Detection of *Mycobacterium tuberculosis* in pediatric stool samples using TruTip technology

AW Mesman[1], annelies_mesman@hms.harvard.edu

M Soto[2], msoto_ses@pih.org

J Coit[1], Julia_coit@hms.harvard.edu

R Calderon[2], rcalderon_ses@pih.org

J Aliaga[2], jaliaga_ses@pih.org

NR Pollock[3], Nira.Pollock@childrens.harvard.edu

M Mendoza, mmendoza_ses@pih.org

FM Mestanza[4], franciscommestanza@gmail.com

CJ Mendoza[4], carlosjose.mendozafox@gmail.com

MB Murray[1], megan_murray@hms.harvard.edu

L Lecca[1,2], llecca_ses@pih.org

R Holmberg[5], RHolmberg@akonni.com

MF Franke[1§], molly_franke@hms.harvard.edu

Affiliations: [1] Harvard Medical School, Department of Global Health and Social Medicine, Boston, USA; [2] Socios En Salud Sucursal (Partners In Health), Lima, Peru; [3] Boston Children's Hospital, Boston, USA; [4]Ministerio de Salud del Peru [5]Akonni Biosystems Inc, Fredrick, USA

[§] Corresponding author

Abstract

Background: Rapid and accurate diagnosis of childhood tuberculosis (TB) is challenging because children are often unable to produce the sputum sample required for conventional tests. Stool is an alternative sample type that is easy to collect from children, and studies investigating the use of stool for molecular detection of *Mycobacterium tuberculosis (Mtb)* have led to promising results. However, tests performed thus far are not able to examine multi-drug resistance. The TruTip workstation (Akonni Biosystems) is an automated lysis and extraction platform that can be integrated with a closed amplification system to detect both *Mtb* and resistance-associated mutations. Our objective here was to evaluate the use of TruTip extraction technology for *Mtb* detection in stool.

Methods: We tested stool samples of 259 children with TB symptoms, ages 0-14 years old, in Lima, Peru. We used the TruTip workstation for sample processing and extraction, followed by IS6110 real-time PCR to detect the presence of *Mtb* DNA. We calculated assay sensitivity in two groups: (1) children with culture confirmed TB (N=22); and (2) children with unconfirmed, clinically diagnosed TB (N=84). We calculated specificity among children in whom TB was ruled out (N=153). Among children with TB, we examined factors associated with a positive stool test.

Results: Overall assay sensitivity was 59% (95% confidence interval 39%-80%) and 1.2% (0.0%-6.5%) in children with culture-confirmed and clinically-diagnosed TB, respectively, and specificity was 97% (93%-99%). The assay detected *Mtb* in stool of 7/7 children with smear-

positive TB [100% sensitivity; (59%-100%)], and in 6/15 [40% (16%-68%)] of children with smear-negative, culture-confirmed TB. Older age, smear positivity, culture positivity and cavitary disease were associated with a positive stool result.

Conclusion: For molecular *Mtb* detection from stool, the TruTip workstation, in combination with IS6110 amplification, led to sensitivity and specificity estimates comparable to other tests such as Xpert. Future optimization is required to also diagnose TB disease in children who now received an unconfirmed diagnosis.

Keywords: Tuberculosis; Children; Molecular diagnostics, TruTip

Background

The World Health Organization estimates that one million new pediatric tuberculosis cases and 194,000 childhood deaths from the disease occurred in 2017 (1). Rapid case detection and treatment initiation is critical to minimizing TB morbidity and mortality in children but is hampered by the absence of a rapid, accurate diagnostic tool in this group. Bacteriologic confirmation of *Mycobacterium tuberculosis (Mtb*) in children is often difficult to achieve because they are frequently unable to expectorate sputum for bacteriologic testing and often have paucibacillary disease that cannot be detected using sputum smear microscopy, culture, and/or molecular testing [e.g. Xpert (Cepheid, Sunnyvale CA, USA)]. Sputum induction and gastric aspiration are two procedures that can be used to obtain respiratory specimens from children unable to expectorate sputum; however, gastric aspiration is invasive and neither procedure is widely implemented in resource-constrained settings. Due to these diagnostic challenges,

bacteriologic confirmation of TB is obtained in only a small minority of children diagnosed with TB in resource-limited clinical settings (2–4).

