Comparison of Xpert MTB/RIF Ultra Results of Stool and Sputum in Children with Presumptive Tuberculosis in Southern Ethiopia

Yohannes Babo *, Bihil Seremolo, Mamush Bogale, Yasin Wabe, Haba Churako, Alemu Bilat, Tamiru Degaga, Ahmed Oumer, Petra Haas, Edine Tiemersma, Degu Jerene

Posted Date: 26 May 2023

doi: 10.20944/preprints202305.1935.v1

Keywords: Simple One-Step (SOS) stool processing method stool; Xpert MTB/RIF (Ultra) assay and children

Article

Comparison of Xpert MTB/RIF Ultra Results of Stool and Sputum in Children with Presumptive Tuberculosis in Southern Ethiopia

Yohannes Babo [1], Bihil Seremolo [1], Mamush Bogale [1], Ahmed Oumer [1], Yasin Wabe [2], Haba Churako [3], Alemu Bilat [4], Tamiru Degaga [5], Petra de Haas [6], Edine Tiemersma [6] and Degu Jerene [6]

[1] KNCV Tuberculosis Foundation, Addis Ababa, Ethiopia; demissieyohanes@gmail.com, bihil.sherefdin@kncvtbc.org, mamush.sahile@kncvtbc.org, ahmed.bedru@kncvtbc.org
[2] Worabe comprehensive specialized hospital, Worabe, Ethiopia; awolyasin30@gmail.com
[3] Wolaita Sodo University hospital, Wolaita, Ethiopia; kotedurehaile@gmail.com
[4] Nigist Eleni Memorial comprehensive specialized hospital, Hossana, Ethiopia; alemuworku11@gmail.com
[5] College of Medicine & Health Sciences, Arba Minch University, Arbaminch, Ethiopia; drtamshib1@gmail.com
[6] KNCV Tuberculosis Foundation, The Hague, The Netherlands;, Petra.deHaas@kncvtbc.org, edine.tiemersma@kncvtbc.org, degu.dare@kncvtbc.org.
* Correspondence: demissieyohanes@gmail.com, yohannes.demissie@kncvtbc.org ; Tel.: +251911804459

Abstract: The introduction of stool as a readily obtainable sample and the recently developed simple-one-step (SOS) stool processing method on Xpert MTB/RIF Ultra (Xpert Ultra) offer an opportunity for TB diagnosis in children. We conducted this study at secondary health facilities in Ethiopia which are the first level referral facilities for childhood TB diagnosis and treatment with the aim to determine if stool-based TB diagnosis can be performed with reasonable level of concordance with sputum tests in using Xpert MTB/RIF Ultra. Eligible children 0-14 years with presumptive pulmonary TB were asked to provide stools in addition to routinely requested sputum samples. We ~~computed Cohen's kappa statistic in SPSS to~~ determine the level of agreement between stool and sputum test results. Of the 373 children included in the study, 61% were <5 years of age and 56% were male. Thirty-six children (9.7%) were diagnosed with TB and all started treatment. The rate of concordance between stool and sputum was high with kappa value of 0.83 (P<0.001). There were more Xpert Ultra positive ~~test~~ results on stool (n=27 (7.2%)) than on sputum/NGA (n=23 (6.2%)). Laboratories in secondary hospitals can perform stool-based TB diagnosis in children with high concordance between stool and sputum test results reaffirming the applicability of the SOS stool method.

Keywords: Simple One-Step (SOS) stool processing method stool; Xpert MTB/RIF (Ultra) assay and children

1. Introduction

The world is lagging in its commitment to achieve the 2018-2022 global TB target set at the UN high-level meeting (UNHLM) in 2018. This may in part be due to the decline in TB notifications and increase in TB deaths due to the interruption of TB services caused by the COVID-19 pandemic; only 1.4 million children were treated for TB between 2018-2020, which is only 41% of the five-year target of 3.5 million [1]. The number of missed TB patients is disproportionally high among young children [2]. Diagnosis of TB is more challenging especially for young children because they often do not produce adequate sputum and often have non-specific symptoms and signs. Furthermore, diagnosis of TB in children is hindered by the low sensitivity and limited accessibility of microbiological testing, and the low probability of obtaining a specimen. Most children with presumptive TB seek care at primary health facilities and face diagnostic delay more than a month during which multiple providers were met and the patients underwent several diagnostic tests, most of them being

 doi:10.20944/preprints202305.1935.v1

inappropriate for TB diagnosis [3]. Alternative specimens, such as gastric aspirates or induced sputum, require specific skills and equipment which are usually not available in the lower-level health care facilities and in remote settings.

