# Evaluation of Xpert MTB/RIF Ultra for the diagnosis of childhood pulmonary tuberculosis using stool specimen

**[Final Project Report]**

**[March 31, 2020]**

**Dr. Sayera Banu**

**Senior Scientist & Head**

**Programme on Emerging Infections**

**Infectious Diseases Division**

**Project Title: Evaluation of Xpert MTB/RIF Ultra for the diagnosis of childhood pulmonary tuberculosis using stool specimen**

**Principal Investigator: Dr. Sayera Banu, Senior Scientist and Head, Programme on Emerging Infections, Infectious Diseases Division**

**Period Covered by This Report: January 2018 to September 2019**

**Supported by: USAID's Research for Decision Maker's Activity**

**Collaborating Partner: National Tuberculosis Control Program, Directorate General of Health Services (DGHS)**

## Disclaimer

**This research study was produced with the support of the United States Agency for International Development (USAID) under the terms of USAID's Research for Decision Makers (RDM) Activity cooperative agreement no. AID-388-A-17-00006. Views expressed herein do not necessarily reflect the views of the U.S. Government or USAID. icddr,b is also grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing unrestricted/institutional support.**

# Table of Contents

## Background:

Tuberculosis (TB) is one of the top ten leading causes of death worldwide. According to World Health Organization (WHO), an estimated 10.0 million people developed TB and 1.45 million died from the disease in 2018 (1). Bangladesh is both a high TB and multi-drug resistant TB (MDR-TB) burden country. With a population of 161 million, Bangladesh, one of the 30 high TB and MDR TB burden countries is a home to an estimated 357,000 new cases per year (1). The incidence of TB (all forms) is estimated at 221 per 100,000 population per year and TB mortality is estimated at 29 per 100,000 population per year. The rate of multi-drug resistant MDR-TB is 1.5% among new cases and 4.9% among retreatment cases (1).

According to the World Health Organization (WHO), in 2019, an estimated one million (11% of the total) children (<15 years) were affected with tuberculosis (TB) globally and 170,000 children died in the same year due to TB (1). In Bangladesh, around 10,000 (4%) of total reported TB cases are children (1). The reasons behind this existing gap may be due to under-diagnosis and under-reporting of childhood TB (child TB) cases.

TB diagnosis always remains challenging in children due to their inability to produce good-quality sputum as sputum is essential for TB diagnosis. Induced sputum and gastric aspirate are although collected as alternative, these are invasive procedures, require well-trained staff, a good laboratory and a good hospital set up. All of these may not be available in low resource settings. Moreover bacteriological confirmations from these specimens are rarely achieved due to paucibacillary nature of disease (2-5). Hence, diagnosis is mostly made clinically (usually involves a tuberculin skin test to diagnose infection, chest radiography, and clinical signs and symptoms) that leads to more chance of over and under diagnosis. To overcome this gap, an alternative easy-to-collect specimen is required for child TB diagnosis.

Children usually swallow sputum. After swallow, *Mycobacterium tuberculosis* (MTB) can pass through the gastro intestinal tract and thus can be detected in stool of patients with pulmonary TB (PTB). Several studies found stool to be a promising specimen for both adults and children that can be tested with different laboratory tests including the more sensitive Xpert MTB/RIF assay (Xpert) (6-12). Recent studies have successfully detected MTB from stool specimen using Xpert assay. However, these studies were performed on a small number of samples. In 2013, a team from South Africa, performed Xpert testing on stool specimens from 115 children with suspected PTB and Xpert detected 47% of bacteriologically confirmed TB cases (n=17) (2). Another study in 2014 in Kenya, used stool specimen from 91 children with suspected PTB and showed 100% (6/6) detection by Xpert

among smear positive cases. They also showed 7.5% detection by Xpert among smear negative cases (11). A recent study from Zimbabwe showed that Xpert detected 68% bacteriologically confirmed cases using stool specimens (8). Moreover, we have optimized the Xpert assay for successful detection of PTB using stool specimens from adult subjects in our laboratory. Xpert assay detected MTB in 89.3% of stool specimens from sputum smear microscopy positive cases, whereas all stool specimens from healthy control were negative by the assay. Compared with the sputum culture positive results, the sensitivity of the Xpert assay was 92.6% (11). The limit of detection (LOD) for Xpert is 131 CFU/ml of sputum specimen therefore any specimen with MTB less than this LOD would be missed by Xpert testing (13). Recently, an updated version, Xpert MTB/RIF Ultra (Xpert Ultra) has been developed with higher sensitivity (LOD of 16 CFU/ml) (13). This updated version can detect as low bacteria as liquid culture and also improved RIF resistance determination. Four newly developed RT-PCR probes replaced the five probes of previous version and can discriminate synonymous mutations. Also, incorporation of probes for IS*1081* along with IS*6110* region, improved the MTB detection from raw specimens (14). Ease of use of Xpert Ultra is also similar to Xpert. So, it is expected that, Xpert Ultra assay should significantly increase TB detection in smear-negative patients and provide more reliable RIF resistance detection. As childhood TB specimens are paucibacillary, Xpert Ultra could be an effective tool for the detection of MTB from the stool specimen (7). Recently, WHO has endorsed Xpert Ultra as initial tests using different specimens for paucibacillary TB detection (child TB, extra pulmonary TB etc.) (15). Limited studies have evaluated Xpert Ultra for child TB diagnosis using respiratory specimen (7), however, no study has been found on stool specimen yet.

In this study, we have evaluated the diagnostic performance of Xpert Ultra for child TB detection using stool specimen.

## Objective:

To evaluate the diagnostic performance of Xpert Ultra using stool in children with symptoms suggestive of pulmonary TB

## *Specific Objectives:*

1. To determine the sensitivity of Xpert Ultra test to diagnose childhood pulmonary TB compared to bacteriologically positive TB cases using stool specimen
2. To determine the specificity of Xpert Ultra test to rule out childhood pulmonary TB compared to bacteriologically negative TB cases using stool specimen

## Methodology:

### *Study design:*

This is a cross-sectional study by design.

### *Study sites and preparation for study:*

1. Presumptive child PTB patients (<15 years of age) were enrolled from four tertiary health care facilities. The study sites were-
2. Sir Salimullah Medical College and Mitford Hospital (SSMCH)
3. Shaheed Suhrawardy Medical College and Hospital (ShSMCH)
4. Dhaka Medical College Hospital (DMCH)
5. icddr,b Dhaka Hospital (icddr,b hospital)

A memorandum of understanding (MOU) was developed between icddr,b and the participating hospitals that included a brief description of the study, methodology, work plan, terms and conditions, recruitment of study physicians and nurses, and other administrative issues. The MoUs were signed with the authorities of all participating hospitals before starting the study activities at those sites.

### *Staff training:*

Field (one field research assistant/FRA and four field assistants/FA) and laboratory staff were trained properly regarding study activities and their responsibilities. One FA was placed at paediatric department of each hospital for carrying out field activities and the FRA was responsible for supervising these activities (Photos 1 & 2).

