, USA, [2]Division of Pulmonary and Critical Care Medicine, University of California–San Francisco, San Francisco, California, USA, [3]Center for Tuberculosis, University of California–San Francisco, San Francisco, California, USA, [4]Mulago National Referral Hospital, Kampala, Uganda, [5]National TB and Leprosy Program, Ministry of Health, Kampala, Uganda, [6]Infectious Diseases Institute, Kampala, Uganda, [7]Division of Pediatric Infectious Diseases, Baylor College of Medicine, Houston, Texas, USA, [8]Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda, [9]Departments of Chemical Engineering and Materials Science Engineering, University of Utah, Salt Lake City, Utah, USA, and [10]Department of Medicine, Center for Vulnerable Populations, University of California–San Francisco, San Francisco, California, USA

Background. Xpert MTB/RIF Ultra (Xpert Ultra) has improved the sensitivity to detect pulmonary tuberculosis (TB) in adults. However, there have been limited prospective evaluations of its diagnostic accuracy in children.

Methods. We enrolled children undergoing assessment for pulmonary TB in Kampala, Uganda, over a 12-month period. Children received a complete TB evaluation and were classified as Confirmed, Unconfirmed, or Unlikely TB. We calculated the sensitivity and specificity of Xpert Ultra among children with Confirmed vs Unlikely TB. We also determined the diagnostic accuracy with clinical, microbiological, and extended microbiological reference standards (MRSs).

Results. Of the 213 children included, 23 (10.8%) had Confirmed TB, 88 (41.3%) had Unconfirmed TB, and 102 (47.9%) had Unlikely TB. The median age was 3.9 years, 13% were HIV-positive, and 61.5% were underweight. Xpert Ultra sensitivity was 69.6% (95% confidence interval [CI]: 47.1-86.8) among children with Confirmed TB and decreased to 23.4% (95% CI: 15.9-32.4) with the clinical reference standard. Specificity was 100% (95% CI: 96.4-100) among children with Unlikely TB and decreased to 94.7% (95% CI: 90.5-97.4) with a MRS. Sensitivity was 52.9% (95% CI: 35.1-70.2) and specificity 95.5% (95% CI: 91.4-98.1) with the extended MRS. Of the 26 positive Xpert Ultra results, 6 (23.1%) were "Trace-positive," with most (5/6) occurring in children with Unconfirmed TB.

Conclusions. Xpert Ultra is a useful tool for diagnosing pulmonary TB in children, but there remains a need for more sensitive tests to detect culture-negative TB.

Key words. child; diagnosis; tuberculosis; Xpert MTB/RIF Ultra.

Despite advances in rapid molecular testing for pulmonary tuberculosis (TB), the microbiologic confirmation of TB in children remains a challenge and has contributed to high morbidity and mortality [1, 2]. Young children in particular have paucibacillary disease, limiting the performance of sputum-based molecular testing [3]. Xpert MTB/RIF (Cepheid, Sunnyvale, USA) is a commonly used semiautomated nucleic acid amplification test (NAAT) with high specificity (98%) [4] but overall has a modest 62% sensitivity in children compared with 85% in adults [4, 5]. The inability to accurately diagnose TB in children delays timely initiation of treatment and contributes to the 55% case detection gap for pediatric TB worldwide [1].

To lower the limit of detection and improve sensitivity, the next generation Xpert MTB/RIF Ultra ("Xpert Ultra") cartridge added 2 different multicopy gene targets, fully nested polymerase chain reactions (PCRs) for *rpoB* and IS*6110*, and a larger reaction chamber [6, 7]. A multicenter study demonstrated a 17% increase in sensitivity compared with Xpert MTB/RIF in adults with smear-negative TB [8]; consequently, the World Health Organization (WHO) has endorsed Xpert Ultra for first-line testing for pulmonary TB disease [7]. However, the few studies that have assessed the diagnostic accuracy of Xpert Ultra in children have key limitations. First, previous studies have been retrospective and used banked specimens [9–13] or invasive sample types such as bronchoalveolar lavage [12]. Second, previous studies used only a microbiological reference standard (MRS) [9, 10], which could inflate sensitivity estimates as only 24% of children with TB are culture positive [14]. Given

Received 17 August 2020; editorial decision 24 November 2020; accepted 1 December 2020; Published online January 8, 2021.

[a]These authors contributed equally to this work.

Corresponding Author: Devan Jaganath, MD, MPH, Division of Pediatric Infectious Diseases, University of California, 550 16th St., 4th Floor, San Francisco, CA, USA. E-mail: devan.jaganath@ucsf.edu.