Ethiopia developed its national childhood TB roadmap in 2015 and last updated in 2019. The key strategies outlined in the roadmap are integrating TB screening, diagnosis, and prevention services into IMNCI (Integrated Management of Newborn and Childhood Illnesses) clinics at the primary health care level and intensifying contact investigations at the TB clinics [4]. This supported the integration of childhood TB services into other childcare services addressing children's peculiar needs. Key achievements were increased national leadership in addressing childhood TB and development of working groups focusing on childhood TB, increasing recognition of the importance of integrating childhood TB into IMNCI clinics and revision of the IMNCI register to include TB screening. A study done in Addis Ababa, Ethiopia to evaluate TB screening integration into IMNCI demonstrated the feasibility of the strategy and resulting in improved TB screening, identification of presumed TB cases, and TB case detection [5].

Despite concerted efforts made to address childhood TB, the lack of child friendly diagnostic samples other than sputum to confirm TB diagnosis and perform drug susceptibility test remains to be a key barrier in operationalizing the strategies in the national roadmap. Since 2020 stool Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, USA) has been recommended by the world health organization (WHO) as an alternative sample to diagnose TB in children [6]. Since 2022 stool was also recommended to be tested using the more sensitive Xpert MTB/RIF Ultra (Xpert Ultra) [7,8]. A simple stool processing method called the Simple-One-Step (SOS) stool method is gaining popularity because of its ease of implementation at peripheral sites [9,10]. The method is as simple as sputum processing, requires no additional equipment or supplies and can be performed at all sites where an Xpert instrument is functional [6,10,11]. The method showed to have similar sensitivity and specificity compared to other stool processing methods [7,9,12].

While we earlier confirmed the utility of the SOS stool method in different settings [13–15], further evidence is needed in its operational feasibility in lower-level health facilities. In this study, we determined if the SOS stool method can be implemented in secondary level health facilities under routine setting and (incremental) yield of using stool as an alternative sample in children. The findings of this study will provide additional evidence on the scalability of stool-based testing using Xpert Ultra cartridge to address the diagnostic difficulty in childhood TB care in a high TB burden and resource limited setting.

2. Materials and Methods

Study design and setting

A cross-sectional study was conducted in four selected hospitals in Southern Nations, Nationalities, People's Region (SNNPR), Ethiopia from October 2021 to December 2021. The study recruited children seeking care in the outpatient clinic and identified in the selected health facilities as presumptive TB patients as per the national algorithm. The four hospitals were selected because of a relatively high TB notification rate among children, serving a catchment population with low socioeconomic status and experience of research participation in addition to availability of a GeneXpert instruments on-site. These hospitals are the first level referral sites for the diagnostic evaluation and treatment of TB in children referred from primary health facility.

2.1. Sample size and sampling

Assuming a positive discordance rate of 10%, a negative discordance rate of 5%, a study power of 90%, at α=5%, the calculated sample size for the current study was 627 children with presumptive TB [16]. Children with presumptive pulmonary TB visiting the selected hospitals during the study period and whose parents consented for participation were recruited consecutively. Since this sample

 doi:10.20944/preprints202305.1935.v1

size was not achieved due to lower enrollment rate than anticipated, we performed power analysis with the sample size achieved which yielded power >90%.

