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World Health Organization

Practical manual of processing stool samples for diagnosis of childhood TB

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About this manual

This practical manual aims to help countries implement stool testing into their tuberculosis (TB) diagnostic and clinical practice. It provides evidence and incorporates recommendations on the use of stool as a sample type for diagnosing TB. It also contains steps for implementation and details on the laboratory process for conducting stool testing using Xpert MTB/RIF and Xpert MTB/RIF Ultra (Xpert Ultra).

Target audience

The target audience for this manual is ministries of health, programme managers, clinicians, front-line health workers (especially in paediatric TB), TB testing site managers, supervisory laboratory staff and GeneXpert users at national, state or provincial and testing site level, as well as implementing partners.

Abbreviations and acronyms

CI	confidence interval
FIND	Foundation for Innovative New Diagnostics
GA	gastric aspirate
GLI	Global Laboratory Initiative
HIV	human immunodeficiency virus
KNCV	KNCV Tuberculosis Foundation
MTBC	*Mycobacterium tuberculosis* complex
NTP	national TB programme
OSF	optimized sucrose flotation
POSEE	Paediatric TB Operational and Sustainability Expertise Exchange
PPE	personal protective equipment
SOP	standard operating procedure
SOS	simple one-step
SPK	stool processing kit
SR	sample reagent
TB	tuberculosis
WHO	World Health Organization

Part A Background

A1 Introduction

Each year, 1.1 million children globally fall ill with tuberculosis (TB), of whom only 400 000 are notified; with the case detection gap being highest in children aged under 5 years. In 2020, this gap was 72.5%, whereas for children aged 5–14 years the gap was 55.4% *(1)*. Obtaining bacteriological confirmation of TB is challenging in children because of the frequent paucibacillary presentation of the disease. Diagnostic specimens have a low bacterial load, which decreases diagnostic test sensitivity. Diagnosis is further complicated by the fact that obtaining a sufficient volume of specimen from children can be difficult. Children, especially young children, generally cannot effectively expectorate and produce a sputum sample. Often, invasive procedures such as sputum induction or gastric aspiration are required to obtain a specimen. In many settings, the equipment and consumables required for sputum induction or gastric aspiration are not available, or clinical staff lack the skills to competently perform these methods. Also, parents or other caregivers may be reluctant to have these invasive procedures performed on their children.

Stool collection is a non-invasive method. *Mycobacterium tuberculosis* complex (MTBC) can be detected in stool specimens because sputum is coughed up and subsequently swallowed, and then passes through the gastrointestinal system. Since 2021, the World Health Organization (WHO) has recommended stool as a new specimen type alongside sputum (expectorated or induced), nasopharyngeal aspirate or gastric aspirate (GA) for both Xpert MTB/RIF and Xpert Ultra as the initial diagnostic test for TB and the detection of rifampicin resistance in children aged under 10 years with signs and symptoms of pulmonary TB *(2, 3)*. The recent WHO consolidated guidelines on TB, Module 5, and the accompanying operational handbook, provide recommendations on the management of TB in children and adolescents, including on the use of stool *(4, 5)*.

Recent systematic reviews for the detection of MTBC on stool using Xpert MTB/RIF found pooled sensitivities of 50% (95% confidence interval [CI]: 44–56), 57% (95% CI: 40–72), 62% (95% CI: 44–76) and 67% (95% CI: 52–79), and pooled specificities of 99% (95% CI: 98–99), 98% (95% CI: 96–99), 99% (95% CI: 97–99) and 99% (95% CI: 98–99) compared with a microbiological reference standard *(4–9)*. A systematic review and meta-analysis of Xpert Ultra data found a sensitivity of 53% (95% CI: 35–70) and a specificity of 98% (95% CI: 93–99) *(10, 11)*. Given the fact that all specimen types have incomplete sensitivity, using two specimens (preferably of two different specimen types) might increase the chance of obtaining a bacterial diagnosis of TB *(12)*. However, there is no WHO recommendation on this to date.

A2 Stool processing methods

Various methods to process stool for Xpert MTB/RIF or Xpert Ultra testing have been described *(13–20)*. The methods vary in terms of technique (or combination of techniques) used to bring stool into suspension (e.g. hand shaking or mechanical shaking using a vortex) and to separate *M. tuberculosis*

bacilli from stool debris (e.g. centrifugation, filtration and sedimentation). Consequently, methods vary in complexity, labour intensity, time investment, and the required equipment, supplies and infrastructure. Annex 1 provides a list of relevant publications, giving the stool processing methods used and the reported sensitivities and specificities against various reference standards.

Several authors have described relatively simple, centrifuge-free methods for stool processing – for example, Banada et al. *(15)*, Walters et al. *(17)* and Andriyoko et al. *(18)*. Other simple methods are the optimized sucrose flotation (OSF) method developed by the TB-Speed consortium *(19)*, and the simple one-step (SOS) method developed by the KNCV Tuberculosis Foundation (KNCV) *(20)*.

A3 Comparison of stool processing methods

Standardized comparison studies are needed owing to the high heterogeneity among stool processing methods and study designs, and consequently among the pooled sensitivity and specificity values from the four systematic reviews *(6–9)*.

Jasumback et al. applied four different stool processing methods *(15, 17, 20, 21)* to stool samples spiked with multiple log concentrations of *Mycobacterium bovis* bacille Calmette-Guérin (BCG) *(22)*. Walters et al. used a method that included centrifugation *(21)*; this resulted in more frequent detection of BCG at lower concentrations (5/5 replicates at 10^3 colony forming units [cfu]/mL and 3/5 replicates at 10^2 cfu/mL) compared with the other three methods – that is, the method used by Banada et al. *(15)* (3/5 replicates at 10^3 cfu/mL and 1/5 replicates at 10^2 cfu/mL), the centrifuge-free swab-based method used by Walters et al. *(17)* (1/5 replicates at 10^3 cfu/mL and 0/5 replicates at 10^2 cfu/mL) and the SOS stool method *(20)* (3/5 replicates at 10^3 cfu/mL and 1/5 replicates at 10^2 cfu/mL). However, numbers were too small to carry out statistical analyses. The SOS stool method was considered to be most suitable for low-resource settings, because of its low error rate, short processing time and minimal requirements regarding biosafety precautions and laboratory equipment *(22)*.