**Photo 1 & 2: Staff training**

### *Orientation programme:*

Orientation programme was arranged at the paediatric department of the selected hospitals to sensitize the physicians regarding the study activities. The orientation programmes were held before initiation of study activities (Photos 3 & 4).


**Photos 3 & 4: Orientation programme at paediatric departments of study hospitals**

### *Enrolment of study participants:*

The presumptive children were enrolled into the study upon receiving informed consent or assent from the participants. Socio-demographic and clinical data were collected from them and, stool and respiratory specimens were collected for smear for AFB microscopy, Xpert Ultra and culture and drug susceptibility testing (DST) in Löwenstein–Jensen (L-J) media. The study activities have been detailed in Figure 1.

### *Case definition:*

***Definition: "Presumptive pulmonary tuberculosis"***

There are some common criteria recommended by "National Guidelines for the Management of Tuberculosis in Children" for suspecting a child to have PTB those were followed by the physicians (16). Those include-

- "persistent, non-remitting cough for >2 weeks not responding to conventional antibiotics, and/or
- persistent documented fever (>38°C/100.4°F) for >2 weeks, and/or

- documented weight loss or not gaining weight during the past 3 months, and/or
- Fatigue, reduced playfulness, decreased activity"

### *Inclusion criteria:*

- Children of 0 to <15 years of age with symptoms suggestive for PTB irrespective of nutritional status
- Children of same age group with informed consent from parents/guardians and assent where applicable (for 11-<15 years old children )

### *Exclusion criteria:*

- Children having other serious co-morbid conditions (patient in Intensive Care Unit, any heart condition etc.) when physician will not be able to collect respiratory specimens
- Children who already have started anti-TB treatment
- Children who are suspected clinically as intestinal TB

### *Study period:*

Presumptives were enrolled between the period of January 2018 and April 2019.

### *Sample size:*

We calculated the sample size using formulas with expected sensitivity and specificity of the diagnostic test evaluated in this study.

The formula using sensitivity was: $n_{Se} = \frac{Z^2\, Se\,(1- Se)}{d^2 \text{ X Prev}}$ , and,

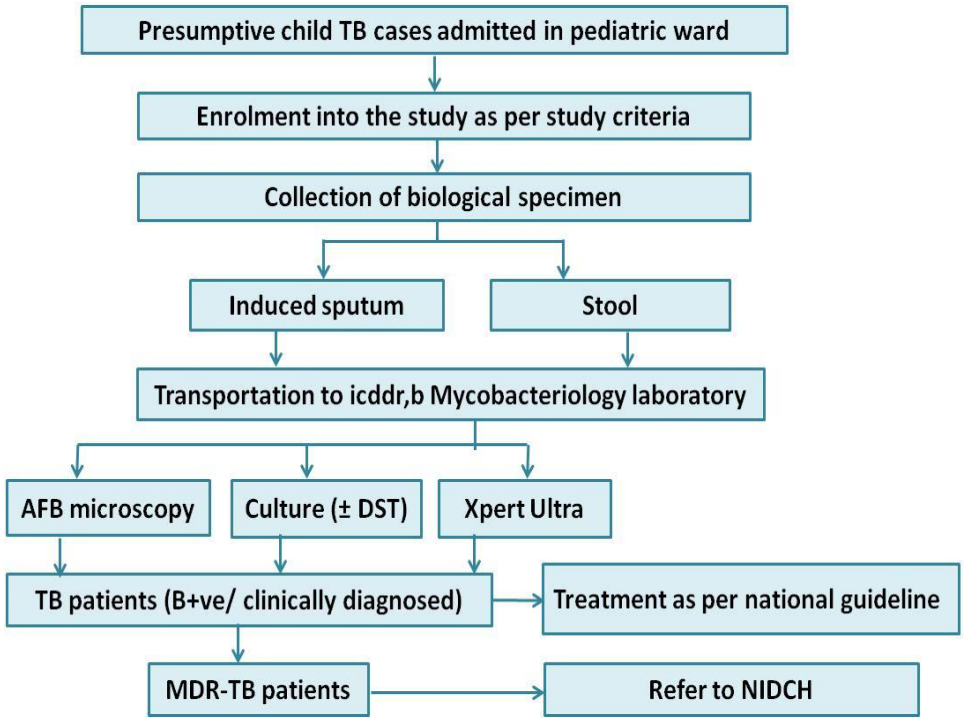
formula using specificity was: $n_{Sp} = \frac{Z^2\, Sp\,(1- Sp)}{d^2 \text{ X } (1- \text{Prev})}$

Here, n= sample size, z= at 95% CI, 1.96; Se= sensitivity, Sp= specificity, d= precision, Prev=prevalence.

To detect sensitivity of 80% and specificity of 95% of Xpert Ultra assay at 10% level of precision when TB prevalence is expected to be 15% among presumptive children, our calculated sample size was 435 by using sensitivity formula and 23 by using specificity formula. Here we considered 5% loss to follow up/incomplete data. We considered the large

calculated sample size or this study. During the study period, 454 presumptive child TB cases were enrolled from the selected health care facilities.