2.2. Study population

We consecutively enrolled children up to 14 years old visiting the selected health care facilities and identified as presumptive pulmonary TB (clinical symptoms suggestive of pulmonary TB (PTB), (i.e., cough for more than two weeks; weight loss/failure to thrive; reduced playfulness; fever/night sweats) and/or contact history with a registered TB case and/or chest X-ray (CXR) suggestive of TB) by the clinician. Critically ill patients (including those in coma, terminally ill due to chronic debilitating co-morbidities, or other conditions determined to be "critical" by the treating physician), and those whose parents/caregivers did not provide informed consent were excluded from the study.

2.3. Study procedures

2.3.1. Participant enrollment

Parents/caregivers of eligible children were informed about the study by the data collectors from the health facility during their medical visit. They were provided with an information sheet containing the details of the study. Written parental consent was taken if they agreed to their child's participation in the study. In addition to routinely requested sputum samples (spontaneous/Naso gastric aspirate (NGA)) and other tests (CXR if available), all children were requested to submit one stool sample. Parents/caregivers were instructed by the laboratory personnel on how to collect sputum and stool samples on site and were provided with stool containers to allow the collection of stool samples for same day testing.

2.3.2. Laboratory procedures

Dedicated laboratory personnel received the sputum and stool samples and recorded the demographic data in the Xpert MTB/RIF register. National standard operating procedures were used to process and test the sputum using Xpert-Ultra assay, which follows the procedure as provided by the manufacturer. Briefly, sample reagent (SR) was added in a 2:1 ratio to sputum specimen. Mixed well twice during the 15 minutes incubation time at room temperature. Two milliliters (ml) of the mixture were transferred in to test cartridge. The test cartridge was loaded into the GeneXpert instrument. *M. Tuberculosis* (MTB) positivity and its rifampicin (RIF) resistance were determined within 2 hours. For stool the SOS stool processing method was followed [17]. In short, 0.8 g of solid, or 2ml of liquid stool was added to the SR bottle which comes with every Xpert-Ultra cartridge, and the mixture was then vigorously shaken by hand for 30 seconds, then incubated for 10 minutes, then shaken again by hand for 30 seconds, and incubated for another 10 minutes to allow debris to settle. Next, 2 ml of the supernatant was transferred to the Xpert-Ultra cartridge and analyzed using the GeneXpert instrument. The semi-quantitative levels of Xpert-Ultra results were trace, very low, low, medium, or high. The non-determinate Xpert-Ultra results (invalid, No result & errors) were repeated once to define the final test outcome. The diagnosis of TB is made when MTB is detected from sputum sample or based on the clinical assessment of the clinician according to the national guidelines.

2.3.3. Data management and statistical analysis

Detailed clinical and demographic information of the patients and Xpert-Ultra test results of their samples were collected using structured paper forms developed for the study. The paper-based records were then entered using a KoBo Toolbox [18] data entry sheet developed for this study. The quality of data entry was validated by cross-checking selected key variables in the database with the information on the paper forms for 10% of the participants. The data were finally analyzed using SPSS (version 20). Descriptive analyses were used for participant characteristics. We further used

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 May 2023 doi:10.20944/preprints202305.1935.v1

Cohen's kappa statistic to measure the level of agreement between sputum and stool Xpert-Ultra testing.

2.3.4. Ethical considerations

The study protocol was approved by the SNNPR Ethical Review Committee (ዩወ6-19/1826). Participants' information was kept confidential. We also didn't include any names or other personal identifying information of the participant in the digital files. The informed consent and paper forms with personal identifying information were stored in a lockable cabinet.

3. Results

Of 373 children with presumptive TB included in the study, 61% were <5 year of age and 56% were male. The median age was 3 (IQR: 1-8). Most patients reported chronic cough (98%), followed by fever (88%) and weight loss or failure to thrive (69%), and 21% had contact history with a known TB patient. HIV status was available for 239 (64%), of whom 4 (1.7%) were HIV infected (Table 1).

Table 1. Baseline characteristics of the study participants (n=373).