In an in vitro study *(20)*, the TB-Speed consortium, in collaboration with KNCV, compared the two-step method described by Andriyoko et al. *(18)* with the SOS stool processing method *(20)*. The comparison was done using stool samples with different consistency spiked with multiple log concentrations of *M. tuberculosis*. The SOS stool method was found to have a higher sensitivity than the two-step method; this was attributed to the fact that the two-step method uses an additional dilution step.

Three stool processing methods have been assessed in parallel as part of two studies led by the Foundation for Innovative New Diagnostics (FIND) and the TB-Speed consortium. The studies were performed at referral laboratories in Africa and Asia and included the disposable stool processing kit (SPK), which resulted from optimization of the methods described by Banada et al. *(15)* and Walters et al. *(17)*; the OSF *(19)*; and the SOS *(20)* stool processing methods.

Pooled data from an interim analysis of the FIND and TB-Speed studies suggest similar performance of the three stool processing methods in terms of sensitivity and specificity *(23, 24)*. Briefly, the sensitivity of Xpert Ultra for TB detection in stool was 52.1% (95% CI: 38.3–65.5) for SOS, 48.3% (95% CI: 35.9–60.8) for SPK and 46.8% (95% CI: 33.4–60.8) for OSF, and the specificity was 97.5% (95% CI: 94.9–98.9) for SOS, 97.1% (95% CI: 94.5–98.5) for SPK and 97.8% (95% CI: 95.1–99.1) for OSF (see Table 1).

The proportion of non-determinate Xpert Ultra results was 8.7% (35/401) for SOS, 11.8% (53/541) for SPK and 10.3% (40/388) for OSF *(23)*. No calculations were planned at this point to determine statistical significance or assess repeat Xpert Ultra test results. A limitation of this interim analysis is the low number of MTBC-positive children included, which resulted in wide confidence intervals around the sensitivity estimates.

Table 1. Pooled interim results of the studies by FIND and TB-Speed

Method	Total number of tests	True positive results	False positive results	False negative results	True negative results	Sensitivity (95% CI)	Specificity (95% CI)
SOS	332	25	7	23	277	52.1% (38.3–65.5)	97.5% (94.9–98.9)
SPK	368	28	9	30	301	48.3% (35.9–60.8)	97.1% (94.5–98.5)
OSF	319	22	6	25	266	46.8% (33.4–60.8)	97.8% (95.1–99.1)

CI: confidence interval; FIND: Foundation for Innovative New Diagnostics; OSF: optimized sucrose flotation; SOS: simple one-step; SPK: stool processing kit.

The FIND and TB-Speed studies also looked at user acceptability and feasibility of the stool methods, and the findings suggest good acceptability of stool as a sample for TB diagnosis in children *(24)*. All methods were found to be easy to process by laboratory staff at reference level, and all had a high median ease-of-use score. However, most users considered that these methods cannot be performed by non-laboratory staff (e.g. nurses and health care workers) in primary health care settings without access to a laboratory. Overall, the SOS stool method appeared to be the preferred method because it does not require additional equipment or supplies compared with sputum Xpert testing *(23, 24)*.

A4 Stool processing methods described in this manual

This practical manual focuses on two stool processing methods: the OSF and SOS stool methods. The disposable SPK assessed during the FIND and TB-Speed studies was a prototype kit; although shown to be accurate, the kit offers no additional benefits over the simpler OSF and SOS stool methods and its development and commercialization will therefore not be advanced. Hence, the SPK and the underlying methods described by Banada et al. *(15)* and Walters et al. *(17)* are not the focus of this manual.

Part B How to perform stool testing

B1 Stool collection and storage

Stool collection is usually done by the caregivers or the patients themselves, depending on the age of the child. Ideally, the collection takes place at the health care facility. However, to obtain a specimen on demand is often challenging; therefore, stool is collected at home and the patient or caregiver needs to return to the facility for specimen submission.

Following the procedures at the local setting, the patient or caregiver is provided with a stool container. Different types of containers can be used; some of these have a small spoon integrated into the screwcap, as shown in Fig. 1. Importantly, the container should be wide-mouthed to make it easy to add the stool, and it should be able to hold at least 3–5 g of stool. Thus, sputum containers can also be used for stool collection.

Fig. 1. Example of a stool container

The stool container should be provided in a plastic bag containing absorbent material, to keep the container clean during transport to and from the child's home. The absorbent material will absorb any substances that may leak out of the container if it is not closed properly.

The patient or caregiver should be instructed by the laboratory staff or other health care worker on how to collect the stool sample. The instructions below can be adapted to country-specific needs. A flyer to provide to the patient or caregiver, showing the steps on how to collect the stool sample and explaining the importance of returning the sample to the clinic, can be useful.

Instructions for the patient or caregiver on how to collect the stool sample:

1. Ideally, collect the stool sample during the first daily bowel movement. If possible, first empty the bladder, to avoid mixing urine with the stool sample.

2. Put some clean plastic sheeting on the spot where the stool will be dropped, to ensure the collection of a clean sample. Avoid contamination of the plastic with soil, detergent or disinfectant from the toilet.

3. If the stool sample needs to be collected from a child that uses a diaper (i.e. a nappy), then either collect the stool directly from the diaper as soon as possible after defecation, or put a plastic sheet in the diaper to avoid (prolonged) contact between the stool and the surface of the diaper (diapers may contain substances that inhibit the test).

4. Fill the stool container with the stool sample up to half full, using (for example) the spoon provided with some types of containers, a clean plastic bag, a clean piece of cardboard or a clean spoon. **Do not fill the container to the brim**. Only a small amount of stool is required for testing (2 g is sufficient for both testing and retesting if the first test is unsuccessful).

5. Close the container tightly, place the container in the plastic bag provided (preferably a self-sealable bag) and close the bag. Leave the absorbent material in the plastic bag so that this material can absorb any substances that may leak out of the container.

6. As soon as the stool sample has been collected, store the plastic bag containing the stool container in a clean, cool place (e.g. in a fridge if possible), avoiding exposure to direct sunlight. Do **not** freeze the sample.

7. Take the plastic bag containing the stool container to the health care facility, preferably on the same day that you collected the stool sample.