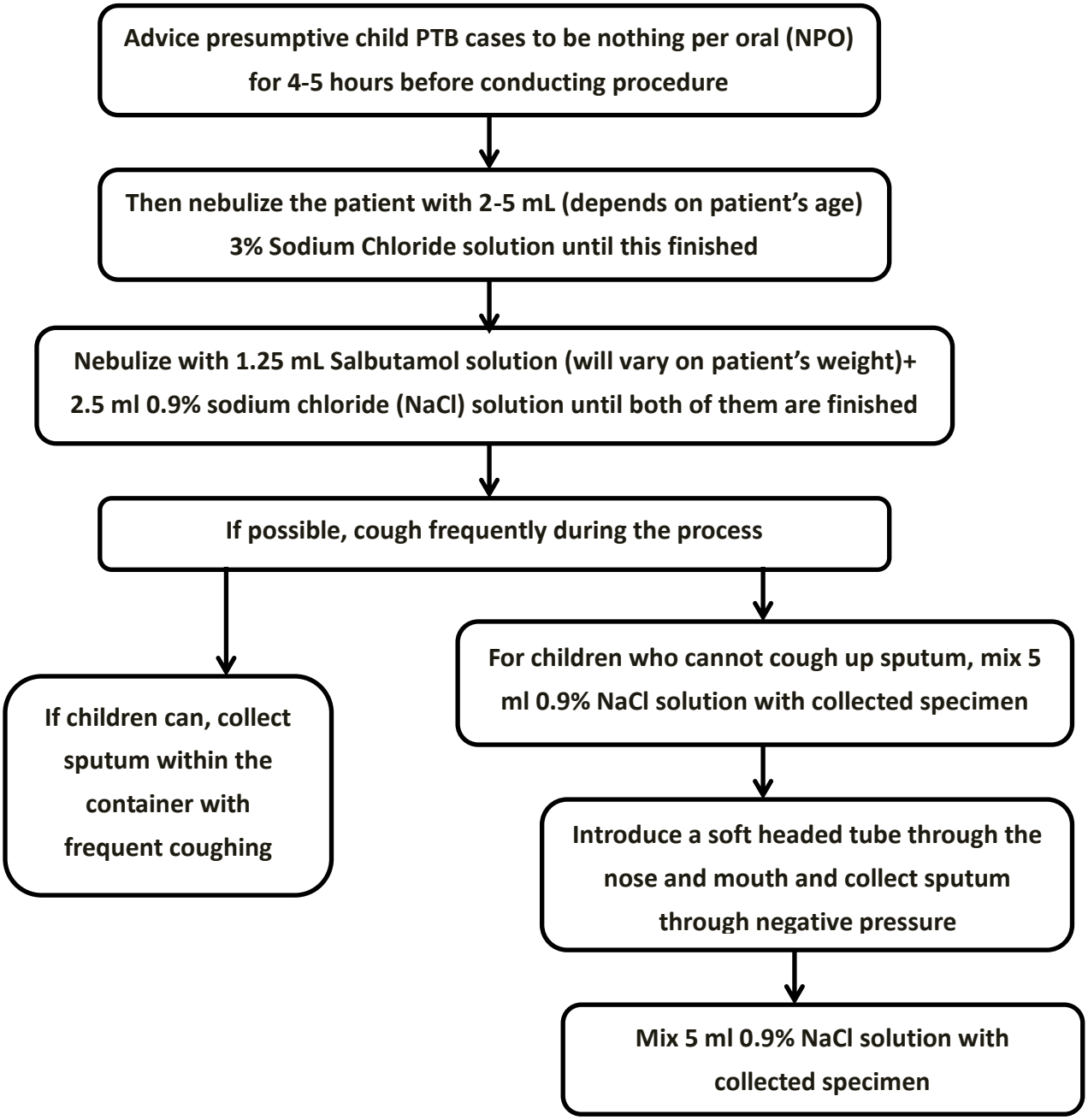
**Figure 1: Study flowchart**




### *Specimen collection and transportation:*

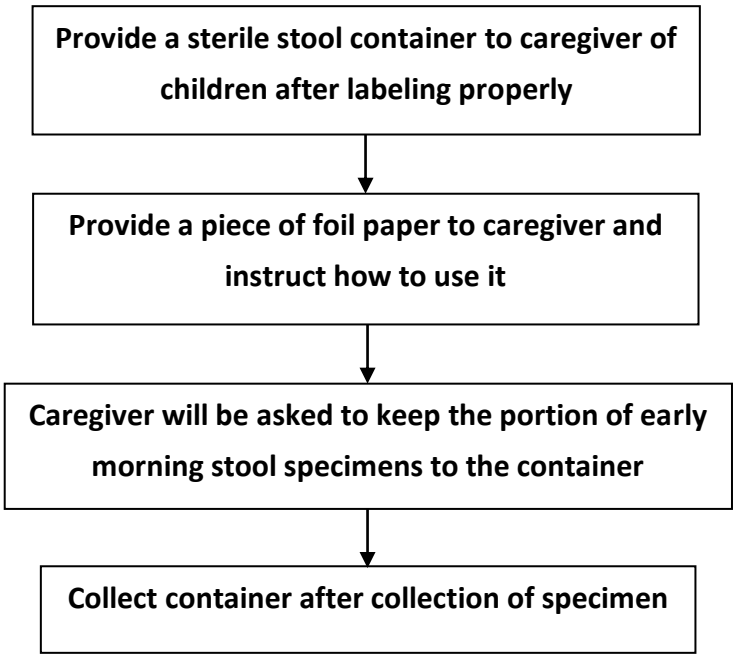
Respiratory specimens were collected from the presumptive child patients in empty stomach. The local physicians were responsible for specimen collection. Upon receiving informed consent or assent, the specimens were collected. The children were instructed to keep nothing per mouth for four to five hours prior to the procedure. Physicians and concerned staff members collected the specimens maintaining the standard procedures. The steps of induced sputum collection are given in Figure 2.

**Figure 2: Steps of induced sputum collection**




For stool specimen collection, the FAs instructed properly and provided a labeled sterile container to the parents/ guardians of the respective child patients after completing the interview. The parents were asked to keep the portion of early morning stool specimens to the container. The next morning FAs collected the specimen from the parents/caregivers (Figure 3).

**Figure 3: Steps of stool specimen collection**



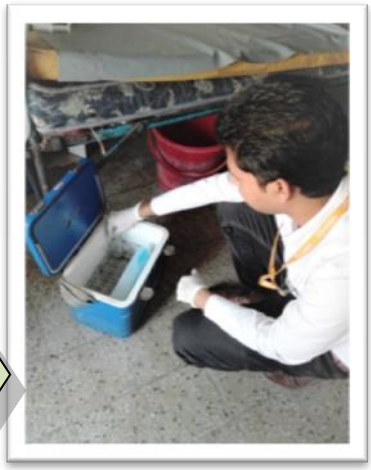

After collection of specimens at hospitals, the FAs stored them in cool box temporarily and then transported them to the mycobacteriology laboratory, icddr,b on the same working day for laboratory test performances (Photos 5-8).

**Photo 5: Taking informed written consent**

**Photo 6: Data collection**

**Photo 7: Collection of induced sputum**

**Photo 8: Temporary storage of specimens in a cool box**



*Laboratory procedures:*

***Induced sputum decontamination and processing***

Induced sputum is decontaminated and concentrated according to the Petroff's NaOH method (14). Briefly, equal volume of NALC-NaOH-Na-citrate solution (0.5% N-acetyl-L-citrate, 4% NaOH, and 2.94% Na-citrate) are added into the centrifuge tube and incubated for 15 minutes at room temperature. The tube are then filled with sterile phosphate buffer saline (PBS) (pH 6.8) up to 45 ml mark and centrifuged at 3000g for 15 minutes. After centrifugation, the supernatant are discarded and the resultant sediments were saved for further testing.

After processing of both stool and respiratory specimen, sediments were used for microscopy. A loop-full of processed sample were stained by Ziehl-Neelsen (ZN) staining following standard procedure.