Characteristic		Number (%)
Age (in years)	<5	226 (60.6)
	5-14	147 (39.4)
	Median (IQR)	3 (1-8)
Sex	Female	163 (43.7)
	Male	210 (56.3)
Presenting symptoms		
	Chronic cough	365 (97.8)
	Fever	327 (87.7)
	Wight loss or Failure to Thrive	258 (69.2)
	Night sweats	205 (55.0)
	Contact history	78 (20.9)
HIV status		
	Negative	235 (63.0)
	Not done	134 (35.9)
	Positive	4 (1.1)

Thirty-six children (9.6% of 373) were diagnosed with TB of whom 23 (64%) had bacteriologically confirmed TB (sputum Xpert-Ultra positive). A valid test result was obtained for 368/373 children both for stool and the sputum/NGA sample with a concordant result for 360 (96.5%, 95% CI 71–98%) children. The rate of concordance between stool and sputum was high with kappa value of 0.83 (Table 2). Discordant results were found in 6 children with Xpert-Ultra stool positivity (3 MTB detected, trace, 2 MTB detected, low, and 1 MTB detected, high) and Xpert-Ultra sputum-negative results. A discordant result was also found in 2 children having Xpert-Ultra stool negative and Xpert-Ultra sputum positive results (both MTB detected, trace).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 May 2023 doi:10.20944/preprints202305.1935.v1

Table 2. Comparison of Xpert-Ultra results of stool and sputum/NGA from children with presumptive TB.

MTB detected from sputum/NGA	MTB detected from stool		
	No	Yes	Total
No	339	6	345
Yes	2	21	23
Total	341	27	368
Kappa value: 0.83; 95% CI 0.71-0.94			

Of the six children with MTB detected in stool only, 4 were children under the age of five. Two children had a TB contact history and several symptoms suggesting TB while the other half had only several symptoms suggesting TB. Out of the six children, only one child was clinically diagnosed with TB and started treatment during the data collection period. The demographic and clinical characteristics of children with MTB detected on stool specimens are depicted in Appendix A.

MTB was detected in both sputum & stool of 1/4 (25%) HIV-infected children (very low on sputum and trace on stool). This child was a one-year-old with cough, fever, weight loss, had contact history with pulmonary TB patients and was started on treatment. In the HIV-uninfected children, stool testing detected MTB in 23/236 (9.7%) while sputum testing detected MTB in 21/236 (8.9%). Among children under the age of 5 years, stool testing detected MTB in 14/27 (51.9%) versus 12/23 (52.2%) MTB detection in the sputum testing (Table 3).

Table 3. Characteristics of children diagnosed with TB by sputum and stool samples.

Characteristics	No of children with MTB detected in sputum (n=23)	No of children with MTB detected in stool (n=27)	p-value
Age, median (IOR)	3 (1-7)	3 (1-7)	
Sex			
Female	10 (43.5%)	11 (40.7%)	0.85
Age			
< 5	12 (52.2%)	14 (51.9%)	0.98
> 5-14	11 (47.8%)	13 (48.1%)	
History of TB contact			
Yes	16 (69.6%)	18 (66.7%)	0.83
HIV status			
Positive	1 (4.3%)	1 (3.7%)	
Presenting symptoms			
Cough	23 (100%)	27 (100%)	
Fever	23 (100%)	26 (96.3%)	
Weight loss	23 (100%)	24 (88.9%)	
Night sweets	**21(91.3%)**	**22 (81.5%)**	**0.56***

 doi:10.20944/preprints202305.1935.v1

*Fisher's exact test.

Of the 371 **Xpert-Ultra tests conducted** on sputum/NGA samples, one (0.27%) resulted in non-determinate test results (invalid)Of the 373 Xpert-Ultra tests on stool samples, 9 (2.4%) resulted in non-determinate results (7 error, 1 invalid, and 1 No result). The error codes for non-determinate stool Xpert-Ultra results were 5011, 2037, 2097, 2005, 2008 or 5007 at initial testing. The difference in the proportions of non-determinate test results for Xpert-Ultra testing of sputum/NGA samples versus testing of stool samples with the SOS stool method was statistically significant (P = 0.048). For six (75%) out of 8 stool samples in which the Xpert-Ultra test was repeated (for the sample with 'no result', the test was not repeated), the repeat test led to valid results.