Journal of the Pediatric Infectious Diseases Society 2021;10(5):586–92

DOI: 10.1093/jpids/piaa159

the challenges of diagnosing TB in children, studies including longitudinal follow-up and multiple reference standards are needed to better understand the performance of Xpert Ultra for diagnosing childhood TB.

We prospectively enrolled children being evaluated for pulmonary TB in Kampala, Uganda, and followed children for up to 6 months to classify children as having Confirmed, Unconfirmed, or Unlikely TB based on clinical, radiographic, and microbiologic information in accordance with consensus recommendations [15]. Our objective was to determine the potential range of Xpert Ultra sensitivity and specificity estimates by using multiple reference standards.

METHODS

Study Design and Participants

We enrolled consecutive children less than 15 years old in Kampala, Uganda. The study was conducted at the Mulago National Referral Hospital Pediatric TB clinic from November 2018 to November 2019. Participants were recruited from inpatient and outpatient wards at Mulago Hospital as well as community clinics and hospitals around Kampala. We included children who had a cough for at least 1 week and at least 2 of the following: (1) unexplained weight loss or failure to thrive, (2) unexplained fever for at least 1 week, (3) unexplained lethargy or reduced playfulness for at least 1 week, (4) an abnormal chest x-ray, or (5) contact with an individual with pulmonary TB disease. Children were excluded if they were initiated on anti-TB treatment or prophylaxis or had completed anti-TB treatment within the past year. We also excluded participants who were unable to provide a respiratory specimen by any of the collection methods. Caregivers completed an informed consent, and children provided assent if aged 8 years or older. The study was approved by the Mulago Hospital Research and Ethics Committee, the Uganda National Council of Science and Technology, and the University of California, San Francisco, Institutional Review Board. The study was also conducted in accordance with the Standards for Reporting of Diagnostic Accuracy Studies (STARD) [16].

Clinical Procedures

At enrollment, trained clinical staff obtained a medical history and anthropometrics and conducted a physical exam to evaluate for signs and symptoms of pulmonary TB. Venipuncture was performed and blood was collected for HIV and cluster of differentiation 4 (CD4) testing using the national testing algorithm [17]. All children received an anteroposterior and lateral chest x-ray (CXR). Tuberculin skin testing (TST) was performed, with 5 units of tuberculin (Tubersol, Sanofi Pasteur, Lyon, France) injected intradermally and induration read at 48–72 hours.

We collected 2 specimens for smear microscopy, Xpert Ultra, and solid and liquid culture before the initiation of anti-TB treatment. If the child could not provide expectorated sputum, induced sputum, gastric aspirate, or nasopharyngeal aspirate was obtained by trained staff. If one method was unsuccessful for the second specimen, a different method was attempted. If still unsuccessful, the first specimen was used for smear and culture, and the remaining sediment was resuspended for Xpert Ultra testing. Beginning in July 2019, urine was also collected by spontaneous void or urine bag for Determine TB-lipoarabinomannan (LAM) (Alere, Waltham, MA, USA) testing.

Clinical providers made the decision to initiate anti-TB treatment based on the national childhood TB guidelines [18]. Xpert Ultra is an approved test in Uganda, and the result was made available to the provider. All children returned at 2 months regardless of treatment initiation, and a clinical examination and repeat CXR were performed. Children on anti-TB medication also returned at 6 months to evaluate treatment response. All children diagnosed with TB were also followed up by the treating facility per the national guidelines, and adherence was assessed at biweekly visits during the intensive phase and monthly during the continuation phase.

Laboratory Testing

All TB testing was done by trained study laboratory technologists at the Mulago National Referral Hospital TB Laboratory and the Makerere University Mycobacteriology Laboratory using standard protocols. For Xpert Ultra, a sample reagent was added to the respiratory sample at a 2:1 ratio, and the mixture was vortexed for 10–15 seconds and incubated for 15 minutes at room temperature. Two milliliters (2 mL) of the liquefied sample were then transferred to the Xpert MTB/RIF Ultra cartridge for testing in a 4-module GeneXpert instrument. Mycobacterial culture was performed by trained staff blinded to Xpert Ultra results. Respiratory specimens were digested and decontaminated using sodium hydroxide and *N*-acetyl-cysteine to a final concentration of 1.5%, neutralized with sterile phosphate-buffered solution, and centrifuged. The resulting sediment was resuspended in a sterile phosphate-buffered solution at pH 6.8. Lowenstein-Jensen (LJ) slant and mycobacterial growth indicator tube (MGIT) were inoculated with a 0.5 mL suspension, and fluorescent acid-fast bacillus (AFB) smear microscopy was performed with the decontaminated samples. MGIT tubes were incubated in a BACTEC MGIT 960 instrument (BD; Franklin Lakes, NJ, USA) for up to 42 days, and LJ slants were incubated at 37°C for up to 8 weeks. Positive cultures were confirmed for *Mycobacterium tuberculosis* complex using SD Bioline strips (SD MPT64 TB Ag kit, South Korea). Determine TB-LAM was performed with standard protocols using 60 μL of urine [19].