For transport and storage of stool specimens, the same conditions apply as for transport and storage of sputum specimens for Xpert testing. Thus, between collection and testing, stool specimens can be kept at a maximum of 35 °C for up to 3 days, followed by a maximum of 7 days at 2–8 °C. Ideally, stool sample containers should be kept at 2–8 °C while being sent to the laboratory and should then be stored in the refrigerator (at 2–8 °C) until testing can be performed. Sample preparation and testing should be started as soon as possible. Research is ongoing on how best to store stool samples *(25, 26)*. Before handling, allow the stool sample to warm up to room temperature.

B2 Stool processing methods

B2.1 OSF stool processing method

Principle

The TB-Speed OSF method is based on the creation of a sucrose density gradient to support separation of *M. tuberculosis* from stool debris *(19)*.

Biosafety requirements

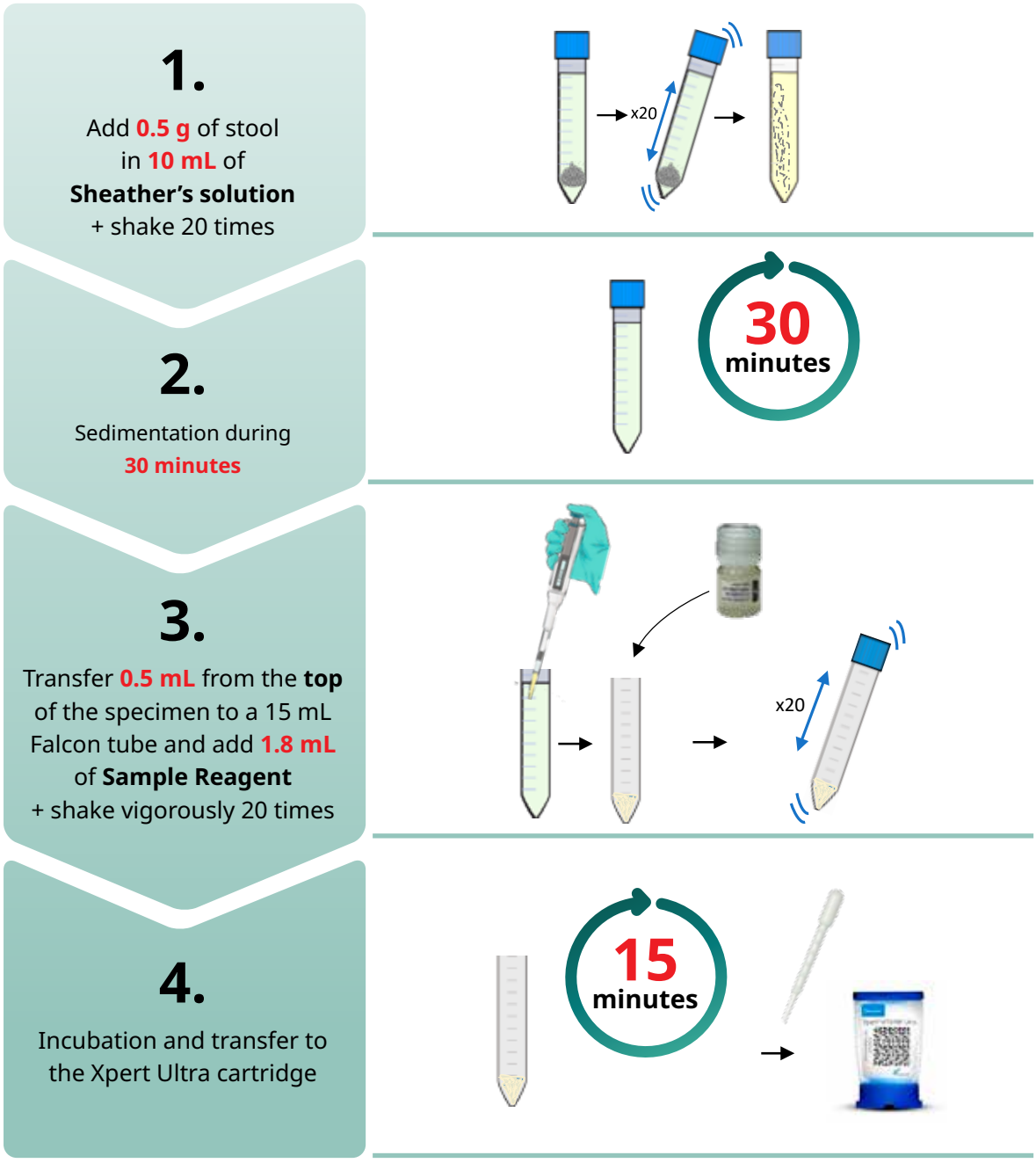
Although the OSF method involves a separation step before inactivation, this step leads to only minimal concentration of the *M. tuberculosis* bacilli. Therefore, the OSF method can be performed in an open, well-ventilated space with appropriate aerosol reduction practices and appropriate use of personal protective equipment (PPE).

Procedure

In brief, 0.5 g of stool is transferred into a 50 mL Falcon tube (for solid stool specimens) or an empty stool container (for liquid stool specimens) (Fig. 2). Next, 10 mL of Sheather's solution (56% sucrose solution) is added to the Falcon tube or stool container, which is then shaken 20 times to homogenize the stool specimen and left to stand for 30 minutes at room temperature, to sediment larger particulate matter. Then 0.5 mL of the resulting supernatant is transferred to another 15 mL Falcon tube, together with 1.8 mL sample reagent (SR) from the Xpert MTB/RIF or Xpert Ultra assay. The mixture is shaken vigorously 20 times then incubated for 15 minutes at room temperature, after which 2 mL of the mixture is transferred to the Xpert MTB/RIF or Xpert Ultra cartridge. The cartridge is then inserted into the GeneXpert instrument. Use of the GeneXpert instrument and interpretation of Xpert results are done according to the manufacturer's instructions.

Detailed information on how to prepare the Sheather's solution and on how to perform the OSF stool processing method can be found in the standard operating procedure (SOP) on the TB-Speed website *(27)*. TB-Speed is a research project sponsored by Inserm (Institut national de la santé et de la recherche médicale, the French National Institute of Health and Medical Research) and funded by Unitaid and L'Initiative *(28)*, with support from ANRS-MIE (an agency within Inserm) *(29)*.

Fig. 2. Schematic overview of the SOP for the detection of *M. tuberculosis* complex and resistance to rifampicin in stool by using the TB-Speed OSF method and the Xpert MTB/RIF or Xpert Ultra assay



OSF: optimized sucrose flotation; SOP: standard operating procedure.