Overall, there were more Xpert-Ultra MTB positive test results on stool (n=27 (7.2%) of all results) than on sputum/NGA (n=23 (6.2%) of all results, p<0.001). The semi quantitative result of the Xpert-Ultra of the stool and the sputum/NGA specimen has no significant difference. The Xpert-Ultra trace rate on stool was 6/27 (22.2%). Six children with MTB trace calls detected in the stool sample were under the age of five years. Out of the six children with trace calls in the stool, four had Xpert-Ultra MTB very low detected in the sputum and two had no MTB detected. The two children had TB suggestive symptoms and one had TB contact history but were not clinically diagnosed to have TB (Table 4).

Table 4. Semi-quantitative categories of Xpert-Ultra assays on sputum and stool specimens among bacteriologically confirmed PTB patients in children from 4 hospitals in SNNPR, Ethiopia, October – December 2021.

Sputum Xpert ultra-result	Stool Xpert MTB/RIF ultra-result					
	Negative	Trace	Very low	Low	Medium	High
Negative		2	0	3	0	1
Trace	2	0	0	0	0	0
Very low	0	4	3	0	0	0
Low	**0**	**0**	**2**	**6**	**0**	**0**
Medium	**0**	**0**	**0**	**2**	**0**	**0**
High	**0**	**0**	**1**	**1**	**1**	**1**

4. Discussion

This study assessed the rate of agreement between sputum/NGA and stool sample results on Xpert-Ultra in four hospitals in Southern Ethiopia. Our study has some key findings. First, the Xpert-Ultra on stool sample had a very good concordance with sputum/NGA sample. Second, there was a low rate of non-determinate results for stool specimens related to stool processing, even though this was slightly higher than for sputum specimens. Third, MTB was more often detected in the stool than in the sputum sample. Fourth, the semi-quantitative Xpert Ultra results of sputum/NGA samples were not different from those measured by Xpert Ultra testing of stool samples.

The very high concordance rate between Xpert-Ultra results on stool and sputum/NGA samples implies that stool sample yields a comparable result with that of sputum/NGA sample when done in a routine setting. A study from China, a high TB burden country reported moderate and good quality of agreement between stool and sputum specimen on Xpert-Ultra assay [19]. The stool processing methods used in the Chinese studies were different from the one we report in the current study. Another small study from a teritiary hospital in Indonesia with a two-step stool processing method showed 89% (95% CI 71–98%) concordant result between stool and respiratory samples [20]. A study from Pakistan reported a comparable performance and good agreement between Xpert testing of

 doi:10.20944/preprints202305.1935.v1

stool and sputum/NGA in a cohort of children with high probability of pulmonary TB though the stool processing method is different including the stool volume used i.e., 0.15 gram of stool [21].

The rate of non-determinate stool Xpert Ultra results was within the acceptable range and repeat testing of initial non-determinate stool results led to a valid result in almost all. There is a need to closely monitor the rate of non-determinate test results of the stool Xpert testing as is done for sputum Xpert testing and take corrective actions. Onsite training and supervision of laboratory staff is critical to improve performance and reduce non-determinate results overtime. One reason for invalid/error results in stool-based testing are the presence of PCR inhibitors that are naturally more present in stool compared to sputum; the other reason may be that when not carefully transferring the supernatant and the sedimentation process in the SOS stool method is disturbed, debris of the stool might take into the cartridge which leads to clotting of the micro fluid filter in the Xpert-Ultra cartridge leading to specific errors like error 2008 and 5006/5007. It is therefore important to distinguish the errors that occurred due to stool processing and error codes that are related to the GeneXpert instrument or cartridge failure. A study by de Haas et al showed a non-significant difference in the stool processing non-determinate results of Xpert testing of NGA versus stool samples, while the rate of non-determinate results decreased over time, probably due to experience with the SOS stool method among lab technicians increased in Vietnam [9]. A study in Southwest Ethiopia in a teaching and referral hospital using the SOS stool method documented 4.6% (7/152) rate of error/invalid results on Xpert assay [22]. The difference could be because Xpert-Ultra is less likely to produce invalid or error results than Xpert, offering a better performance and makes it a better choice for diagnosis of tuberculosis. Another study in China found a higher frequency of invalid test results from stool samples than from GA. The processing method used in this study, which involved using phosphate buffer for stool processing, may have inhibited the PCR reactions [19].