Definitions

Underweight was defined as a weight-for-age z-score <−2 if a child was under 5 years old, or body mass index < 18.5 kg/m^2 if a child was aged 5 years or above. CXRs were digitized and read by 2 independent readers using a standardized form [20]. Readers classified CXRs as normal or abnormal, and if abnormal, whether TB was likely or equivocal. A third reader was used for discrepant classifications. TST was defined as positive if the measured induration was ≥10 mm (≥5 mm if HIV positive). Determine TB-LAM was defined as positive if grade 1 or greater.

Index and Reference Standards

The index test was Xpert Ultra. An Xpert Ultra "trace" result was defined as positive per the WHO recommendations for children [7]. TB status was classified by 2 independent childhood TB experts as Confirmed intrathoracic TB (positive solid or liquid culture), Unconfirmed TB (negative cultures, but signs and symptoms of TB and clinical evidence of TB including response to anti-TB treatment), or Unlikely TB (negative cultures, no clinical evidence of TB, and no anti-TB treatment given or no improvement) in accordance with the National Institutes of Health (NIH) consensus definitions [15]. A third reviewer was used to resolve discrepant classifications. We excluded participants who could not be classified because they had a negative mycobacterial culture, were not started on treatment, and did not return at 2 months to assess clinical status.

For the primary analysis, we defined Confirmed TB as a positive solid or liquid culture and determined sensitivity and specificity among children with Confirmed and Unlikely TB, respectively. In order to present the range of potential bias in sensitivity and specificity estimates, we also used alternative reference standards that incorporated children with Unconfirmed TB [21]. The clinical reference standard (CRS) included children with Unconfirmed TB in the TB group and the MRS included Unconfirmed TB in the Not TB group. The extended MRS (eMRS) was similar to the MRS, except that children with Unconfirmed TB who had positive smear microscopy or Determine TB-LAM results were defined as having TB.

Statistical Analysis

Baseline clinical and demographic characteristics were described using summary statistics. The sensitivity, specificity,