B2.2 SOS stool processing method

Principle

The SOS stool processing method uses one step to release *M. tuberculosis* from stool. Particulate matter is sedimented by gravity, and it is assumed that this allows TB bacilli to float to the top of the watery solution because of their lipid-containing cell wall *(20)*.

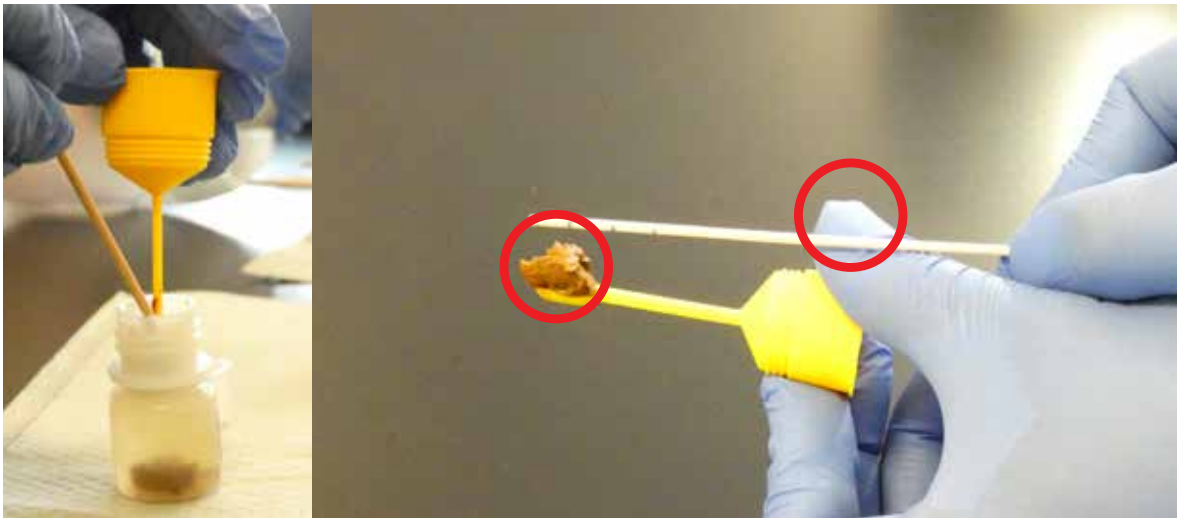
Biosafety requirements

In the SOS stool method, stool is added directly into the SR bottle provided in the Xpert kit; this results in immediate inactivation of the bacteria. Therefore, the SOS stool method can be performed in an open, well-ventilated space with appropriate aerosol reduction practices and appropriate use of PPE.

Procedure

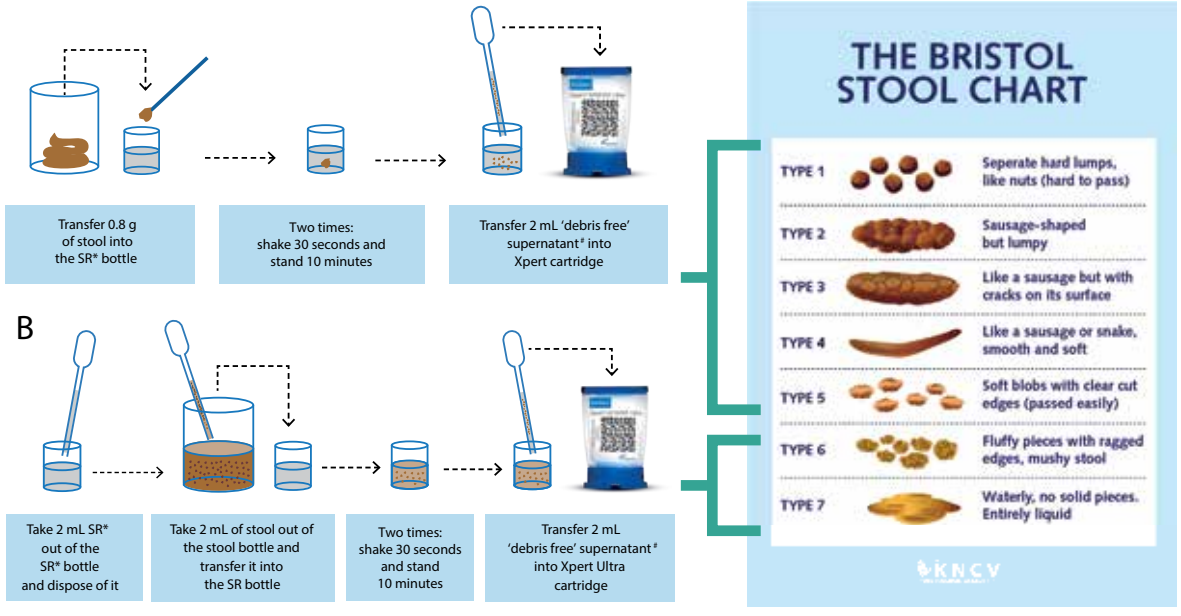
Before stool processing, the consistency of the stool specimen is assessed using the Bristol stool scale *(30)*. For stool with the appearance of Bristol type 1 to 5 (formed stool), 0.8 g or a thumbnail size amount of stool (Fig. 3) is directly transferred from the stool container into the SR bottle using a wooden stick or applicator (Fig. 4). For stool with the appearance of Bristol type 6 and 7 (liquid stool), 2 mL SR is removed from the SR bottle, then 2 mL of stool is transferred to the SR bottle using a balloon pipette (Fig. 3). For all types of stools, the SR is shaken vigorously for 30 seconds and then incubated for 10 minutes at room temperature. This step is repeated once. After carefully ensuring that solid particles and debris have settled, 2 mL of the supernatant is then transferred from the SR bottle to the Xpert MTB/RIF or Xpert Ultra cartridge. The cartridge is then inserted into the GeneXpert instrument. Use of the GeneXpert instrument and interpretation of Xpert results is done according to the manufacturer's instructions. A detailed SOP on how to perform the SOS stool processing method can be found in the KNCV stool toolbox on the KNCV website *(31)*.

Fig. 3. In the SOS stool processing method, 0.8 g or an amount of stool equal to the size of a thumbnail is used



SOS: simple one-step.

Fig. 4. Schematic overview of the SOP of the SOS stool processing method and the Xpert MTB/RIF or Xpert Ultra assay for different types of stools[a]



SOP: standard operating procedure; SOS: simple one-step; SR: sample reagent.