In this study, stool specimens yield a higher proportion of MTB detected than sputum specimen. This could be since stool samples may contain MTB DNA from sites other than the lungs, such as the abdominal lymph nodes, intestines, and pleura. Moreover, this can be explained by the low quantity and/or poor quality of the sputum/NGA samples taken from children in the younger age group. A similar finding was reported by de Haas et al in Ethiopia and Vietnam, where children had MTB detected in their stool but not in their sputum/NGA sample [9,11]. There is no notable difference in the performance of stool testing as compared to sputum testing by age, TB contact history, presenting symptoms and HIV status of children (the number of HIV infected children is very low to make comparison in this study). Though not statistically significant, studies from South Africa [23] and Zimbabwe [24] reported that Xpert MTB/RIF on stool was more sensitive in HIV-infected than in HIV-uninfected children.

Another important finding in our study was that the semi quantitative Xpert-Ultra results were not different between the stool and sputum samples. Out of the six trace calls in the stool, four had very low MTB in the sputum and two had no MTB detected. The two children had multiple TB suggestive symptoms and were not clinically diagnosed to have TB. A study from Bangladesh reported that the proportion of trace call was higher in stool specimens compared to induced sputum [21] while a study by Sun from China found that the semi-quantitative scale used for Xpert-Ultra on stool samples correlated with that of Xpert-Ultra using gastric aspirate [20]. Detecting MTB from swallowed sputum in the stool in the lower scale of the semiquantitative scale (low, very low and trace call) could be due to the paucibacillary nature of TB in children. In addition, more dilution of MTB in intestines as sputum gets mixed with food, PCR inhibition due to substances in stool and less easy to "free" the MTB bacilli from stool than from sputum.

One limitation of our study was that we have no follow-up data for those children with MTB detected in stool reporting clinical symptoms of TB. Thus, we do not know if they were diagnosed at a later point in time. This was because stool was not yet recommended as an initial sample for testing in Ethiopia at the time of the study. This study on the SOS stool processing method was successfully implemented in peripheral laboratories under the routine conditions of the resource limited setting.

5. Conclusions

The SOS stool method on Xpert-Ultra for TB detection yields a comparable result with sputum Xpert-Ultra testing. Stool can be used as an alternative to respiratory specimen and showed to be a non-invasive rule in test for patients who cannot provide sputum. Hence the SOS stool method is applicable to expand childhood TB services at secondary health care levels. Further studies on the applicability of stool-based testing for TB diagnosis among HIV infected children are warranted as these children benefit most from this non-invasive and innovative method.

Author Contributions: "Conceptualization, D.J. and Y.B.; methodology, D.J., P.H., E.T. and Y.B.; software, D.J.; validation, D.J. and Y.B.; formal analysis, D.J. and Y.B.; investigation, Y.W., H.C., A.B., T.D., B.S. and M.B.; resources, D.J.; data curation, D.J. and Y.B.; writing—original draft preparation, Y.B.; writing—review and editing, Y.B., Y.W., H.C., A.B., T.D., B.S., M.B., A.O., P.H., D.J.; visualization, D.J. and Y.B.; supervision, D.J. and A.O.; project administration, Y.B.; funding acquisition, D.J. All authors have read and agreed to the published version of the manuscript."

Funding: "This research was funded by St Antonius Stichting Projects (**SAS-P**), a philanthropic foundation based in the Netherlands."

Institutional Review Board Statement: "The study was conducted in accordance with the Declaration of Helsinki and approved by the SNNPR Ethical Review Committee (protocol code ꝑ⍵6-19/1826) and date of approval on 12 May 2021)."