Stool is a sample type that can be easily obtained from most children, and from which *Mtb* can be detected using Xpert (5–10) or other laboratory-developed PCR assays (11–13). However, isolation and amplification of DNA using extraction kits and in-house tests is an often laborious, multistep procedure. In the case of Xpert, the process is automated, but detection of drug resistance is currently limited to rifampin-associated resistance mutations in rpoB. An ideal test would be an automated point-of-care workstation with integrated capacity for both *Mtb* and expanded drug resistance testing -criteria listed in the product profile for novel TB diagnostics in low resource settings (14). The TruTip workstation is a multiplex automated platform for nucleic acid extraction (Akonni Biosystems, Frederick, MD, USA) (15,16). The platform can be connected to a closed amplicon system for amplification and microarray-based detection of *Mtb* as well as a number of drug resistance-associated mutations (17–19). TruTip has been used for nucleic acid isolation from a variety of pathogens and sample types, and has demonstrated excellent *Mtb* DNA recovery from raw sputum (15,20). The aim of the present study was to estimate sensitivity and specificity of *Mtb* detection in stool from children with symptoms compatible with intrathoracic TB in Lima, Peru, using this novel technology with IS6110 PCR.

Methods

Ethics

Study participants' guardians provided written informed consent to participate, and children eight years of age and older provided written assent. Consent for publication was not applicable.

All study procedures were approved by the Ethics Committee of Peru's National Institute of Health and the Office of Human Research Administration at the Harvard Medical School.

Study population

Between May 2015 and February 2018, we consecutively recruited children to participate in a pediatric TB diagnostics study. Eligible children were less than 15 years of age, had a history of contact with an adult with TB in the last two years, and presented to a participating public sector health center in Lima, Peru with symptoms compatible with TB (i.e., persistent cough for more than two weeks; unexplained weight loss; unexplained fever for more than one week; and/or unexplained fatigue or lethargy) (21). For this analysis, we included the subset of children with culture-confirmed TB or clinically-diagnosed unconfirmed TB who had at least one stool sample available. We also included a subset of the children in whom TB had been ruled out (i.e, controls), matching on age and sample collection date when possible.

Study procedures and sample collection

Children were evaluated for TB per Peruvian National Tuberculosis Strategy guidelines (22). In brief, children provided up to two gastric aspirate (GA) and/or sputum samples (expectorated or induced) for smear and culture, and Ministry of Health pediatric pulmonologists considered these results as well as medical history, physical examination, chest X-ray findings and tuberculin skin testing (TST) results when diagnosing or ruling out TB. We additionally requested two stool samples from all children for research purposes before the child initiated treatment. Stool collection, five gram per sample, took place at home or the health center, and contact between stool and diapers in young children was prevented. Following collection, samples were cold-

chain transported to the laboratory and stored at -80°C until testing. Because testing of stool did not occur in real time and because the assay was experimental, stool testing results were not shared with families or healthcare providers. HIV testing was not performed because the HIV prevalence among children in the study setting is <0.1% (23).

Laboratory procedures

Sample processing and testing was performed in the BSL-3 and BSL-2 facilities at the Socios En Salud Sucursal Laboratory in Lima. GA samples were neutralized to a pH of 6.8-7.2 upon collection, and GA as well as sputum samples were transported under cold chain conditions to the laboratory. Here, we centrifuged and decontaminated all samples in 2% NaOH/0.25% n-acetyl-L-cysteine (NALC). GA and sputum samples were analyzed by Ziehl-Neelsen staining/smear microscopy and liquid culture (BACTEC MGIT 960, BD Franklin Lakes, USA).

Lysis and DNA extraction procedures using the TruTip workstation were similar to previously described methods for sputum samples (20). In brief, 500 milligrams of thawed stool was homogenized in TE buffer, in a magnetically-induced vortexing (MagVor) element, and after a 20 minute heating step at 56°C, the samples were transferred to the TruTip workstation for DNA extraction (16). *Mtb* DNA was amplified in duplicate by real-time PCR using a Roche 480 Lightcycler (Basel, Switzerland) with primers targeting the insertion element IS6110 (24). We considered a sample positive for *Mtb* DNA if the cycle threshold (Ct) value for both PCR replicates was <37 and the change in fluorescence was >6 units. We repeated the PCR step in duplicate for discordant replicates and considered the sample to be indeterminate for *Mtb* DNA if

the two replicates remained discrepant after a second PCR run. Laboratory staff were blinded to participants' clinical and demographic information.