[a] The upper panel shows the procedure for stool of Bristol type 1–5 (i.e. formed stool) and the lower panel for stool of Bristol type 6 and 7 (i.e. liquid stool).

B2.3 Characteristics summary of OSF and SOS stool processing methods

Table 2 summarizes the characteristics of the OSF and SOS stool processing methods. The table includes additional materials required, preparation time *(32)*, incubation time and biosafety requirements.

Table 2. Characteristics of the OSF and SOS stool processing methods

	SOS	OSF
Additional supplies[a]	Applicator to transfer stool (wooden stick), balloon pipettes[b]	Applicator to transfer stool, sample preparation tubes, distilled water, sucrose (Difco), balloon pipets
Additional equipment[c, d]	None	Electronic balance, heating magnetic stirrer and bar magnet, screw cap glass bottle (1 L), graduated cylinder (1 L), autoclave
Median preparation time (range)	23 (20–30) minutes	56 (45–87) minutes
Incubation time	Incubation 10 minutes Sedimentation 10 minutes	Sedimentation 30 minutes Incubation 15 minutes

OSF: optimized sucrose flotation; SOS: simple one-step.

[a] Additional supplies are supplies that are required in addition to what is provided in the Xpert MTB/RIF or Xpert Ultra kit (the kit includes cartridges, sample reagent buffer and balloon pipettes).

[b] Additional balloon pipettes might be required when a high load of liquid stool is processed and the balloon pipets provided in the Xpert kit are not sufficient to cover the work.

[c] Additional equipment is equipment required in addition to the GeneXpert instrument.

[d] Preparation of the Sheather's solution will be done in batches at central level and distributed to the sites, meaning that the equipment listed is for the central level, not for the GeneXpert site.

Part C Implementation of stool testing

This section describes the requirements for the implementation of stool testing. It follows the 10 steps outlined in the chapter on implementation of new diagnostics in the *WHO operational handbook on tuberculosis Module 3: Diagnosis (3)*.

C1 Where to place stool testing in the tiered laboratory network

Introduction of stool testing for diagnosis of paediatric TB adds a new sample type to an existing diagnostic platform. Currently, WHO recommends that stool be tested using the Xpert MTB/RIF and Xpert Ultra assays. Thus, it is logical to incorporate stool testing in the existing laboratory network for Xpert MTB/RIF or Xpert Ultra testing, and to use the GeneXpert instruments and network already present in-country. When scaling up GeneXpert instruments, the placement strategy needs to consider access for specific key populations benefiting from stool testing (e.g. children) and an effective sample transportation system.

Ultimately, countries should be able to perform stool testing at any GeneXpert site; for example, in the same laboratory performing sputum testing for TB. However, when resources are limited, the use of stool should mainly be promoted where it would be of most value, and it is important to consider that stool testing might be most cost-effective in settings with higher prevalence of TB *(32)*. Furthermore, the choice of specimen type (GA, induced sputum or stool) collected for children depends on the acceptability for children, parents and caregivers, health care workers and other stakeholders; on the feasibility of collecting and preparing specimens in the local context; and on local test availability.

To help clinicians decide on whether a child should be started on TB treatment, the WHO operational handbook on the management of TB in children and adolescents provides guidance on treatment decision algorithms that integrate a risk assessment for rapid progression of TB disease, bacteriological confirmation where possible and available (including stool testing as relevant), history of contact, clinical signs and symptoms and findings on chest radiography *(5)*. The Pediatric TB Operational and Sustainability Expertise Exchange (POSEE) is a task force under the Child and Adolescent TB Working Group and an entity of the Stop TB Partnership. POSEE has written a position paper that clearly describes the position of the microbiological diagnosis in the diagnostic pathway *(12)*.

C2 Steps and processes for implementation

C2.1 Policies and planning

Globally, countries have adopted either Xpert MTB/RIF or Xpert Ultra (or both) in their national guidelines as an initial test to diagnose TB and detect resistance to rifampicin. However, most countries do not include stool specimens as a sample type for diagnosis of TB among children. Therefore, national guidelines and policies need to be adapted and updated to include stool for the diagnosis of TB in children, in line with the new WHO recommendations *(3)*.

The in-country technical working group leads the review of the national guidelines and policies, including the implementation of stool testing. Generally, a situational analysis plan contains a map of the health care centres that have a GeneXpert instrument and linkages to referring and referral laboratories. To prepare a costed operational plan with timelines and milestones for the introduction of stool testing, the working group can use the existing situation analysis if one is available or can undertake a new analysis. The POSEE task force has developed a budget tool for specimen collection for TB diagnosis to support national TB programmes (NTPs) in budget forecasting, and has included stool as one of the possible specimens. The budget tool and other relevant tools are available from the Child and Adolescent TB Working Group page on the Stop TB Partnership website *(33)*.

The best approach is a phased implementation, starting with a pilot implementation at a few selected sites that have a high TB notification rate or are actively involved in diagnosis of paediatric TB. These sites can subsequently train other sites, ensuring that all provinces achieve access to Xpert stool testing and have trained staff to perform the tests. To determine which sites to prioritize for the implementation of stool Xpert testing, the number of children seen with signs and symptoms of TB (presumptive TB) can be used as an indicator. Alternatively, the number of children with respiratory infections, pneumonia or malnutrition can be considered. However, it may be difficult to retrieve this information as NTPs often do not systematically collect such data – the number of children clinically or radiographically diagnosed with TB is a good alternative for selecting the sites.

C2.2 Regulatory processes

No registration specific for stool testing is needed, because stool is a sample type that is already collected in routine settings for other diseases and laboratory tests. Diagnosis of TB from stool samples uses GeneXpert instruments, which are already registered for this purpose.

C2.3 Equipment and site preparation

For site preparation, equipment additional to the GeneXpert instruments might be needed, depending on the stool processing method to be implemented. Implementation of the SOS stool method requires no additional equipment other than the equipment currently used for Xpert MTB/RIF or Xpert Ultra sputum testing. Implementation of the OSF method does require some additional equipment, but it is low cost (see Table 2).