***Stool processing***

Stool specimen is processed according to laboratory optimized method. Approximately, 2 gm of stool from each subject is taken into a 50 ml centrifuge tube. Equal volume of sterile normal saline (0.9% NaCl) is added to the stool specimen and mixed well by vortexing. Then normal saline is added up to 30 ml mark in the centrifuge tube and incubated at room temperature for 30 minutes. After incubation, 10 ml of supernatant is transferred into a new 50 ml centrifuge tube and then decontaminated and concentrated following the

Petroff's NaOH method (15). Briefly, equal volume of NALC-NaOH-Na-citrate solution (0.5% N-acetyl-L-citrate, 4% NaOH, and 2.94% Na-citrate) is added into the centrifuge tube and incubated for 20 minutes at room temperature. The tube is then filled with sterile phosphate buffer saline (PBS) (pH 6.8) up to 40 ml mark and centrifuged twice at 3000g for 20 minutes. After centrifugation, the supernatant is discarded and the resultant sediment is saved for smear microscopy, Xpert Ultra, and culture tests.

***Smear microscopy***

After processing of both stool and respiratory specimen, sediment was used for microscopy. A loop-full of processed sample was stained by Ziehl-Neelsen (ZN) staining following standard procedure.

***Culture and susceptibility testing***

Culture and antibiotic susceptibility testing of *M. tuberculosis* strains were performed according to conventional methods (17). The processed specimens were inoculated on 2 Lowenstein Jensen (L-J) slants. The L-J slants were incubated at 37°C for 8 weeks and examined once every week for any growth of visible mycobacterial colony as well as contamination. After getting sufficient culture growth, a standard suspension of *M. tuberculosis* isolates were inoculated onto L-J media containing antimicrobial agents and also onto control L-J media without any antimicrobial agent. Isolates were considered resistant to a particular concentration of drug when 1% or more colonies grow on the drug-containing medium when compared to the drug-free medium.

***Xpert MTB/RIF Ultra assay***

A portion of processed stool and IS were tested with Xpert MTB/RIF Ultra cartridges according to manufacturer's instruction. The test integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection. Sample reagent buffer were added to the aliquoted sample in a 2:1 ratio. The closed container containing sample and buffer mixture were manually agitated twice during a 15 minutes of incubation period at room temperature before 2 ml of the inactivated mixture were transferred to the Xpert test cartridge. Cartridges were inserted into the test platform and the automatically generated results were read.

According to the WHO Technical Expert Consultation findings on Xpert® MTB/RIF Ultra assay "trace calls" should be considered to be true positive results for use in clinical decisions in children. However, it has been recommended that, in case of "trace call" result, a fresh specimen from the patient needs to be collected for repeat testing. If the repeat result is "positive", it will be considered as "positive" to diagnosis of PTB and if it is "negative" it will be considered as negative.

### *Delivery of laboratory test Results, TB diagnosis and management:*

All the laboratory test reports were provided to the local physicians and to the parents/guardians of the child patients upon their availability. However, all the diagnoses were made by the local physicians and the treatment were initially started at the respective hospitals' DOTS facility as per national guideline. After discharge, the patients were referred to the nearest DOTS centres to continue the TB treatment and follow up as per national guideline.

### *Statistical analysis:*

We analyzed data using SPSS version 20. We used proportions to summarise the socio-demographic details, nutritional status and symptom profile.

The children who were bacteriologically positive in any of the tests performed (smear microscopy/culture/Xpert Ultra) on either induced sputum or stool specimens were considered as 'bacteriologically confirmed'. The children who were positive on induced sputum were considered as 'bacteriologically confirmed on induced sputum' and on stool specimens were considered as 'bacteriologically confirmed on stool specimen'. We used Venn-diagram to depict the bacteriological confirmation using smear microscopy, culture and Xpert Ultra on induced sputum and stool specimens.

We calculated the sensitivity and specificity with 95% confidence interval (CI) for detecting PTB for Xpert Ultra using stool specimen with 'bacteriologically confirmed on induced sputum' as reference (4). This was done as culture positivity on induced sputum might be very low in children due to paucibacillary nature of TB and also use of solid culture which has relatively lower yield than the liquid culture (7). We also calculated the sensitivity and specificity of Xpert Ultra on stool specimen considering 'trace call' on Xpert Ultra as negative. We have also presented the above results using 'bacteriologically confirmed on induced sputum culture' as the reference test.

We calculated proportion of children with presumptive PTB positive for individual and combination of tests against 'bacteriologically confirmed', 'diagnosed as TB' and 'all enrolled'.

## Results:

### *Baseline characteristics:*

Among 454 presumptive child TB patients, seven (1.4%) could not provide stool specimens (Figure 4). However, laboratory reports of their induced sputum were delivered to the respective physicians and parents/caregivers and, all were negative.

Among 447 included, 296 (66.2%) were aged less than five years, 254 (56.8%) were males, 143 (48.3%) of under five years old children were severely malnourished and 44 (29.1%) children aged 11-14 years were severely thin (Table 1). During enrolment, 321 (71.8%) had cough for >2 weeks, 306 (68.5%) had fever for >2 weeks, 381 (85.2%) had significant weight loss and 114 (25.5%) had contact with TB patient in family within last one year (Table 1). The demographic, nutritional status and clinical profiles of children with presumptive PTB, PTB and non TB patients have been detailed in Table 1.

**Table 1: Demographic, nutritional status and clinical profile of children (<15 years) with presumptive PTB and PTB and non TB patients enrolled from selected four tertiary care hospitals of Dhaka, Bangladesh during January-2018 to April-2019, N=447**