Data analysis

Data were analyzed using SAS (Version 9.4. SAS Institute Inc., Cary, NC, USA) and Graphpad Prism (Version 7 Graphpad software Inc., La Jolla, CA, USA). We analyzed the data both at the child-level (including all samples from each child) and at the sample-level. We calculated sensitivity and specificity and 95% confidence intervals (CI). Sensitivity of *Mtb* detection from stool was calculated separately for children with confirmed TB (i.e., based on a positive culture from sputum or GA) and clinically-diagnosed unconfirmed TB. Specificity was calculated among those in whom TB had been ruled out. Among children with TB, we conducted chi-square tests to examine the associations between a positive stool test and the following variables: age, sex, smear and culture results, and cavitation on chest x-ray. A p-value of <0.05 was considered statistically significant. Numerical precision for all percentages, p-values and 95% CIs is presented based on the evidence-based recommendations proposed by Cole (25).

Results

Study population

Among 628 children enrolled in the study, 136 children were diagnosed with TB [either culture confirmed (n = 33) or unconfirmed, clinically-diagnosed TB (n = 103)]. From this larger cohort, we selected 259 children based on stool sample availability among the TB cases, with a median age 5.1 years, of whom 48% were female (Fig 1). Nearly all children (255/259; 98%) had at least one respiratory sample collected. Of the remaining four with no respiratory sample, three

received a clinical diagnosis and one received no TB diagnosis. The cohort included 22 children with culture-confirmed TB and 84 children with unconfirmed, clinically diagnosed TB (Fig 1). Children between the ages of 11 and 14 years old were more likely to have a culture-confirmed diagnosis, and all seven children with smear-positive sputum belonged to this older age group (Table 1). Thirteen of 22 children with confirmed TB were diagnosed based on expectorated sputum analysis and the remaining nine on gastric aspirate analysis.

Table 1: Demographic characteristics of children, stratified by TB status.

Variable		All children (N=259) n (%)	SM+, CU+ TB[a] (N=7) n (%)	SM-, CU+ TB[a] (N=15) n (%)	All CU+ TB (N=22) n (%)	Unconfirmed clinically diagnosed TB (N=84) n (%)	No TB diagnosis (N=153) n (%)
Age group (years)	0-4	125 (48)	0 (0)	8 (53)	8 (36)	42 (50)	75 (49)
	5-10	37 (14)	0 (0)	5 (33)	5 (23)	34 (40)	58 (38)
	11-14	97 (37)	7 (100)	2 (13)	9 (41)	8 (10)	20 (13)
Female sex		124 (48)	5 (71)	6 (40)	11 (50)	47 (56)	66 (43)

[a]CU: culture, SM: smear

From the 259 children, we collected 484 stool samples. Most children had two stool samples; however 34 children (13%) had only one sample available. Seventeen samples of children diagnosed with TB were collected after TB treatment initiation (median time from treatment initiation to collection: 3 days; range [1 – 11 days]). Collection after >72 hour of treatment start occurred eight times, and happened four of eight times among children with smear-positive sputum because they were eligible to initiate TB treatment immediately, resulting in a narrow window between enrollment and treatment initiation during which pre-treatment samples could be collected.

Per sample analyses

Based on a small comparison study between extraction methods, including the QIAamp DNA Mini Kit (Qiagen), the MoBio Powerfecal DNA Kit (Qiagen), and the TruTip workstation (Akonni Biosystems), in which TruTip yielded the best *Mtb* DNA recovery from stool measured by real-time PCR of IS6110 (data not shown), we decided to use this method for extraction in our study. The assay detected *Mtb* in 25/484 samples. Only one sample, from a child in the control group, had an indeterminate result, which we excluded in further analyses. Among 13 stool samples from seven children with smear positive sputum, 100% (95% confidence interval [CI]: 59%-100%) were positive by the stool assay (Table 2). Sensitivity was lower in the smear-negative culture-confirmed group (23%; 95% CI: 5.6%-41%, Table 2). We detected *Mtb* in 1.4% of samples from control children, resulting in a specificity of 99% (95% CI: 97-99.8%).

Table 2: Per sample analyses of *Mtb* stool assay in 259 children. 95% CI are adjusted for multiple samples per patient.