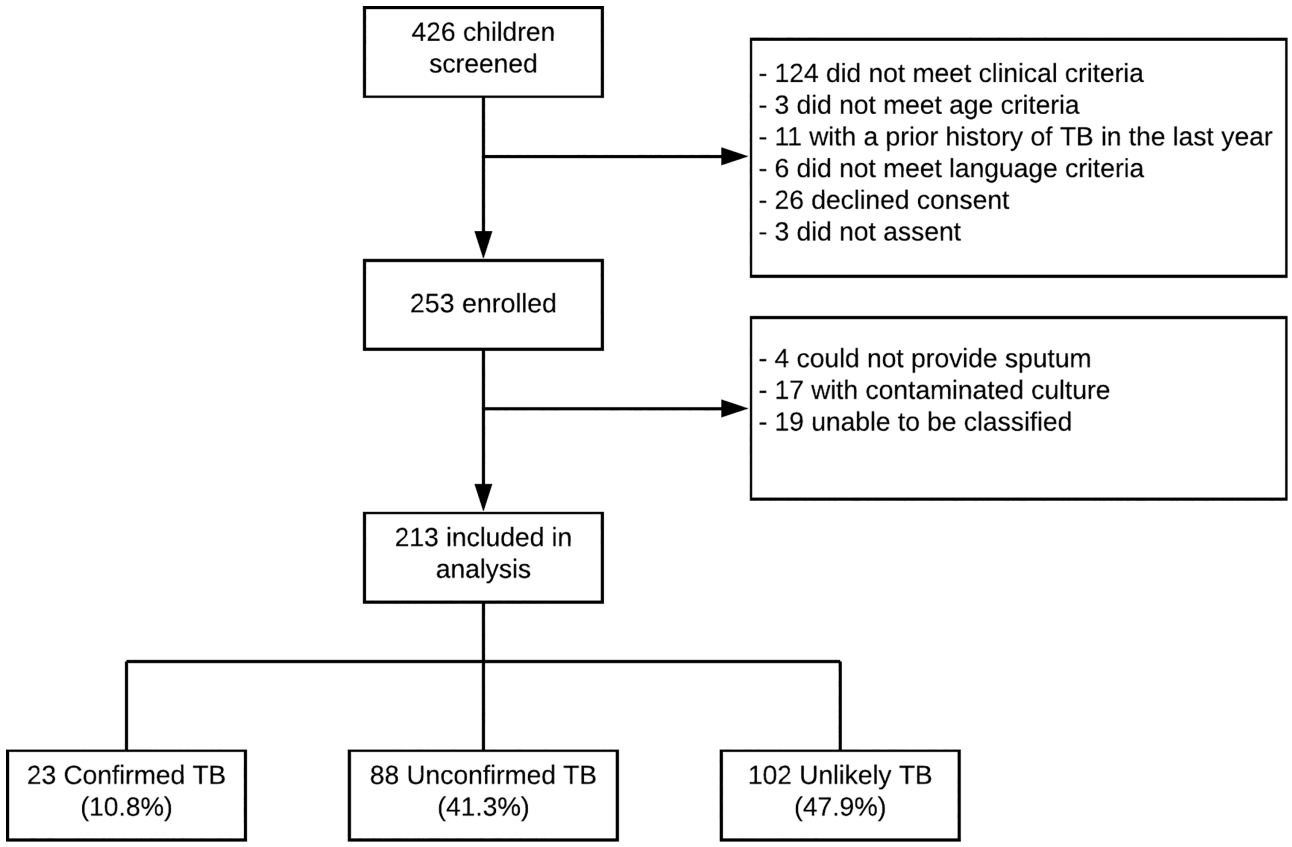

Figure 1. Flowchart of participants.

positive predictive value (PPV), and negative predictive value (NPV) of Xpert Ultra were calculated with exact binomial 95% confidence intervals (CIs) using the primary and secondary reference standards. In addition, sensitivity was evaluated in subgroups defined by age (<5 vs ≥5 years), AFB smear status, and induced/expectorated sputum vs gastric or nasopharyngeal aspirate and compared using proportion testing with 95% CIs for difference. Significance was defined as *P*-value < .05. Analyses were performed using STATA version 16 (StataCorp, College Station, TX, USA).

RESULTS

Participant Enrollment and Clinical Characteristics

We screened 426 children and enrolled 253 over 12 months (Figure 1). Forty children were excluded for an inability to obtain a respiratory sample (n = 4), an inability to classify TB status (n = 19), or contaminated liquid and solid cultures (n = 17). Of the 213 children included in the analysis, 23 (10.8%) had Confirmed TB, 88 (41.3%) had Unconfirmed TB, and 102 (47.9%) had Unlikely TB. Among children with Confirmed TB, the MGIT median time to positivity was 8 days (interquartile range [IQR] of 5-17), and, for LJ, the median time was 45.5 days (IQR: 26-57). Fourteen children (6.6%) were AFB smear positive (13 with Confirmed and 1 with Unconfirmed TB) and 13 children of 83 tested (15.7%) were Determine TB-LAM positive (3 with Confirmed and 10 with Unconfirmed TB).

Key demographic and sample characteristics are summarized in Table 1. The median age at enrollment was 3.9 years (IQR: 1.5-7), with 131 (61.5%) children under 5 years old. HIV status was available in 192 (90.1%) children, and 25 (13.0%; 95% CI: 8.6-18.6) were HIV positive, with a median CD4 count of 724 cells/μL (IQR 394-981). Nearly two-thirds of children were underweight (131/213, 61.5%, 95% CI: 54.6-68.1).

Table 1. Sample Characteristics (N = 213)

Characteristic	n (%) or Median (IQR)[a]
Age	3.9 years (1.5-7)
<5 years	131 (61.5)
5-9 years	63 (29.6)
10-14 years	19 (8.9)
Male sex	111 (52.1)
HIV Positive	25/192 (13.0)
CD4 cell count (cells/μL) (n =21)	724 (394-981)
BCG vaccine	188/200 (94.5)
TST positive[b]	97/202 (48.0)
CXR read at baseline	
Normal	102/203 (50.2)
Abnormal—Equivocal	54/203 (26.6)
Abnormal—Likely TB	47/203 (23.2)
History of TB Contact	95 (44.6)
Past history of pulmonary TB (>1 year ago)	3 (1.4)
Underweight	131/213 (61.5)
Two samples taken for Xpert Ultra and culture	157 (73.7)
Sample type used for Xpert Ultra	
Expectorated sputum	38 (17.8)
Induced sputum	87 (40.9)
Gastric aspirate	55 (25.8)
Nasopharyngeal aspirate	33 (15.5)
Same sample type for Xpert Ultra and culture	53 (24.9)
Xpert Ultra semi-quantitative results (n = 26)	
High	7 (26.9)
Medium	5 (19.2)
Low	4 (15.4)
Very Low	4 (15.4)
Trace	6 (23.1)
Rifampin resistance	0
Smear microscopy positive	14 (6.6)
Determine TB-LAM positive	13/83 (15.7)