Informed Consent Statement: "Informed consent was obtained from all participants involved in the study."

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

Acknowledgments: We would like to thank the study participants, and parents/caregivers for their participation in the study. We would also like to thank the staff from all the healthcare facilities in the study area.

Conflicts of Interest: "The authors declare no conflict of interest."

Appendix A: Demographic and clinical characteristics of children with MTB detected on stool only.

Age in years	Sex	Cough	Fever	Weight loss or FTT	Contact history	Was TB diagnosed clinically?	Was TB treatment started?	Xpert Ultra result
2	Male	Yes	Yes	Yes	Yes	Yes	Yes	MTB detected low
9	Female	Yes	Yes	No	Yes	No	No	MTB detected low
0	Male	Yes	No	No	Yes	No	No	MTB detected trace
0	Male	Yes	Yes	Yes	No	No	No	MTB detected low
3	Female	Yes	Yes	Yes	No	No	No	MTB detected trace
13	Male	Yes	Yes	No	No	No	No	MTB detected high

References

1. Global tuberculosis report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
2. Rapid communication on updated guidance on the management of tuberculosis in children and adolescents. World Health Organization, Geneva, Switzerland. 2021.
3. Raizada N, McDowell A, Parija D, Sachdeva KS, Khaparde SD, Rao R, Pavani TN, Sudha S, Tyagi H, Rebecca YM, Huddart S, Salhotra VS, Nair SA, Denkinger CM, Chadha SS, Sarin S, Kalra A. Pathways to diagnosis of pediatric TB patients: A mixed methods study from India. Indian J Tuberc. 2021 Jul;68(3):363-373. doi: 10.1016/j.ijtb.2020.12.011. Epub 2020 Dec 31. PMID: 34099202.
4. Ministry of Health of the Federal Demographic Republic of Ethiopia. Roadmap towards ending Childhood and Adolescent TB in Ethiopia.2nd Edition. Addis Ababa, Ethiopia. Available at National Roadmap towards ending Childhood and Adolescent TB in Ethiopia (iifphc.org). Accessed 30 March 2023.)
5. Ketema L, Dememew ZG, Assefa D, Gudina T, Kassa A, Letta T, Ayele B, Tadesse Y, Tegegn B, Datiko DG, Negeri C, Bedru A, Klinkenberg E. Evaluating the integration of tuberculosis screening and contact

investigation in tuberculosis clinics in Ethiopia: A mixed method study. PLoS One. 2020 Nov 19;15(11):e0241977. doi: 10.1371/journal.pone.0241977. PMID: 33211710; PMCID: PMC7676707.

6. WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detention, 2021 update. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
7. WHO. WHO operational handbook on tuberculosis. Module 5: management of tuberculosis in children and adolescents. Geneva, Switzerland: World Health Organization; 2022.
8. Kay AW, Ness T, Verkuijl SE, Viney K, Brands A, Masini T, González Fernández L, Eisenhut M, Detjen AK, Mandalakas AM, Steingart KR, Takwoingi Y. Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children. Cochrane Database Syst Rev. 2022 Sep 6;9(9):CD013359. doi: 10.1002/14651858.CD013359.pub3. PMID: 36065889; PMCID: PMC9446385.
9. de Haas P, Nhung N, Hng N, Hoà N, Loan, N,; Thanh, N et al. Introduction of the Simple One-step stool Xpert-Ultra method to detect TB in children and adults in Vietnam. IJTLD Volume 27, Number 1, 1 January 2023, pp. 19-27(9)
10. Practical manual of processing stool samples for diagnosis of childhood TB. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO
11. de Haas P, Yenew B, Mengesha E, et al. The Simple One-Step (SOS) Stool Processing Method for Use with the Xpert MTB/RIF Assay for a Child-Friendly Diagnosis of Tuberculosis Closer to the Point of Care. J Clin Microbiol. 2021;59(8):e0040621. doi:10.1128/JCM.00406-21
12. Jasumback CL, Dlamini Q, Kahari J, Maphalala G, Dlamini MG, Dube GS, et al. Laboratory comparison of stool processing methods for Xpert((R)) Ultra. Public health action. 2021;11(2):55-7.
13. Orikiriza P, Smith J, Ssekyanzi B, Nyehangane D, Taremwa IM, Turyashemererwa E, et al. Tuberculosis diagnostic accuracy of stool Xpert MTB/RIF and urine AlereLAM in vulnerable children. European Respiratory Journal. 2022;59(1).
14. Kabir S, Rahman SM, Ahmed S, Islam MS, Banu RS, Shewade HD, et al. Xpert Ultra assay on stool to diagnose pulmonary tuberculosis in children. Clinical Infectious Diseases. 2021;73(2):226-34.
15. MacLean E, Sulis G, Denkinger CM, Johnston JC, Pai M, Ahmad Khan F. Diagnostic Accuracy of Stool Xpert MTB/RIF for Detection of Pulmonary Tuberculosis in Children: a Systematic Review and Meta-analysis. J Clin Microbiol. 2019;57(6).
16. StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC. Available online: https://www.stata.com/support/faqs/resources/citing-software-documentation-faqs/ (accessed on 25 June Month 2021).
17. KNCV Tuberculosis Foundation. Available online: https://www.kncvtbc.org/uploaded/2021/03/Stoolbox-SOP1.pdf (accessed on 15 May 2021)
18. Kobo Toolbox. Available online: https://support.kobotoolbox.org/kobocollect_on_android_latest.html (accessed on 18 May 2021)
19. Sun L, Liu Y, Fang M, Chen Y, Zhu Y, Xia C, Jia J, Quan S, Wang Y, Tian X, Shi Y, Duan L, Shi X, Liao Q, Wan C, Shen A. Use of Xpert MTB/RIF Ultra assay on stool and gastric aspirate samples to diagnose pulmonary tuberculosis in children in a high-tuberculosis-burden but resource-limited area of China. Int J Infect Dis. 2022 Jan;114:236-243. doi: 10.1016/j.ijid.2021.11.012. Epub 2021 Nov 10. PMID: 34774781.
20. Senjuti Kabir, S M Mazidur Rahman, Shakil Ahmed, Md Shamiul Islam, Rupali Sisir Banu, Hemant Deepak Shewade, Pruthu Thekkur, Sayeeda Anwar, Nazneen Akhter Banu, Rumana Nasrin, Mohammad Khaja Mafij Uddin, Sabrina Choudhury, Shahriar Ahmed, Kishor Kumar Paul, Razia Khatun, Mohammod Jobayer Chisti, Sayera Banu, Xpert Ultra Assay on Stool to Diagnose Pulmonary Tuberculosis in Children, Clinical Infectious Diseases, Volume 73, Issue 2, 15 July 2021, Pages 226–234, https://doi.org/10.1093/cid/ciaa583
21. Hasan Z, Shakoor S, Arif F, Mehnaz A, Akber A, Haider M, Kanji A, Hasan R. 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 8;10(1):473. doi: 10.1186/s13104-017-2806-3. PMID: 28886729; PMCID: PMC5591572.
22. Dubale M, Tadesse M, Berhane M, Mekonnen M, Abebe G (2022) Stool-based Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children at a teaching and referral hospital in Southwest Ethiopia. PLoS ONE 17(5): e0267661. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0267661
23. Nicol MP, Spiers K, Workman L, Isaacs W, Munro J, Black F, Zemanay W, Zar HJ. Xpert MTB/RIF testing of stool samples for the diagnosis of pulmonary tuberculosis in children. Clin Infect Dis. 2013 Aug;57(3):e18-21. doi: 10.1093/cid/cit230. Epub 2013 Apr 11. Erratum in: Clin Infect Dis. 2014 Jul 1;59(1):145. PMID: 23580738; PMCID: PMC3703104.
24. 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 Feb 1;21(2):161-166. doi: 10.5588/ijtld.16.0357. PMID: 28234079; PMCID: PMC5234433.

 doi:10.20944/preprints202305.1935.v1