	Positive stool result (n/N)	Indeterminate stool result (n/N)	Sensitivity (95% CI[a])	Specificity (95% CI)
All samples	25/484	1/484		
Confirmed TB (SM+)[b]	13/13	0/13	100% (59%-100%)	
Confirmed TB (SM-)	7/30	0/30	23.3% (5.6%-41%)	
Confirmed TB (all smear grades)	20/43	0/43	47% (27%-66%)	
Unconfirmed, clinically diagnosed TB	1/159	0/159	0.6% (0.00%-1.9%)	
No TB diagnosis	4/282	1/282		99% (97%-100%)

[a]CI: confidence interval, [b]SM: smear

Per child analyses

In child-level analyses sensitivity of the stool assay was 55% (95% CI: 33%-75%) in the confirmed TB cases based on the results of each child's first sample. Analyzing both sample

results led to identification of one additional confirmed TB case and a sensitivity of 59% (95% CI: 39%-80%) (Table 3). While the majority (n=11, 79%) of positive stool samples among TB cases occurred in children in whom TB was confirmed based on culture of expectorated sputum, two children in whom TB was confirmed based on gastric aspirate and one child with an unconfirmed clinical diagnosis had *Mtb* detected in their stool. All three children were under five years of age.

Table 3: Child-level analyses of *Mtb* stool assay in 259 children.

		Positive stool result (n/N)	Sensitivity (95% CI[a])	Specificity (95% CI)
First sample	All children	16/259		
	Confirmed TB (SM+)[b]	7/7	100% (59%-100%)	
	Confirmed TB (SM-)	5/15	33% (13%-61%)	
	Confirmed TB (all smear grades)	12/22	55% (33%-75%)	
	Unconfirmed, clinically diagnosed TB	1/84	1.2% (0.0%-6.5%)	
	No TB diagnosis	3/153		98% (94%-99.5%)
Both samples	All children	18/259		
	Confirmed TB (SM+)	7/7	100% (59%-100%)	
	Confirmed TB (SM-)	6/15	40% (16%-68%)	
	Confirmed TB (all smear grades)	13/22	59% (39%-80%)	
	Unconfirmed, clinically diagnosed TB	1/84	1.2% (0.0%-6.5%)	
	No TB diagnosis	4/153		97% (93-99.3%)

[a]CI: confidence interval, [b]SM: smear

Factors associated with stool test positivity among TB cases

Signs of adult-like TB (i.e., a positive smear result, a positive culture result, the ability to spontaneously produce sputum and cavitary TB), were associated with detection in stool and

more common in the 11-14 year age group (Fig 2; Table 4). There was no association between stool positivity and sex.

Table 4: Factors associated with *Mtb* stool assay positivity among children with culture-confirmed or unconfirmed, clinically diagnosed TB (N=106).

Variable		Positive stool result (N=14) n (%)	Negative stool result (N=92) n (%)	P-value
Age group (years)	0-4	3 (6.0)	47 (94)	0.0008[a]
	5-10	4 (10)	35 (90)	
	11-14	7 (41)	10 (59)	
Sex	female	7 (12)	51 (88)	0.8[b]
	male	7 (15)	41 (85)	
Smear result	positive	7 (100)	0 (0.0)	<0.0001[b]
	negative	7 (7.3)	89 (93)	
Culture result	positive	13 (59)	9 (41)	<0.0001[b]
	negative	1 (1.2)	83 (99)	
Cavitary disease (x-ray)	yes	5 (100)	0 (0.0)	<0.0001[b]
	no	9 (8.9)	92 (91)	
Ability to produce sputum	Yes	12 (27)	33 (73)	0.0006[a]
	no	2 (3.4)	56 (97	
Culture-confirmation based on sputum	Yes	11 (85)	2 (15)	0.007[b]
	no	2 (22)	7 (78)	

[a]Chi-square test [b]Fisher's Exact test

Discussion

Our study shows that the TruTip workstation, in combination with IS6110 real-time PCR amplification, had 100% sensitivity for *Mtb* detection in stool among children with smear-positive confirmed TB and 40% sensitivity in children with smear-negative confirmed TB. Importantly, we detected *Mtb* in stool of three children below the age of five that were not able to expectorate sputum (two of whom were diagnosed based on GA samples and one who was clinically diagnosed). The overall sensitivity (59%) and specificity (97%) of this assay relative to

liquid culture of respiratory samples was comparable to previous pediatric studies using Xpert for stool testing (7,26). Moreover, it is comparable to the 62% estimated sensitivity of Xpert for pediatric sputum samples compared to culture (27). Unfortunately, similar to other studies (6,28–30), we rarely detected TB in children with culture negative, clinically diagnosed TB.