| Characteristics | | Total (N=447) | | Bacteriologically confirmed on induced sputum (N=29) | | Bacteriologically confirmed on stool but negative on induced sputum (N=43) | | Clinically diagnosed (N=39) | | Non TB (N=336) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number | (%)[$] | Number | (%)[$] | Number | (%)[$] | Number | (%)[$] | Number | (%)[$] |
| Age (in years) | | | | | | | | | | | |
| | 0-4 | 296 | (66.2) | 15 | (51.7) | 29 | (67.4) | 22 | (56.4) | 230 | (68.5) |
| | 5-9 | 105 | (23.5) | 8 | (27.6) | 11 | (25.6) | 14 | (35.9) | 72 | (21.4) |
| | 10-14 | 46 | (10.3) | 6 | (20.7) | 3 | (7.0) | 3 | (7.7) | 34 | (10.1) |
| Gender | | | | | | | | | | | |
| | Male | 254 | (56.8) | 14 | (48.3) | 24 | (55.8) | 25 | (64.1) | 191 | (56.8) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | 193 | (43.2) | 15 | (51.7) | 19 | (44.2) | 14 | (35.9) | 145 | (43.2) |
| Nutritional status* | | | | | | | | | | | |
| <5 years (n=296)¥ | No malnutrition | 77 | (26) | 2 | (13.3) | 6 | (20.7) | 3 | (13.6) | 66 | (28.7) |
| | Moderate malnutrition | 76 | (25.7) | 5 | (33.3) | 8 | (27.6) | 6 | (27.3) | 57 | (24.8) |
| | Severe malnutrition | 143 | (48.3) | 8 | (53.3) | 15 | (51.7) | 13 | (59.1) | 107 | (46.5) |
| ≥5 years (n=151) ¶ | Overweight and obesity | 4 | (2.6) | 0 | (0.0) | 0 | (0.0) | 0 | (0.0) | 4 | (3.8) |
| | Normal | 55 | (36.4) | 2 | (14.3) | 8 | (57.1) | 4 | (23.5) | 41 | (38.7) |
| | Thinness | 48 | (31.8) | 6 | (42.9) | 2 | (14.3) | 6 | (35.3) | 34 | (32.1) |
| | Severe | 44 | (29.1) | 6 | (42.9) | 4 | (28.6) | 7 | (41.2) | 27 | (25.5) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | thinness | | | | | | | | | |
| Cough | | | | | | | | | | |
| | No cough | 17 | (3.8) | 0 | (0.0) | 2 | (4.7) | 1 | (2.6) | 14 | (4.2) |
| | Less than 2 weeks | 109 | (24.4) | 8 | (27.6) | 12 | (27.9) | 7 | (17.9) | 82 | (24.4) |
| | More than 2 weeks | 321 | (71.8) | 21 | (72.4) | 29 | (67.4) | 31 | (79.5) | 240 | (71.4) |
| Fever | | | | | | | | | | |
| | No fever | 17 | (3.8) | 0 | (0.0) | 1 | (2.3) | 2 | (5.1) | 14 | (4.2) |
| | Less than 2 weeks | 124 | (27.7) | 1 | (3.4) | 14 | (32.6) | 7 | (17.9) | 102 | (30.4) |
| | More than 2 weeks | 306 | (68.5) | 28 | (96.6) | 28 | (65.1) | 30 | (76.9) | 220 | (65.5) |
| Other | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| symptoms[#] | | | | | | | | | | |
| | Loss of appetite | 399 | (89.3) | 27 | (93.1) | 36 | (83.7) | 37 | (94.9) | 299 | (89.0) |
| | Night sweats | 278 | (62.2) | 21 | (72.4) | 27 | (62.8) | 28 | (71.8) | 202 | (60.1) |
| | Significant weight loss | 381 | (85.2) | 26 | (89.7) | 37 | (86.0) | 36 | (92.3) | 282 | (83.9) |
| | Decreased activity | 419 | (93.7) | 28 | (96.6) | 41 | (95.3) | 36 | (92.3) | 314 | (93.5) |
| | Nutritional oedema | 12 | (2.7) | 0 | (0.0) | 1 | (2.3) | 0 | (0.0) | 11 | (3.3) |
| | Previous history of TB | 11 | (2.5) | 0 | (0.0) | 1 | (2.3) | 1 | (2.6) | 9 | (2.7) |
| | History of TB contact in family | 114 | (25.5) | 8 | (27.6) | 10 | (23.3) | 18 | (46.2) | 78 | (23.2) |

* Nutritional status as assessed using WHO z scores based on height, weight and age

[#] As reported by the parents / care giver and multiple answers are possible

[$] Percentage calculated out of 'N'

[¥] Any of height for age (HAZ), weight for age (WAZ) and weight for height (WAZ) measuring: up to -2: no malnutrition, <-2 to -3: moderate malnutrition and <-3 as severe malnutrition was considered.

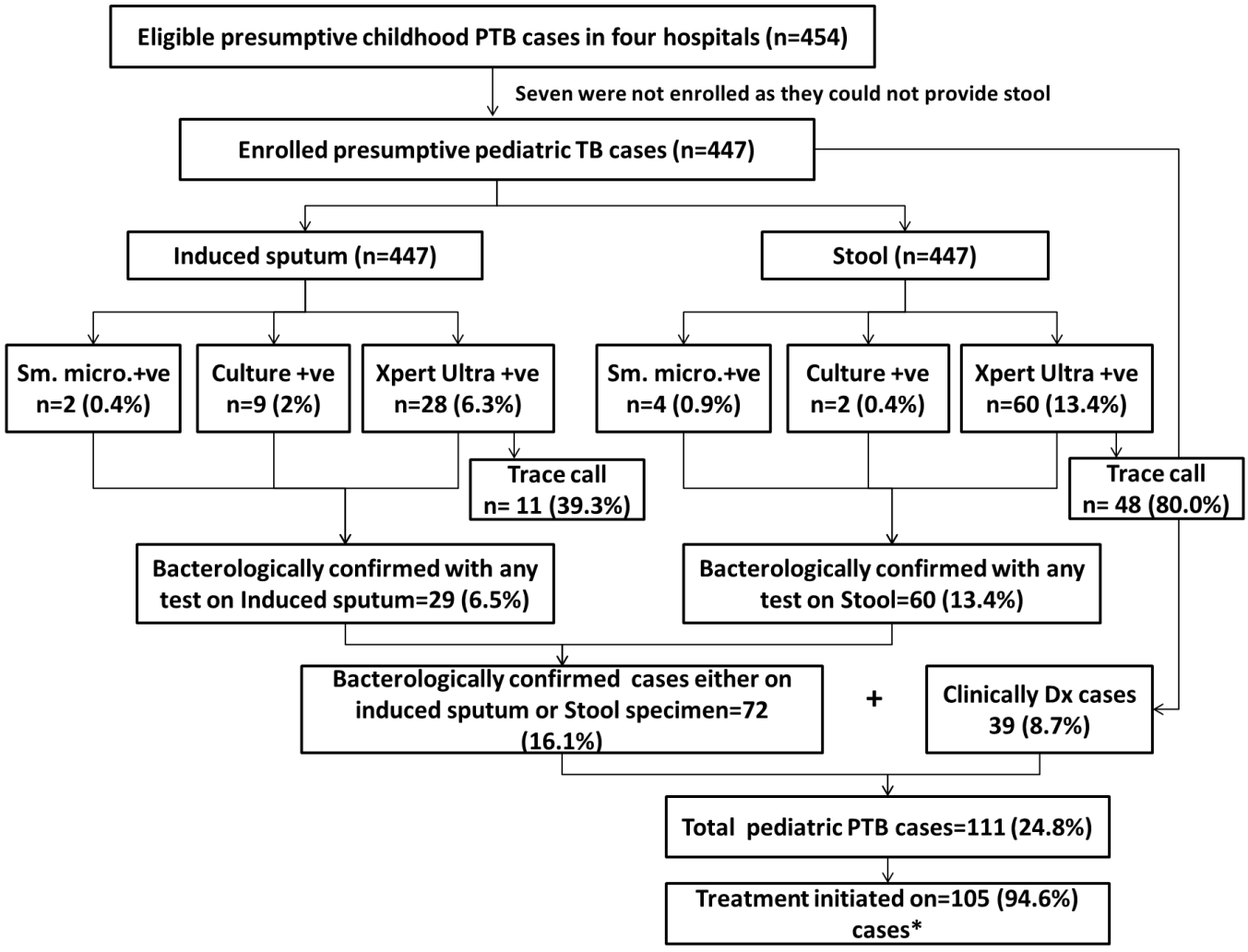
¶ BMI for age was measured and categorized as per WHO growth card for 5 to 19 years old children