The introduction of stool testing will increase the use of the GeneXpert platform in each site. Therefore, for each site enrolled in stool testing, the expected workload needs to be estimated and matched with the diagnostic capacity of the available GeneXpert instruments. The number of broken or in other ways non-functional, modules should be considered, because this naturally reduces the

capacity per site. The costed operational plan for the site preparation will include provision of stool collection and processing, workload analysis, staff mapping, training of staff and other logistical needs. Annex 2 provides an example of what is needed to start stool testing; it can be adapted to country specifics and used to check the readiness of the sites to implement stool testing.

C2.4 Supply chain

For implementation of stool testing, a few additional supplies might be needed on top of those that are routinely used for sputum Xpert testing (see Table 2). Most important for supply management is the increase in the number of Xpert cartridges used. For each site, the incremental workload needs to be estimated and matched with the number of cartridges to be supplied. Owing to its higher sensitivity, Xpert Ultra is preferable to the Xpert MTB/RIF assay for the detection of TB in children.

Another requirement is stool containers, which can vary in type, as discussed in Section B1.

Transportation of stool samples should use the same transport network that is used for sputum Xpert testing; ideally, a cold chain should be maintained, with samples kept at low temperature until testing. Each stool container should be packed in a ziplocked plastic bag containing absorbent material with a biohazard label on the outside, as for sputum transportation. If such bags are not available, filled stool containers should at least be wrapped in toilet paper and placed separately in a simple plastic bag, to avoid any cross contamination should leakage occur during transport to the laboratory.

C2.5 Procedures

The most up to date SOPs of the OSF stool processing method can be found on the TB-Speed website *(27)*, and a detailed SOP for the SOS stool method can be found on the KNCV website *(31)*. The SOPs should be customized to the country requirements, and translated into local languages where necessary.

C2.6 Digital data

When stool testing is implemented, instructions need to be provided to the laboratory staff on what relevant details should be added when collecting and analysing the data. For example, when starting the test run in the GeneXpert instrument, "stool" should be entered within the field for sample type. This allows the test runs of stool samples to be separated from test runs of other sample types when measuring the positivity rate or other quality indicators.

If the GeneXpert instrument is linked to a connectivity system (e.g. GXAlert or DataToCare) that allows additional data to be entered, it is useful to add information about the stool sample or patients *(34)*. Such information could include, for example, the consistency of the stool sample (e.g. as listed in the Bristol chart, or grouped as "formed", "semi formed" or "liquid") or the age or other characteristics of the child patient – capturing this type of information can be useful for operational research purposes.

C2.7 Quality assurance control and assessment

Targets of quality indicators (e.g. error and invalid rates) that are set for sputum Xpert testing might need to be adjusted when performing Xpert testing on stool. Current studies suggest that the initial non-determinate rate for stool is slightly higher than for sputum Xpert testing.

In comparison with sputum, stool naturally contains a high load of solid particles, which may lead to errors linked to issues with the sample transfer through the microfluid filter in the cartridge (e.g. if the supernatant containing the bacteria is not well separated from the debris). Current studies on stool testing show that the most common error code obtained is "error 2008", which is linked to this phenomenon. Also, stool contains other organic substances not present in sputum that might inhibit the polymerase chain reaction, resulting in invalid Xpert results. Repeating the test once using the same sample decreases the rate of non-determinate Xpert results *(35)*. More studies are needed to provide better insights on these aspects.

C2.8 Recording and reporting

For the implementation of stool testing, the recording and reporting tools need to be carefully reviewed and adapted to include stool as a sample type. For analysis of both the number of notified cases and quality indicators, it is important that the outcome of the test can be stratified by sample type, including stool.

C2.9 Training and competency assessment

The most cost-effective approach is to first train staff already competent with sputum Xpert testing on how to perform the specific steps for stool processing, using either the OSF or SOS processing method; these staff are already familiar with the use of the GeneXpert instrument. For staff who are not competent with sputum Xpert testing, a more extensive training is required. As observed for sputum Xpert testing, it takes time for the laboratory staff to become familiar with the use of stool samples and with the processing method. Therefore, it might be that, when initially implementing the use of stool, there will be slightly more non-determinate Xpert results; however, this would be expected to decrease as staff gain more experience. This situation should be considered when ordering Xpert cartridges.

In parallel with the training on stool processing for the laboratory staff, all involved health care providers should be trained to perform the stool collection procedure and to explain the procedure to parents and caregivers. Generally, at most health care centres, stool is routinely collected for other diseases (e.g. parasitology). Thus, health care staff are likely to already have the knowledge and the tools to collect this specimen type. However, staff working in TB facilities may be less familiar with stool collection, and thus may need specific training on stool collection and on the development and distribution of a stool collection flyer. Furthermore, all health care providers involved in stool sample collection should be trained on the treatment decision algorithms that include stool as a primary specimen.

C2.10 Monitoring and evaluation

Once staff have been trained and have started using stool as a primary specimen to diagnose TB in children, it is essential to closely monitor and supervise their stool-related work on a weekly or fortnightly basis, at least for the first 2 months. Troubleshooting should address the rate of non-determinate results, as discussed in Section C2.7. For stool testing, additional indicators need to be added to the standard laboratory monitoring and supervision checklist for Xpert MTB/RIF and Xpert Ultra testing *(36)*. Annex 3 provides a list of the stool-specific indicators to consider. These indicators can be added to the standard list of indicators used for supervision and monitoring of sputum Xpert testing.

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Annex 1. Overview of publications on stool processing for TB detection until December 2021

For each publication, the following are provided if applicable and if reported: patient age, number of patients included in the analysis/ all patients eligible, study population, stool processing method used and sensitivities and specificities against various reference standards.