In line with previous reports (8,31) we found strong associations between a positive stool test and signs of more adult-like TB (i.e., cavitary disease; positive sputum culture; positive sputum smear; the ability to produce sputum), which were most common in the oldest age group. In our study, in contrast to DiNardo et al (12), there was no negative association between stool positivity and collection of stool > 72 hours post treatment initiation, which likely was related to the fact that late collection often occurred among (older) children with smear-positive respiratory samples. This emphasizes the impact of age and smear status on the outcomes of stool testing. This has critical implications for the reporting of pediatric TB diagnostic studies because stool assay sensitivities may vary widely based on the percentage of children in the cohort with adult-like TB and/or high bacterial burden. Because children with adult-like TB are often able to produce a sputum sample for testing, a stool assay will only begin to fill the pediatric TB diagnostic gap if it is sensitive in children who cannot already spontaneously expectorate sputum. To facilitate an understanding of stool assay performance and its utility across settings, we recommend stratifying stool sensitivity results by some measure of disease severity, ideally smear status, and reporting results according to whether the child was able to spontaneously expectorate sputum.

The TruTip workstation had a number of attractive attributes for *Mtb* DNA extraction from stool. Extraction procedures required limited pipetting steps and transfer of larger volumes compared to commercially available kits, even though in the present study the TruTip workstation was not yet in its final automated (sample-to-answer) form. Eventually, the TruTip will be integrated with a microarray-based detection of *Mtb* as well as a number of drug resistance-associated mutations (17–19), and additionally it will have the advantage that the extracted DNA eluate can be isolated for other purposes, such as sequencing. It was a limitation that we had no access yet to the fully automated TruTip workstation here, and could not evaluate its use. Another limitation in our study was that we had no follow-up data for the children in the control group, who all had a houdehold contact with TB disease, or data about clinical improvement of the children receiveing treatment. In the absence of a reliable gold standard test for pediatric TB, treatment responses and clinical data ideally are taken into account for the final diagnosis.

Our stool test protocol required a relatively small input volume (500 mg), which may minimize the impact of PCR inhibitors in stool that can impede the detection of *Mtb*. In fact, several studies show reduced sensitivity with larger sample volumes. For example, Banada et al showed higher sensitivity and less invalid test results when stool input volume was decreased from 1.2 to 0.6 grams (5). Recently, DiNardo et al used only 50 mg of stool and measured 67% sensitivity compared to culture, with a limit of detection for *Mtb* of 96 colony forming units, demonstrating that even smaller volumes can be sufficient to reach high sensitivity. Another consideration to optimize sensitivity and specificity would include increasing the number of aliquots tested per sample.

Conclusion

In conclusion, the results of our study show that the TruTip workstation, in combination with realtime IS6110 PCR, can be used to detect *Mtb* in pediatric stool samples, and its performance is comparable to other platforms. Further optimization could increase test sensitivity and contribute to a test that would fill a gap of diagnosis of children not able to produce sputum

Figure legends

Figure 1. Flowchart of cohort enrollment and stool study inclusion.

Figure 2. TB diagnostic criteria and stool results, by age group.
Bars indicate the percentage of children meeting each criterion.

Abbreviations

AFB – acid fast bacilli; Ct – cycle threshold; DST – drug sensitivity testing; Mtb – Mycobacterium tuberculosis; PCR – Polymerase Chain Reaction; TB – tuberculosis; TST – tuberculin skin test; WHO – World Health Organization

Declarations

Acknowledgements

We are grateful for all children and guardians who participated in this study. We thank Socios En Salud for contribution and dedication to health equity and all the health care workers who contribute to this study and treating patients. We thank Zibiao Zhang for data management support.

Availability of data and materials

Reported data are available on request to the corresponding author

Conflicting interests

RH is an employee Akonni Biosystems inc. The company was not involved in study design.

Author's contributions

AM: led the data analysis and interpretation and wrote the manuscript. MS: implementation of the study and interpretation of results. JC: analysis and interpretation of results. JA: implementation and data analysis. RC: study design and data analysis. NP: study design and interpretation of results. MM: implementation of the study. FM: implementation of the study. CM: implementation of the study. MM: study design and interpretation of results, LL: study design and interpretation of results. RH: study design, implementation and interpretation of results. MF conceptualized and designed the study, interpreted the results, and contributed to writing and editing. All authors critically reviewed the manuscript and approved the final version.