PTB-pulmonary tuberculosis; TB-tuberculosis

*Results of laboratory investigations, diagnosis of TB and treatment:*

With induced sputum, two (0.4%) were positive by microscopy, nine (2%) by culture and 28 (6.3%) by Xpert Ultra (Figure 4). Of the 28 Xpert Ultra positive specimen, 11 (39.3%) had 'trace call'. In total, 29 (6.5%) were bacteriologically confirmed on induced sputum (Figure 4), of which, 20 (69%) were exclusively diagnosed with Xpert Ultra (Figure 5). With stool specimen, four were positive by microscopy (0.9%), two (0.4%) by culture and 60 (13.4%) by Xpert Ultra (Figure 4). Of the Xpert Ultra positive specimens, 48 (80%) had 'trace call'. In total, 60 (13%) were bacteriologically confirmed on stool specimen (Figure 4), of which, 56 (93%) were exclusively diagnosed with Xpert Ultra (Figure 5). One induced sputum specimen was positive by culture which was negative by any test using both specimens.

**Figure 4: Flow Chart depicting the enrollment, investigation results and treatment of children (<15 years) with presumptive PTB admitted in selected four tertiary care hospitals of Dhaka, Bangladesh during January-2018 to April-2019, N=447**



PTB: pulmonary tuberculosis

* One stool specimen showed invalid result on Xpert MTB/RIF Ultra

In total, 72 (16.1%) were bacteriologically confirmed (Figure 4), of which, 43 (59.7%) were exclusively detected through Xpert Ultra on stool specimen (Table 2).

**Table 2: Diagnostic validity of Xpert MTB/RIF Ultra assay on stool specimen compared with bacteriological confirmation with induced sputum specimen among children (<15 years) with presumptive PTB enrolled from selected four tertiary care hospitals of Dhaka, Bangladesh during January-2018 to April-2019, N=446[#]**

| Tests on Stool Specimen | | Bacteriological confirmation with induced sputum | | | | Sensitivity (%) (95% CI) | Specificity (%) (95% CI) |
|---|---|---|---|---|---|---|---|
| | | Pos | | Neg | | | |
| | | N | (%)* | n | (%)* | | |
| Total | | 29 | (100.0) | 417 | (100.0) | | |
| Xpert MTB/RIF Ultra | Pos | 17 | (58.6) | 43 | (10.3) | 58.6 (40.7-74.5) | 89.7 (86.4-92.3) |
| | Neg | 12 | (41.4) | 374 | (89.7) | | |
| Xpert MTB/RIF Ultra (trace call as negative) | Pos | 11 | (37.9) | 1 | (0.2) | 37.9 (22.7-56.0) | 99.8 (98.7-100.0) |
| | Neg | 18 | (62.1) | 416 | (99.8) | | |

* Column percentage

# One stool specimen showed invalid result on Xpert Ultra

PTB-pulmonary tuberculosis; Pos-positive; Neg-negative

A total of 39 (8.7%) children were diagnosed clinically. Of 111 (bacteriologically positive-72; clinically diagnosed-39) children diagnosed with TB, 105 (94.6%) were initiated on anti-TB treatment (Figure 4). Six children (5.4%) positive in Xpert Ultra on stool specimen did not receive anti-TB treatment. Five of them were not treated based on physicians' suggestion and parents of another child refused treatment. All the five children who were not initiated

on anti-TB treatment based on physicians' suggestion were followed up over phone for the six months and they did not develop active TB.

***Diagnostic validity of Xpert Ultra assay on stool specimen***

The sensitivity and specificity of Xpert Ultra were found 58.6% (95% CI, 40.7-74.5) and 89.7% (95% CI, 86.4-92.3) respectively when compared to the 'bacteriologically confirmed on induced sputum' children with Xpert Ultra as reference standard. On considering 'trace call' as negative on Xpert Ultra, the sensitivity was found 37.9% (95% CI, 22.7-56.0) (Table 2).

Compared to 'culture on induced sputum' as reference test, the sensitivity and specificity of Xpert Ultra on stool specimen were 88.9% (95% CI, 56.5-98.0) and 88.1% (95% CI, 84.7-90.8) respectively. The sensitivity was 77.8% (95% CI, 97.4-99.5) on considering 'trace call' as negative on Xpert Ultra, (Table 3).

**Table 3: Diagnostic validity of Xpert MTB/RIF Ultra assay on stool specimen compared with culture results of induced sputum specimen among children (<15 years) with presumptive PTB enrolled from selected four tertiary care hospitals of Dhaka, Bangladesh during January-2018 to April-2019, N=446[#]**