Abbreviations: BCG, Bacillus Calmette–Guérin; CXR, chest X-ray; HIV, human immunodeficiency virus; LAM, lipoarabinomannan; TB, tuberculosis; TST, tuberculin skin testing; IQR, interquartile range.
[a]N = 213 unless missing data, and denominator indicated.
[b]Defined as ≥10mm if HIV negative, ≥5 mm if HIV positive.

Diagnostic Performance of Xpert Ultra

The most common Xpert Ultra sample type was induced sputum (40.9%), followed by gastric aspirate (25.8%) (Table 1). By age group, the most common sample type was induced sputum (50.4%) in children under 5 years, expectorated and induced sputum in children 5–10 years (34.9% and 28.6%, respectively), and expectorated sputum in children above 10 years (79.0%).

Among children with Confirmed vs Unlikely TB, the sensitivity and specificity of Xpert Ultra were 69.6% (95% CI: 47.1-86.8) and 100% (95% CI: 96.4-100), respectively (Figure 2 and Supplementary Table 1). The PPV was 100% (95% CI: 79.4-100), and the NPV was 93.6% (95% CI: 87.2-97.4). Utilizing the CRS, the sensitivity decreased to 23.4% (95% CI: 15.9-32.4), and, with the MRS, specificity decreased to 94.7% (95% CI: 90.5-97.4). When smear microscopy and Determine TB-LAM results were considered (eMRS), sensitivity was 52.9% (95% CI: 35.1-70.2) and specificity was 95.5% (95% CI: 91.4-98.1). There were no cases of rifampin resistance.

In subgroup analyses (Table 2), Xpert Ultra was more sensitive in AFB smear-positive children than in AFB smear-negative children (92.3% vs 40%, +52.3% difference, 95% CI: 18.7-85.9, *P* = .01). The sensitivity of Xpert Ultra was lower in children less than 5 years old, underweight children, and gastric/nasopharyngeal aspirate specimens, but results were not statistically significant.

Trace Results

Of the 26 positive Xpert Ultra results, 6 (23.1%) were "trace" positive (Supplementary Table 2). The majority (4/6) were in children under 5 years old, all were in HIV-negative children without a prior history of TB, and 2 children had microbiological evidence of TB (one with a positive culture and one with positive Determine TB-LAM result). Five of the 6 children (83%) with "trace" results were classified as Unconfirmed TB and increased the yield of Xpert Ultra in children with

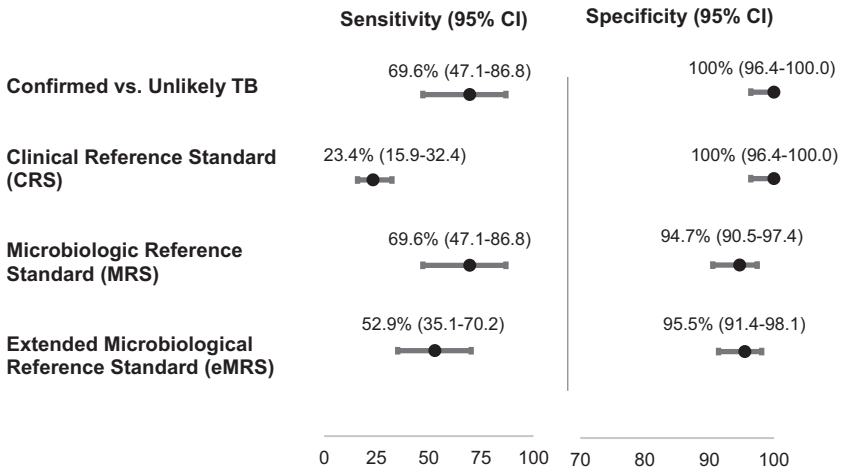

Figure 2. The Diagnostic Accuracy of Xpert Ultra in Children Across Reference Standards, Kampala, Uganda. Abbreviations: TB, tuberculosis; CI, confidence interval.

Unconfirmed TB by 5.7% (95% CI: 1.9-12.8). The one trace result among children with Confirmed TB increased the yield of Xpert Ultra by 4.3% (95% CI: 0.1-21.9).