Ethics Approval and Consent

Study participant guardians' provided written informed consent, and children eight years of age and older provided written assent. All study procedures were approved by the Ethics Committee of Peru's National Institute of Health and the Office of Human Research Administration at the Harvard Medical School.

Funding

This work was supported by the National Institutes of Health (NIH) under the Center of Excellence in Translational Research (CETR) grant U19 AI109755.

References

1. World Health Organization. Global Tuberculosis Report. 2018.
2. Starke JR. Pediatric tuberculosis: Time for a new approach. Tuberculosis. 2003;83(1–3):208–12.
3. Newton S, Brent A, Anderson S, Whittaker E, Kampmann B. Paediatric Tuberculosis. Lancet Infect Dis. 2008;8(8):498–510.
4. DiNardo AR, Detjen A, Ustero P, Ngo K, Bacha J, Mandalakas AM. Culture is an imperfect and heterogeneous reference standard in pediatric tuberculosis. Tuberculosis. 2016;101:S105–8.
5. Banada PP, Naidoo U, Deshpande S, Karim F, Flynn JL, O'Malley M, et al. A novel sample processing method for rapid detection of tuberculosis in the stool of pediatric patients using the Xpert MTB/RIF assay. PLoS One. 2016;11(3):1–13.
6. Nicol MP, Spiers K, Workman L, Isaacs W, Munro J, Black F, et al. Xpert MTB/RIF testing of stool samples for the diagnosis of pulmonary tuberculosis in children. Clin Infect Dis. 2013 Aug;57(3):e18-21.
7. Chipinduro M, Mateveke K, Makamure B, Ferrand RA, Gomo E. Stool Xpert MTB / RIF test for the diagnosis of childhood pulmonary tuberculosis at primary clinics in Zimbabwe. Int J Tuberc Lung Dis. 2017;21(2):161–6.

8. Walters E, Scott L, Nabeta P, Demers A-M, Reubenson G, Bosch C, et al. Molecular detection of mycobacterium tuberculosis from stools in young children by use of a novel centrifugation-free processing method. J Clin Microbiol. 2018 Sep;56(9).

9. Walters E, Gie RP, Hesseling AC, Friedrich SO, Diacon AH, Gie RP. Rapid diagnosis of pediatric intrathoracic tuberculosis from stool samples using the xpert MTB/RIF assay: A pilot study. Pediatr Infect Dis J. 2012 Dec;31(12):1316.

10. Hasan Z, Shakoor S, Arif F, Mehnaz A, Akber A, Haider M, et al. Evaluation of Xpert MTB/RIF testing for rapid diagnosis of childhood pulmonary tuberculosis in children by Xpert MTB/RIF testing of stool samples in a low resource setting. BMC Res Notes. 2017 Sep;10(1):473.

11. Wolf H, Mendez M, Gilman RH, Sheen P, Soto G, Velarde AK, et al. Diagnosis of pediatric pulmonary tuberculosis by stool PCR. Am J Trop Med Hyg. 2008 Dec;79(6):893–8.

12. DiNardo AR, Kay AW, Maphalala G, Harris NM, Fung C, Mtetwa G, et al. Diagnostic and treatment monitoring potential of a stool-based quantitative polymerase chain reaction assay for pulmonary tuberculosis. Am J Trop Med Hyg. 2018 Aug;99(2):310–6.

13. Oberhelman R a, Soto-Castellares G, Caviedes L, Castillo ME, Kissinger P, Moore D a J, et al. Improved recovery of Mycobacterium tuberculosis from children using the microscopic observation drug susceptibility method. Pediatrics. 2006;118(1):e100-6.

14. Denkinger CM, Dolinger D, Schito M, Wells W, Cobelens F, Pai M, et al. Target product profile of a molecular drug-susceptibility test for use in microscopy centers. J Infect Dis. 2015;211(Suppl 2):S39–49.

15. Chandler DP, Griesemer SB, Cooney CG, Holmberg R, Thakore N, Mokhiber B, et al.

Rapid , simple influenza RNA extraction from nasopharyngeal samples. J Virol Methods. 2012;183(1):8–13.

16. Thakore N, Garber S, Bueno A, Qu P, Norville R, Villanueva M, et al. A bench-top automated workstation for nucleic acid isolation from clinical sample types. J Microbiol Methods. 2018;148(January):174–80.

17. Chandler D, Bryant L, Griesemer S, Gu R, Knickerbocker C, Kukhtin A, et al. Integrated Amplification Microarrays for Infectious Disease Diagnostics. Microarrays. 2012;1(3):107–24.