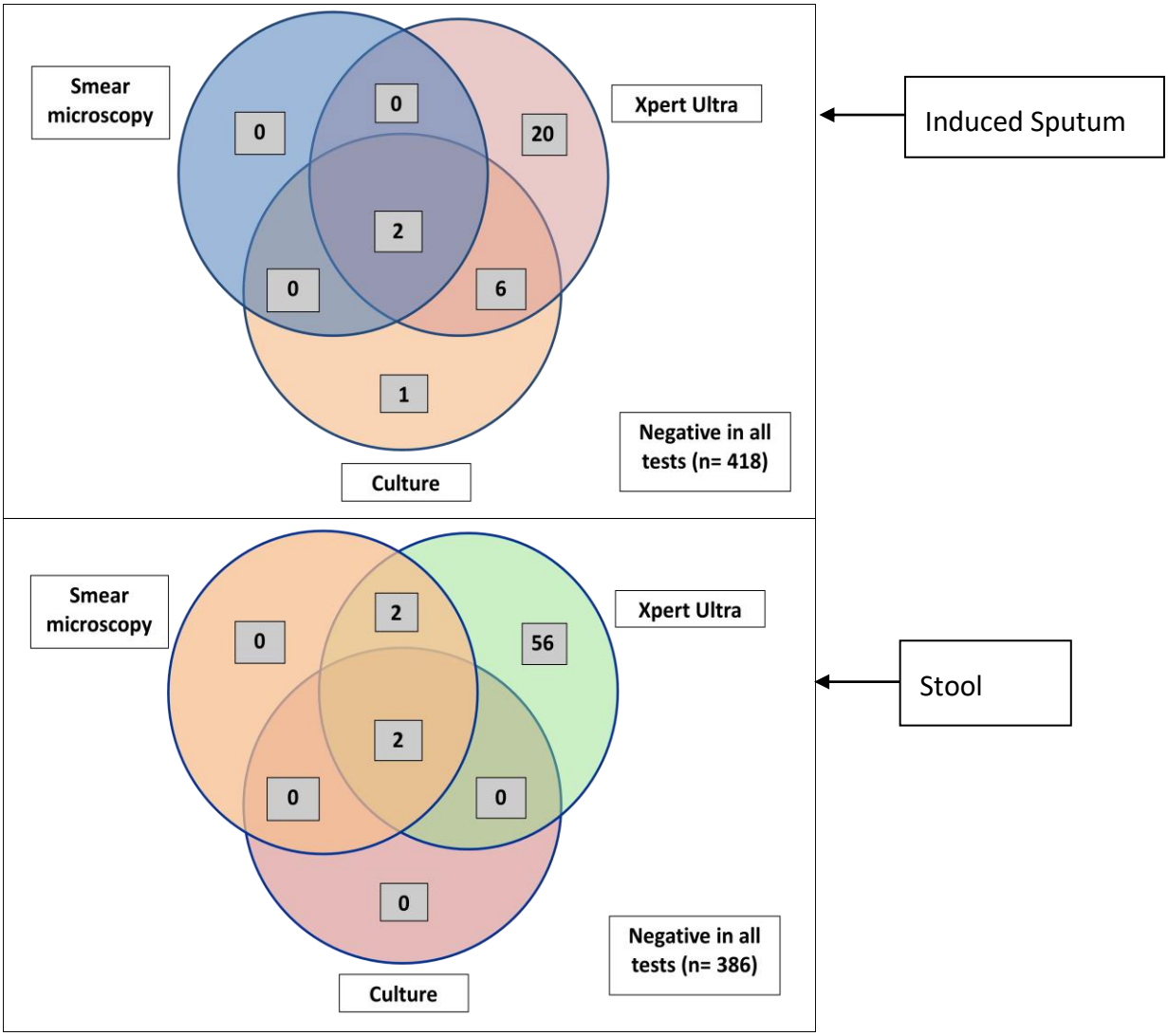
| Investigation | | Culture on induced sputum | | | | Sensitivity (%) (95% CI) | Specificity (%) (95% CI) |
|---|---|---|---|---|---|---|---|
| | | Pos | | Neg | | | |
| | | n | (%)* | n | (%)* | | |
| Total | | 9 | (100.0) | 438 | (100.0) | | |
| Xpert MTB/RIF Ultra | Pos | 8 | (88.9) | 52 | (11.9) | 88.9 (56.5-98.0) | 88.1 (84.7-90.8) |
| | Neg | 1 | (11.1) | 385 | (88.1) | | |
| Xpert MTB/RIF Ultra (trace call as negative) | Pos | 7 | (77.8) | 5 | (1.1) | 77.8 (45.3-93.7) | 98.9 (97.4-99.5) |
| | Neg | 2 | (22.2) | 432 | (98.9) | | |

* Column percentage

# One stool specimen showed invalid result on Xpert Ultra

PTB-pulmonary tuberculosis; Pos-positive; Neg-negative

**Figure 5: Venn diagram showing the bacteriological confirmation using smear microscopy, culture and Xpert MTB/RIF Ultra assay on induced sputum ans stool specimens of presumptive cases (n=447)**




***Additional detection of MTB by Xpert Ulra on stool specimen***

Among bacteriologically confirmed specimens (n=71), 43 were exclusively detected by stool and 11 were exclusively detected by induced sputum. It means that, if we would perform Xpert Ultra on induced sputum only, we could miss 43 cases and if we did Xpert Ultra on

stool only, we could miss to detect 11 PTB cases. Xpert Ultra on stool detected 7.2% additional PTB cases in this study (Table 4).

**Table 4. Comparison of Xpert Ultra on induced sputum and stool specimens tested from presumptive children with PTB, n=446[#]**

| Xpert Ultra (Stool) | Xpert Ultra (IS) | | Total |
|---|---|---|---|
| | +ve (%) | -ve (%) | |
| +ve (%) | 17 (60.7) | 43 (10.3) | 60 (13.5) |
| -ve (%) | 11 (39.3) | 375 (89.7) | 386 (86.5) |
| Total | 28 (100.0) | 418 (100.0) | 446 (100.0) |

# One stool specimen showed invalid result on Xpert Ultra

Among bacterilogically confirmed PTB cases (n=71), positivity of Xpert Ultra on induced sputum was 39% and stool was 85% which was significantly different (p<0.001).

## Discussion:

This is the first study globally where the diagnostic performance of Xpert Ultra assay has been assessed on stool specimen for the diagnosis of PTB among children. We have some key findings: i) the sensitivity of Xpert Ultra on stool specimen was higher compared to the previously conducted studies on Xpert on stool specimen; ii) majority (about eight out of ten cases) of positive case on Xpert Ultra on the stool specimen had 'trace call'. On considering 'trace call' as negative, the sensitivity of Xpert Ultra reduced, however, specificity improved; iii) a high proportion of stool specimens positive by Xpert Ultra were negative on induced sputum; iv) a high percentage of bacteriologically confirmed PTB were positive with Xpert Ultra on stool specimen.

***Detail of the key findings and their programmatic implications:***

The sensitivity of Xpert Ultra on stool specimen was higher than the reported sensitivity of 33% to 59% (with culture on induced sputum as reference) for Xpert on stool specimen in

previous studies (4, 18). The potential reason for such high sensitivity of Xpert Ultra might be due to low limit of detection and the ability to detect 'trace call'. The specificity of Xpert Ultra on stool specimen was lower than that reported for Xpert previously (4, 18). However, the specificity of Xpert Ultra on stool specimen cannot be judged due to the low yield of induced sputum.

A high proportion of stool specimens positive with Xpert Ultra had 'trace call'. The proportion of 'trace call' was higher in stool specimen compared to induced sputum. Also, considering 'trace call' as negative reduced the sensitivity of Xpert Ultra. The possible reasons of having 'trace call' in the specimen may be due to the presence of non-replicating TB bacilli in specimens of people with recent anti-TB treatment or due to self-cured (incipient TB resolved without treatment) TB or due to laboratory cross-contamination (19). In our study, none of the children with 'trace call' had previous history of TB and as all children had symptoms suggestive of active TB, it is less likely to be 'incipient TB' (20). Therefore, we feel that, the detected MTB in stool specimen might be due to having active TB disease among these children. However, in spite of adhering to standard operating procedures to reduce cross-contamination in the laboratory, the possibility of cross-contamination during stool processing cannot be ruled out.

A high proportion of stool specimens positive by Xpert Ultra (including 'trace call') were negative by Xpert Ultra on induced sputum. This can be either due to ability of Xpert ultra on stool specimen to detect true positives over and above tests on induced sputum or due to false positive results because of high percentage of 'trace calls'. Also, none of the five children with 'trace call' on stool specimen with Xpert Ultra not initiated on treatment, showed signs of active TB during six months of follow-up. However, the speculation of false positivity of Xpert Ultra with 'trace call' can't be ruled out with finding of these five untreated children. We strongly recommend future research to systematically assess clinical progression and response to treatment among children with and without 'trace call'. This assessment on clinical evolution could provide a better insight into the utility of 'trace call' of Xpert Ultra on stool specimen for diagnosis of PTB in children.