Study, year/ country (reference)	Age (years) range, median [IQR]	Included in analysis/ all eligible	Population	Amount of stool	Stool processing method includes:				No. micro-biologically confirmed (%)	No. clinically confirmed (%)	Reference standard	Stool Xpert performance	
					dilution in	vortexing	centrifugation	filtration				Sensitivity (95% CI)	Specificity (95% CI)
Ainan, 2021/ Tanzania *(1)*	NR, 2.17 [1.16–5.19]	225/258	Children with presumptive TB in 6 health facilities in Dar es Salaam	2 cm^3	Distilled water and SR	Yes	No	No	8 (3.6)	42 (18.7)	Xpert and/or solid culture on sputum or GA	62.5% (25–92%)	100% (98–100%)
Andriyoko, 2019/ Indonesia *(2)*	0–14, 1.4 [0.4–6.5]	36/NR	Laboratory study in 1 hospital; consecutive stool specimens submitted for TB diagnosis	0.8–1 g	PBS and SR	No	No	No	6 (17)	–	Xpert on GA or induced sputum	100%	87.5%
Banada, 2016/ South Africa *(3)*	0–15, NR	37/40	20 MTB+ and 20 MTB on induced sputum or GA Xpert	0.6 g	Commercial buffer	Yes, with glass beads	No	Yes	20 (54)	–	Xpert on GA or induced sputum	85% (62–97%)	100% (98–100%)
Chipinduro, 2017/ Zimbabwe *(4)*	5–16, 10.6 [8–13]	218/218	Presenting with presumptive TB in 8 PHCs: TB symptoms or history of close contact with TB patient	0.15 g	PBS and SR	Yes	Yes	No	19 (8.7)	–	LJ culture/Xpert on induced sputum	68% (43–87%)	98% (95–99%)

Study, year/ country (reference)	Age (years) range, median [IQR]	Included in analysis/ all eligible	Population	Amount of stool	Stool processing method includes:				No. micro-biologically confirmed (%)	No. clinically confirmed (%)	Reference standard	Stool Xpert performance	
					dilution in	vortexing	centrifugation	filtration				Sensitivity (95% CI)	Specificity (95% CI)
Chipinduro, 2017/ Zimbabwe *(4)*	5–16, 10.6 [8–13]	32/218	Presenting with symptoms of TB in 8 PHCs: TB symptoms or history of close contact with TB patient	0.15 g	PBS and SR	Yes	Yes	No	–	32	Clinical	53% (35–71%)	NR
de Haas, 2021/ Ethiopia *(5)*	NR	123/147	Children presenting with presumptive TB in selected health facilities, for whom routine nasoGA was requested upon clinical examination	0.8–1 g	SR	No	No	No	9 (7.3)	–	Xpert and/or LJ culture and/or MGIT culture on nasoGA	78%	–
Hanrahan, 2019/ South Africa *(6)*	<10, 21.4 months [12.3–42.9]	119	Children with signs and symptoms of TB presenting at a primary-care clinic	NR	PBS, NALC-NaOH and SR	NR	Yes	No	4 (3)	–	Smear, culture or Xpert on any one of the samples collected	0/4 microbiologically confirmed TB patients had a positive test on stool	0/15 patients unlikely to have TB had a positive test on stool
Hanrahan, 2019/ South Africa *(6)*	<10, 21.4 months [12.3–42.9]	119	Children with signs and symptoms of TB presenting at a primary-care clinic	NR	PBS, NALC-NaOH and SR	NR	Yes	No	–	100 (84)	At least 2 of the following: CXR consistent with TB, positive clinic response to anti-TB treatment, documented exposure to TB or a positive TST	0/100 clinically confirmed TB patients had a positive test on stool	0/15 patients unlikely to have TB had a positive test on stool

Study, year/ country (reference)	Age (years) range, median [IQR]	Included in analysis/ all eligible	Population	Amount of stool	Stool processing method includes:				No. micro-biologically confirmed (%)	No. clinically confirmed (%)	Reference standard	Stool Xpert performance	
					dilution in	vortexing	centrifugation	filtration				Sensitivity (95% CI)	Specificity (95% CI)
Hasan, 2017/ Pakistan *(7)*	0–15, 6.8 [2–9]	49/50 of 64 children with clinical symptoms	Presenting with symptoms of pulmonary TB in 2 tertiary hospitals	0.15 g	PBS and SR	Yes	Yes	No	11 (22)	–	LJ culture/ Xpert on GA or sputum	82% (48–98%)	95% (82–99%)
Hasan, 2017/ Pakistan *(7)*	0–15, 6.8 [2–9]	49/50 of 64 children with clinical symptoms	Presenting with symptoms of pulmonary TB in 2 tertiary hospitals	0.15 g	PBS and SR	Yes	Yes	No	–	17 (35)	Clinical	59% (33–82%)	100% (89–100%)
LaCourse, 2018/ Kenya *(8)*	0–12, 2 [1.1–4.8]	147/165	HIV-positive children eligible for ART, hospitalized for acute illness in 4 hospitals	NR	PBS, NALC-NaOH, SR	No	Yes	No	11 (7.5)	–	MGIT culture/ Xpert on sputum or GA	70% (35–93%)	100% (97–100%)
LaCourse, 2018/ Kenya *(8)*	0–12, 2 [1.1–4.8]	165/165	HIV-positive children eligible for ART, hospitalized for acute illness in 4 hospitals	NR	PBS, NALC-NaOH, SR	No	Yes	No	–	85 (52)	Clinical	9% (4–19%)	100% (95–100%)
Lounnas, 2020/ France *(9)*	NA.	NA.	NA.	0.5 g	sucrose solution and SR	No	No	No	NA.	NA.	NA.	NA.	NA.
Marcy, 2016/ Burkina Faso, Cambodia, Cameroon, Viet Nam *(10)*	0–13, 7.2 (4.1–7.2)	272/272	HIV-positive children presenting with presumptive pulmonary TB in 8 tertiary/paediatric hospitals	0.5 g	Sucrose solution and SR	No	Yes	Yes	27 (10)	–	MGIT/LJ culture on GA, induced sputum, NGA, string sample	67% (46–83%)	100% (98–100%)