18. Linger Y, Knickerbocker C, Sipes D, Golova J, Franke M, Calderon R, et al. Genotyping Multi-Drug Resistant M. tuberculosis from Primary Sputum and Decontaminated Sediment with an Integrated Microfluidic Amplification Microarray. J Clin Microbiol. 2018;(January):JCM.01652-17.

19. Linger Y, Kukhtin A, Golova J, Perov A, Lambarqui A, Bryant L, et al. Simplified microarray system for simultaneously detecting rifampin,isoniazid, ethambutol, and streptomycin resistance markers in mycobacterium tuberculosis. J Clin Microbiol. 2014;52(6):2100–7.

20. Thakore N, Norville R, Franke M, Calderon R, Lecca L, Villanueva M, et al. Automated TruTip nucleic acid extraction and purification from raw sputum. PLoS One. 2018;1–12.

21. Cuevas LE, Browning R, Bossuyt P, Casenghi M, Cotton MF, Cruz AT, et al. Evaluation of tuberculosis diagnostics in children: 2. Methodological issues for conducting and reporting research evaluations of tuberculosis diagnostics for intrathoracic tuberculosis in children. Consensus from an expert panel. J Infect Dis. 2012;205(SUPPL. 2):209–15.

22. MINSA. Norma técnica de la salud para el control de la Tuberculosis. Peru: Ministerio de

Salud. 2013.

23. UNAIDS. UNAIDS Data 2017. 2017.

24. Savelkoul PHM, Catsburg A, Mulder S, Oostendorp L, Schirm J, Wilke H, et al. Detection of Mycobacterium tuberculosis complex with Real Time PCR□: Comparison of different primer-probe sets based on the IS6110 element. J Microbiol Methods. 2006;66:177–80.

25. Cole TJ. Too many digits: The presentation of numerical data. Arch Dis Child. 2015;100(7):608–9.

26. Lacourse SM, Pavlinac PB, Cranmer LM, Njuguna IN, Mugo C, Gatimu J, et al. Stool Xpert MTB/RIF and urine lipoarabinomannan for the diagnosis of tuberculosis in hospitalized HIV-infected children. Aids. 2018;32(1):69–78.

27. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, 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(6):451–61.

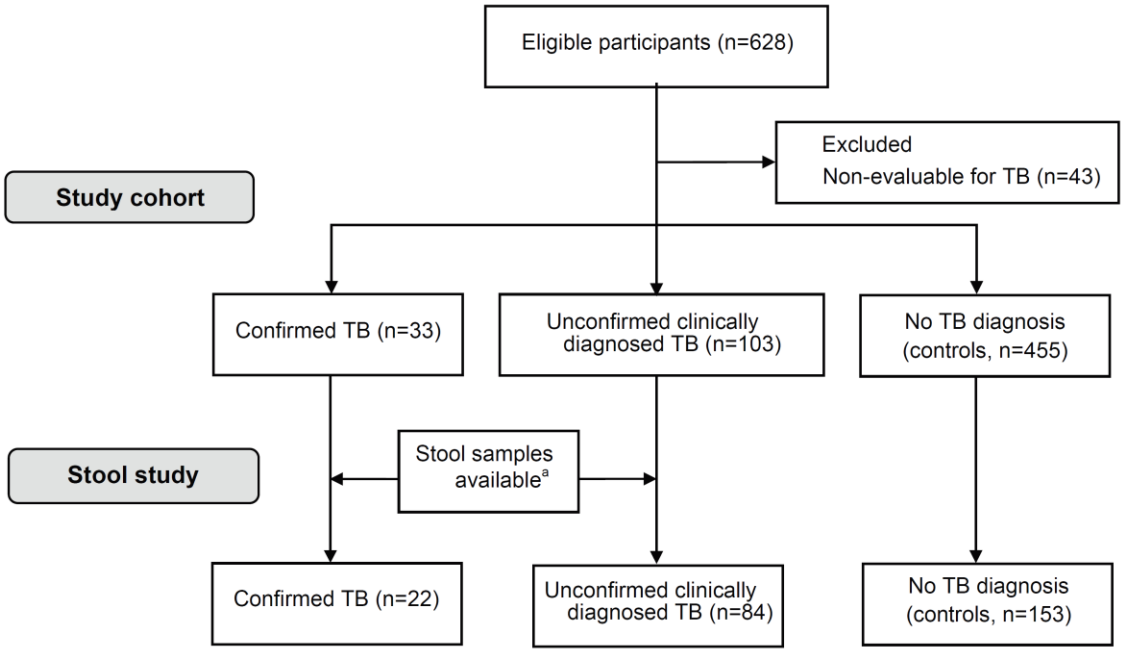
28. Walters E, van der Zalm MM, Palmer M, Bosch C, Demers A-M, Draper H, et al. Xpert MTB/RIF on Stool Is Useful for the Rapid Diagnosis of Tuberculosis in Young Children with Severe Pulmonary Disease. Pediatr Infect Dis J. 2017 Sep;36(9):837–43.