DISCUSSION

Novel diagnostics with improved sensitivity are needed to increase the detection of pediatric TB. We conducted the first known prospective study on Xpert Ultra for children and utilized multiple reference standards based on the NIH consensus definitions. In the primary analysis of children with Confirmed and Unlikely TB, the sensitivity and specificity of Xpert Ultra were 69.6% and 100%, respectively. When clinical, microbiological, and extended MRSs were used, sensitivity ranged from 23.4% to 69.6%, and specificity ranged from 94.7% to 100%. Thus, we found that Xpert Ultra is an important tool for the diagnosis of pulmonary TB in children, but gaps still remain in diagnosing culture-negative TB.

The Xpert Ultra cartridge is expected to improve sensitivity in comparison to the older generation Xpert MTB/RIF cartridge. A meta-analysis found that Xpert MTB/RIF was 62%–66% sensitive and 98% specific for childhood TB based on a MRS [5]. For Xpert Ultra, we found slightly higher sensitivity (69.6%) and slightly lower specificity (94.7%) using a MRS. This is consistent with other findings that support the increased sensitivity of Xpert Ultra comes at the expense of a small decrease in specificity [11]. As expected, sensitivity decreased with the use of a CRS, but the 23.4% sensitivity observed in our study was higher than the 8% reported in Tanzania using Xpert MTB/RIF [22]. Moreover, Xpert Ultra led to microbiological confirmation of TB in 10 of the 88 (11.4%) children classified as having Unconfirmed TB, including 5 children with "trace" results. While we did not directly compare Xpert MTB/RIF to Xpert Ultra in our study, our findings support that Xpert Ultra may increase the yield of pediatric TB diagnosis over Xpert MTB/RIF. Xpert Ultra also allows same-day results as compared with the delay and infrastructure needed for culture-based testing. This overall supports the WHO recommendation for Xpert Ultra in the initial testing for pulmonary TB and replacement over Xpert MTB/RIF [7].

Our diagnostic estimates for Xpert Ultra were within the range of previous studies using banked specimens, with sensitivities ranging from 58% to 90% and specificities ranging from 93% to 100% [9, 11–13, 23]. Variation may be explained by the type of reference standard used, sample type, use of stored specimens, clinical characteristics, and number of tests performed. Notably, Sun et al [12] found that the performance of Xpert Ultra improved when bronchoalveolar lavage fluid was used instead of sputum, with 90% sensitivity among children with a positive culture or AFB smear. Sabi et al [11] reported slightly lower sensitivity and specificity estimates (64.3% and 98.1%, respectively), although more than half of their participants were HIV infected, compared with 13% of our cohort. Zar et al [10] reported a higher yield of cases when multiple respiratory specimens were tested with Xpert Ultra, with up to 87.5% sensitivity reported against the microbiologic reference standard. Zar et al also found that sputum type affected the performance of Xpert Ultra, with 74.3% sensitivity when a single induced sputum sample was used and 46% sensitivity when a single nasopharyngeal aspirate was used. While we also found that the sensitivity of Xpert Ultra was higher in patients who provided expectorated sputum or induced sputum (80%) than in patients who provided gastric or nasopharyngeal aspirate (50%), our findings were not statistically significant, likely due to the smaller sample size of the subgroups. The only other published study in Uganda by Ssengooba et al [23] used banked samples from children with minimal TB and found similar diagnostic estimates (58%

Table 2. Subgroup Analysis of Xpert Ultra on Children in Kampala, Uganda

	Confirmed TB[a]	Unlikely TB[a]	Sensitivity n/N, % (95% CI)	Specificity n/N, % (95% CI)	Difference in Sensitivity % (95% CI)	*P*-value[b]
Age under 5 years	11	63	7/11, 63.6% (30.8 to 89.1)	63/63, 100% (94.3 to 100)	11.4% (–26.2 to 48.9)	.55
Age 5-14 years	12	39	9/12, 75.0% (42.8 to 94.5)	39/39, 100% (91.0 to 100)		
Smear microscopy positive	13	0	12/13, 92.3% (64 to 99.8)	—	52.3% (18.7 to 85.9)	.01
Smear microscopy negative	10	102	4/10, 40% (12.2 to 73.8)	102/102, 100% (96.4 to 100)		
Underweight	19	56	13/19, 68.4% (43.4 to 87.4)	56/56, 100% (93.6 to 100)	6.6% (–40.7 to 53.9)	.79
Normal weight	4	46	3/4, 75% (19.4 to 99.4)	46/46, 100% (92.3 to 100)		
Expectorated/induced sputum	15	61	12/15, 80% (51.9 to 95.7)	61/61, 100% (94.1 to 100)	30% (–10.1 to 70.1)	.14
Gastric/nasopharyngeal aspirate	8	41	4/8, 50% (15.7 to 84.3)	41/41, 100% (91.4 to 100)		

Abbreviations: TB, tuberculosis; CI, confidence interval.
[a]Defined per the NIH consensus definitions [15].
[b]*P*-value determined using proportion testing.

sensitivity and 94% specificity) against a MRS. However, clinical information was not available to classify children per the NIH consensus definitions.