A high percentage of bacteriologically confirmed PTB was positive with Xpert Ultra on stool specimen. The majority were exclusively diagnosed through Xpert Ultra. Moreover, the positivity of Xpert Ultra was significantly higher on stool than induced sputum among the bacteriologically confirmed PTB. Considering 'trace call' as positive, Xpert Ultra on stool specimen detected additional 7% PTB cases. Xpert Ultra on stool specimen has potential to

be adopted as the first-line screening tool in children with presumptive PTB subject to consistent findings of high sensitivity from studies similar to this among ambulatory patients with low pre-test probability and to follow up of children with 'trace call' demonstrating improvement in clinical status with anti-TB treatment. If future studies show consistent results, the Xpert Ultra on stool specimen would be beneficial to reduce delays in diagnosis and invasive procedures especially among ambulatory children referred from peripheral health facilities. The feasibility of Xpert Ultra on stool specimen for diagnosis of children with presumptive PTB from peripheral health facilities and resource poor settings needs to be explored. These explorations could help to get an insight on challenges in collecting and processing stool specimens at such settings. Moreover, the current stool processing method applied in this study is resource intensive and might not be feasible where optimum resources are not available. Further studies could be conducted to simplify and optimize the collection, processing and transportation of samples to facilities with Xpert platforms for testing.

## Conclusions:

In children, Xpert Ultra on stool has better sensitivity compared to the reference test. Moreover, stool has been found to be a superior specimen to induced sputum in diagnosis of child PTB by using Xpert Ultra. A high proportion of Xpert Ultra positive on stool had 'trace call'. Future longitudinal studies on clinical evolution are required to suggest on the management of children with 'trace call'.

## Recommendations:

- Stool has been found to be a superior specimen to IS in child TB diagnosis by using Xpert Ultra
- Stool presents a great opportunity to improve child TB detection when respiratory specimen collection is difficult
- Longitudinal studies on clinical evolution are required to suggest on the management of children with 'trace call'

## Limitations:

1. We included only children admitted to the hospitals and could have had a severe degree of symptoms, the spectrum of disease and a higher pretest probability of

having PTB. Therefore, the overall positivity rate in the study sample might be high and it could have affected the sensitivity and specificity (19, 20). Hence, the study results might not be generalizable to the ambulatory presumptive PTB children with low positivity rate.

2. We did not repeat the test to confirm positivity with 'trace call' results on Xpert Ultra. Thus, we might have overestimated the sensitivity and underestimated the specificity.
3. We had a smaller sample size for calculating sensitivity as the proportion of children with 'bacteriologically confirmed PTB on induced sputum' was less than that anticipated. This could be due to use of solid culture on induced sputum over the liquid culture with better yield. Hence, our estimation of sensitivity is not precise.
4. Physicians made clinical diagnosis only when there was no bacteriological confirmation. Thus, the proportion with positive Xpert Ultra results among those the physicians otherwise would have diagnosed clinically is unknown and the utility of Xpert Ultra in ascertaining bacteriological confirmation among children diagnosed clinically can't be commented.

## Acknowledgements:

This study was conducted in collaboration with the National Tuberculosis Control Program, Directorate General of Health Services (DGHS). We acknowledge their support at every step of our activities. We are grateful to the authorities and physicians of participating hospitals to support us in performing research activities at their facilities especially for patients' enrolment, data collection and ensuring specimen collection.

## References:


1. Organization WH. Global Tuberculosis Report. 2019.

2. Graham SM, Marais BJ, Gie RP. Clinical features and index of suspicion of TB in children. 2009.

3. Kabir S, Uddin MKM, Chisti MJ, Fannana T, Haque ME, Uddin MR, et al. Role of PCR method using IS6110 primer in detecting Mycobacterium tuberculosis among the clinically diagnosed childhood tuberculosis patients at an urban hospital in Dhaka, Bangladesh. International Journal of Infectious Diseases. 2018;68:108-14.

4. 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. Clinical Infectious Diseases. 2013;57(3):e18-e21.

5. Sabi I, Rachow A, Mapamba D, Clowes P, Ntinginya NE, Sasamalo M, et al. Xpert MTB/RIF Ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study. Journal of Infection. 2018;77(4):321-7.

6. Abaye GE, Abebe T, Worku A, Tolessa D, Ameni G, Mihret A. Detection of Mycobacterium tuberculosis from the stool of HIV sero-positive individuals suspected of pulmonary tuberculosis. PloS one. 2017;12(5):e0177529.

7. Atherton RR, Cresswell FV, Ellis J, Kitaka SB, Boulware DR. Xpert MTB/RIF ultra for tuberculosis testing in children: a mini-review and commentary. Frontiers in pediatrics. 2019;7.

8. Chipinduro M, Mateveke K, Makamure B, Ferrand R, Gomo E. Stool Xpert® MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis at primary clinics in Zimbabwe. The International Journal of Tuberculosis and Lung Disease. 2017;21(2):161-6.

9. Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, et al. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. The Lancet Infectious Diseases. 2018;18(1):76-84.

10. Kendall EA, Schumacher SG, Denkinger CM, Dowdy DW. Estimated clinical impact of the Xpert MTB/RIF Ultra cartridge for diagnosis of pulmonary tuberculosis: a modeling study. PLoS medicine. 2017;14(12).

11. Rahman SM, Maliha UT, Ahmed S, Kabir S, Khatun R, Shah JA, et al. Evaluation of Xpert MTB/RIF assay for detection of Mycobacterium tuberculosis in stool samples of adults with pulmonary tuberculosis. PloS one. 2018;13(9).

12. Welday SH, Kimang'a AN, Kabera BM, Mburu JW, Mwachari C, Mungai E, et al. Stool as appropriate sample for the diagnosis of Mycobacterium tuberculosis by Gene Xpert test. Open Journal of Respiratory Diseases. 2014;2014.

13. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. Journal of clinical microbiology. 2010;48(7):2495-501.

14. Alland D, Rowneki M, Smith L, Ryan J, Chancellor M, Simmons AM, et al., editors. Xpert MTB/RIF Ultra: a new near-patient TB test with sensitivity equal to culture. Conference on Retroviruses and Opportunistic Infections; 2015.

15. Organization WH. Molecular assays intended as initial tests for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children: rapid communication. 2020.

16. National guidelines for the management of tuberculosis in children. 2nd ed2016.

17. Canetti G, Fox W, Khomenko Aa, Mahler H, Menon N, Mitchison D, et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bulletin of the World Health Organization. 1969;41(1):21.

18. Mesman AW, Soto M, Coit J, Calderon R, Aliaga J, Pollock NR, et al. Detection of Mycobacterium tuberculosis in pediatric stool samples using TruTip technology. BMC infectious diseases. 2019;19(1):563.

19. Organization WH. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. World Health Organization, 2017.

20. Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, et al. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. Clinical microbiology reviews. 2018;31(4):e00021-18.