29. Memon SS, Sinha S, Sharma S, Kabra S, Lodha R, Soneja M. Diagnostic accuracy of xpert MTB/RIF assay in stool samples in intrathoracic childhood tuberculosis. J Tuberc Ther. 2018;3(2).

30. Orikiriza P, Nansumba M, Nyehangane D, Bastard M, Mugisha IT, Nansera D, et al. Xpert MTB/RIF diagnosis of childhood tuberculosis from sputum and stool samples in a high TB-HIV-prevalent setting. Eur J Clin Microbiol Infect Dis. 2018 Aug;37(8):1465–73.

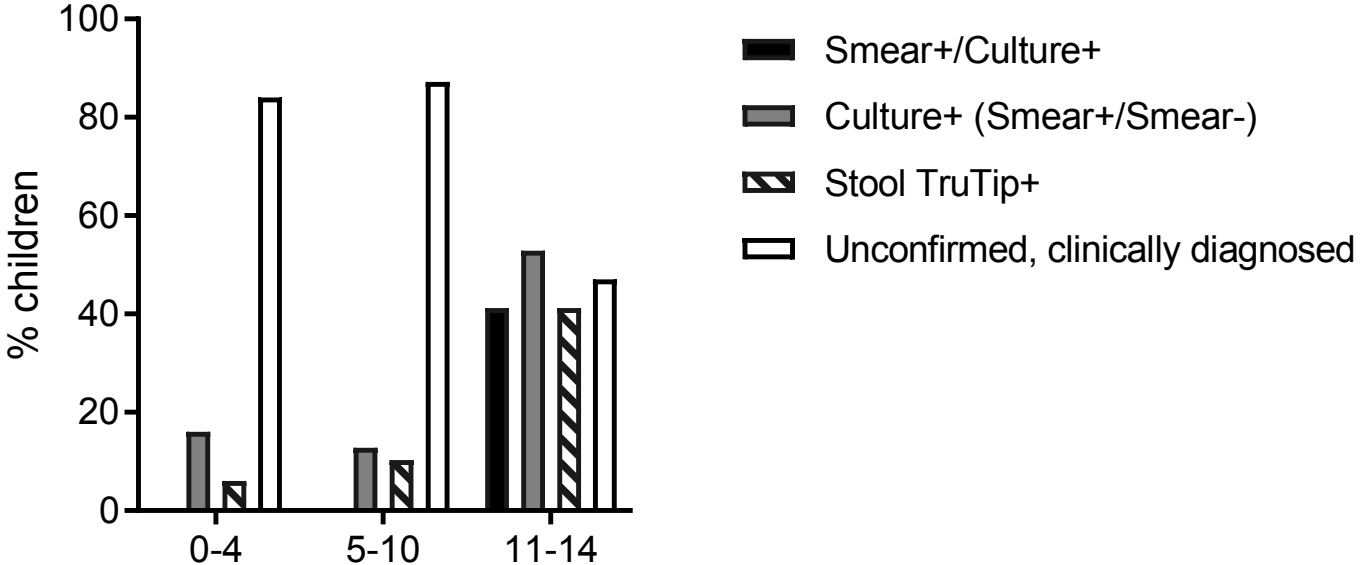
31. Walters E, Demers A-M, van der Zalm MM, Whitelaw A, Palmer M, Bosch C, et al. Stool culture for the diagnosis of pulmonary tuberculosis in children. J Clin Microbiol. 2017;55(12):JCM.00801-17.

Figure 1



[a]Stool samples from a number of TB cases had been used in pilot experiments (data not shown)

Figure 2


100
80
60
40
20
0
% children
0-4
5-10
11-14
Smear+/Culture+
Culture+ (Smear+/Smear-)
Stool TruTip+
Unconfirmed, clinically diagnosed