By including children with Unconfirmed TB (41% of the sample), the alternative reference standards demonstrated a wider range of performance. When the CRS was used, we found that the sensitivity of Xpert Ultra reduced to 23.4%, similar to the 24.4% yield of culture in pediatric studies [14]. This is consistent with the comparable limits of detection for liquid culture (10-100 CFU/mL) and Xpert Ultra (15.6 CFU/mL) [6, 24]. When the MRS was used, there was a decrease in specificity due to potential false positivity from Unconfirmed TB cases. The eMRS attempts to correct some of the bias by adding non-culture microbiological data, although the lower specificity of LAM testing and smear microscopy may overestimate TB cases. Each standard has limitations and may overestimate sensitivity (MRS) or specificity (CRS) [21]. However, by providing a performance range against different reference standards, we sought to reflect a more realistic perspective on the accuracy of Xpert Ultra in children and highlight continued gaps in diagnosis that should be addressed with future assays.

Xpert Ultra added a new "trace" category given the lower limit of detection [6]. In our study, diagnostic yield increased due to the detection of an additional 10 clinically diagnosed, culture-negative cases by Xpert Ultra, and half of which had a "trace" result. This is comparable to a multicenter study of Xpert Ultra in adults, which reported that 44% of culture-negative patients with a positive Xpert Ultra test result had a semiquantitative read of trace [8]. While there is concern that these represent false-positive results in adults, all our Unconfirmed cases with trace findings had signs and symptoms concerning for TB, did not have prior TB in the last year, and all had clinical improvement after initiating anti-TB treatment. Thus, the high proportion of trace results suggests that the more sensitive cartridge in Xpert Ultra is particularly valuable in increasing TB diagnostic yield in children.

This is the first known prospective study to characterize the performance of Xpert Ultra in children using multiple reference standards. All children were symptomatic and underwent comprehensive TB evaluation and classification. Participants were also recruited from both tertiary and community hospitals and outpatient clinics across Kampala, which increased the generalizability of our findings to other high TB burden settings where Xpert Ultra is likely to be used. There are some limitations. The sample size may have prevented the detection of significant differences in subgroup analyses, and an HIV subgroup analysis was not able to be performed. In addition, Determine TB-LAM testing was introduced halfway through the study, and so it is possible that we underestimated sensitivity and overestimated specificity in the eMRS group as not all children received this test. Respiratory specimens were not tested using Xpert MTB/RIF, so we were unable to compare its performance to Xpert Ultra. In the majority

of cases, different specimen types were used for Xpert Ultra and culture testing. This may have biased performance, although all sample types used are recommended under the current guidelines for TB evaluation in children [25]. There were no cases of rifampin resistance, and further assessment of the performance of resistance testing in children is needed.

In conclusion, Xpert Ultra is a useful tool for the diagnosis of pulmonary TB in children and may provide additional yield even compared with mycobacterial culture. Despite the additional benefit of Xpert Ultra, the sensitivity of molecular sputum-based diagnostics remains low in children, especially in those with culture-negative TB, and, therefore, does not exclude TB. This further highlights the need for rapid and more sensitive assays for screening and diagnosing pulmonary TB in children.

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online (http://jpids.oxfordjournals.org).

Notes

Acknowledgments. We thank the patients, families, and staff at Mulago National Referral Hospital, Kampala Capital City Authority Clinics, and Infectious Diseases Institute (IDI) clinics. We thank the Foundation for Innovative New Diagnostics (FIND) for providing Determine TB-LAM testing.

Financial support. This work was supported by grants from the National Heart, Lung, and Blood Institute (grant number R01HL139717 to A. C.) and the National Institute of Child Health and Human Development (grant number K12HD000850 to D. J.).

Potential conflicts of interest. The authors do not have a commercial or other association that might pose a conflict of interest. All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. World Health Organization. *Roadmap Towards Ending TB in Children and Adolescents*. Geneva: World Health Organization; **2018**. Accessed January 18, 2020. https://www.who.int/tb/publications/2018/tb-childhoodroadmap/en/
2. Zar HJ, Nicol MP. Strengthening diagnosis of pulmonary tuberculosis in children: the role of Xpert MTB/RIF Ultra. Pediatrics **2019**; 144:e20192944.
3. Dunn JJ, Starke JR, Revell PA. Laboratory diagnosis of *Mycobacterium tuberculosis* infection and disease in children. J Clin Microbiol **2016**; 54:1434–41.
4. Horne DJ, Kohli M, Zifodya JS, et al. Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev **2019**; 6:Cd009593.
5. Detjen AK, DiNardo AR, Leyden J, et al. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med **2015**; 3:451–61.
6. Chakravorty S, Simmons AM, Rowneki M, et al. The new Xpert MTB/RIF Ultra: improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. mBio **2017**; 8:e00812–7.
7. World Health Organization. *WHO Meeting Report of a Technical Expert Consultation: Non-Inferiority Analysis of Xpert MTB/RIF Ultra Compared to Xpert MTB/RIF*. Geneva: World Health Organization; **2017**. Accessed January 18, 2020. https://www.who.int/tb/publications/2017/XpertUltra/en/
8. Dorman SE, Schumacher SG, Alland D, et al. Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis **2018**; 18:76–84.
9. Nicol MP, Workman L, Prins M, et al. Accuracy of Xpert Mtb/Rif Ultra for the diagnosis of pulmonary tuberculosis in children. Pediatr Infect Dis J **2018**; 37:e261–3.
10. Zar HJ, Workman LJ, Prins M, et al. Tuberculosis diagnosis in children using Xpert Ultra on different respiratory specimens. Am J Respir Crit Care Med **2019**; 200:1531–8.
11. Sabi I, Rachow A, Mapamba D, et al. Xpert MTB/RIF Ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study. J Infect **2018**; 77:321–7.
12. Sun L, Qi X, Liu F, et al. A test for more accurate diagnosis of pulmonary tuberculosis. Pediatrics **2019**; 144:e20190262.
13. Sun L, Zhu Y, Fang M, et al. Evaluation of Xpert MTB/RIF Ultra assay for the diagnosis of childhood tuberculosis: a multicenter accuracy study. J Clin Microbiol **2020**; 58:e00702–20.
14. DiNardo AR, Detjen A, Ustero P, Ngo K, Bacha J, Mandalakas AM. Culture is an imperfect and heterogeneous reference standard in pediatric tuberculosis. Tuberculosis (Edinb) **2016**; 101S:S105–8.
15. Graham SM, Cuevas LE, Jean-Philippe P, et al. Clinical case definitions for classification of intrathoracic tuberculosis in children: an update. Clin Infect Dis **2015**;61(Suppl 3):S179–S87.
16. Cohen JF, Korevaar DA, Altman DG, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. BMJ Open **2016**;6:e012799.
17. Uganda Ministry of Health. *Consolidated Guidelines for Prevention and Treatment of HIV in Uganda. Kampala*, Uganda: Ministry of Health; **2018**.
18. Uganda Ministry of Health. *Management of Tuberculosis in Children: A Health Worker Guide*. Kampala, Uganda: National TB and Leprosy Programme; **2015**.
19. World Health Organization. Lateral *Flow Urine Lipoarabinomannan Assay (LF-LAM): for the Diagnosis and Screening of Active Tuberculosis in People Living with HIV*. Geneva: World Health Organization, **2016**. Accessed January 27, 2020. https://www.who.int/tb/publications/factsheet_lf_lam.pdf
20. Den Boon S, Bateman ED, Enarson DA, et al. Development and evaluation of a new chest radiograph reading and recording system for epidemiological surveys of tuberculosis and lung disease. Int J Tuberc Lung Dis **2005**;9:1088–96.
21. Drain PK, Gardiner J, Hannah H, et al. Guidance for studies evaluating the accuracy of biomarker-based nonsputum tests to diagnose tuberculosis. J Infect Dis **2019**;220(Suppl_3):S108–15.
22. Bacha JM, Ngo K, Clowes P, et al. Why being an expert – despite Xpert –remains crucial for children in high TB burden settings. BMC Infect Dis **2017**;17(1):123.
23. Ssengooba W, de Dieu Iragena J, Nakiyingi L, et al. Accuracy of Xpert Ultra in the diagnosis of pulmonary tuberculosis among children in Uganda: a sub-study from the SHINE trial. J Clin Microbiol **2020**;58:e00410–20.
24. Davies PDO, Gordon SB, Davies G. *Clinical Tuberculosis*: Boca Raton, FL: CRC Press; **2014**.
25. Lewinsohn DM, Leonard MK, LoBue PA, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis **2017**;64:111–5.