Study, year/ country (reference)	Age (years) range, median [IQR]	Included in analysis/ all eligible	Population	Amount of stool	Stool processing method includes:				No. micro-biologically confirmed (%)	No. clinically confirmed (%)	Reference standard	Stool Xpert performance	
					dilution in	vortexing	centrifugation	filtration				Sensitivity (95% CI)	Specificity (95% CI)
Marcy, 2016/ Burkina Faso, Cambodia, Cameroon, Viet Nam *(10)*	0–13, 7.2 (4.1–7.2)	272/272	HIV-positive children presenting with presumptive pulmonary TB in 8 tertiary or paediatric hospitals	0.5 g	sucrose solution and SR	No	Yes	Yes	–	245 (90)	Clinical	11% (8–16%)	96% (81–100%)
Memon, 2018/ India *(11)*	0.5–15, 11	100/100	Children attending the paediatric TB clinic of a tertiary care hospital	0.2 g	PBS, NALC-NaOH, SR	No	Yes	No	26 (26)	–	MGIT culture on induced sputum or GA	11.5% (2.4–30.1%)	98.6% (92.7–99.9%)
Moussa, 2016/ Egypt *(12)*	>1 to <15, NR	115/115	Presenting with clinical signs of pulmonary TB in a tertiary care hospital	2 g	distilled water, PBS, NALC-NaOH, SR	No	Yes	No	36 (31)	–	LJ culture on sputum or induced sputum	83% (67–94%)	99% (93–100%)
Ngadaya, 2020/ Tanzania *(13)*	1–95, 35 [21–47]	590/NR	Presumptive TB patients >1 year old, 7 primary health facilities and 5 tertiary health facilities, **Xpert on stool conducted at CTRL**	2 cm^3	Distilled water and SR	Yes	Yes	No	75 (12.7)	–	LJ culture on sputum	84% (81.0–87.0%)	93.4% (98.5–99.9%)
Ngadaya, 2020/ Tanzania *(13)*	1–95, 35 [21–47]	590/NR	Presumptive TB patients >1 year old, 7 primary health facilities and 5 tertiary health facilities, **Xpert on stool conducted at peripheral laboratories**	2 cm^3	Distilled water and SR	Yes	Yes	No	75 (12.7)	–	LJ culture on sputum	63.0% (47.8–76.1%)	76.7% (72.1–81.4%)

Study, year/ country (reference)	Age (years) range, median [IQR]	Included in analysis/ all eligible	Population	Amount of stool	Stool processing method includes:				No. micro-biologically confirmed (%)	No. clinically confirmed (%)	Reference standard	Stool Xpert performance	
					dilution in	vortexing	centrifugation	filtration				Sensitivity (95% CI)	Specificity (95% CI)
Nicol, 2013/ South Africa *(14)*	1-<15, 2.6 [1.6–4.8]	115/115	PHC and tertiary pediatric hospital	0.15 g (FLOQ swabs[1])	PBS and SR	No	Yes	No	17 (15)	–	MGIT culture on induced sputum	47% (23–72%)	99% (94–100%)
Orikiriza, 2018/ Uganda *(15)*	1 M–14, NR	349/357	Patients starting on TB treatment in a regional referral hospital	NR	Saline solution, NALC-NaOH, PBS and unspecified buffer	No	Yes	No	9 (2.6)	–	LJ & MGIT Culture on sputum or induced sputum	56% (21–86%)	98% (90–100%)
Walters, 2012/ South Africa *(16)*	0 to <14, 17 months [NR]	23/28 (14 with both GA and stool, 6 only GA, 3 only stool)	Presenting with presumptive pulmonary TB in 2 hospitals	NR	Saline solution, NALC-NaOH, PBS and SR	No	Yes	No	4 (17)	–	MGIT culture/ Xpert on GA	NR (combination of stool and GA Xpert versus MGIT culture: 75%)	NR
Walters, 2012/ South Africa *(16)*	0 to <14, 17 months [NR]	23/28 (14 with both GA and stool)	Presenting with presumptive pulmonary TB in 2 hospitals	NR	saline solution, NALC-NaOH, PBS and SR	No	Yes	No	–	12 (52)	Clinical	NR (combination of stool and GA Xpert versus clinical Dx: 25%)	NR
Walters, 2017/ South Africa *(17)*	0 to <13, 1.3 [0.8–2.4]	379/379	Presenting with presumptive pulmonary TB in 2 referral hospitals	<5 g and 1–4 g	PBS, NALC-NaOH, SR	No	Yes	No	72 (19)	–	MGIT culture on GA, induced sputum, NGA, string sample	32% (21–44%)	100% (98–100%)
Walters, 2017/ South Africa *(17)*	0–13, 1.3 [0.8–2.4]	351/379	Presenting with presumptive pulmonary TB in 2 referral hospitals	<5 g and 1–4 g	PBS, NALC-NaOH, SR	No	Yes	No	–	242 (69)	Clinical	10% (6–14%)	100% (97–100%)

[1] For more info, please see FLOQSwabs, COPAN Diagnostics Inc.

Study, year/ country (reference)	Age (years) range, median [IQR]	Included in analysis/ all eligible	Population	Amount of stool	Stool processing method includes:				No. micro-biologically confirmed (%)	No. clinically confirmed (%)	Reference standard	Stool Xpert performance	
					dilution in	vortexing	centrifugation	filtration				Sensitivity (95% CI)	Specificity (95% CI)
Walters, 2018/ South Africa [17]	1.3 [0.9–2.4]	280/302	Presenting with presumptive pulmonary TB in 2 referral hospitals	0.6 g and swab	PBS and SR	Yes, with glass beads	No	Yes	23 (8.3)	–	MGIT/Xpert on expectorated or induced sputum and GA if <5y (subset only)	44.4% (13.7–78.8%) Xpert as RS 25.0% (7.3–52.4%) culture as RS	99.1% (96.8–99.9%) Xpert as RS 99.5% (97.5–100%) culture as RS
Walters, 2018/ South Africa *(18)*	NR, 1.3 [0.9–2.4]	280/302	Presenting with presumptive pulmonary TB in 2 referral hospitals	0.6 g and swab	PBS and SR	Yes, with glass beads	No	Yes	–	88 (31)	Clinical	44.4% (13.7–78.8%) Xpert as RS 25.0% (7.3–52.4%) culture as RS	99.1% (96.8–99.9%) Xpert as RS 99.5% (97.5–100%) culture as RS
Welday, 2014/ Kenya *(19)*	0 to <15, NR	53/91	Laboratory study in 2 hospitals including children referred for TB testing by a clinician	0.15 g	PBS only versus PBS and SR	No	No versus yes	No	6 (11.3)	–	ZN sputum smear microscopy	100%. Direct method did yield more cases than indirect method	89%

ART: antiretroviral therapy; CI: confidence interval; CTRL: central TB reference laboratory; CXR: chest X-ray; GA: gastric aspirate; HIV: human immunodeficiency virus; IQR: interquartile range; LJ: Löwenstein–Jensen; MGIT: mycobacteria growth indicator tube; MTB: Mycobacterium tuberculosis; NA: not applicable; NALC-NaOH: N-acetyl-L-cysteine–sodium citrate-sodium hydroxide; NGA: nasopharyngeal aspirate; NR: not reported; PBS: phosphate-buffered saline; PHC: primary health centre; RS: reference standard; SR: Xpert sample reagent; TB: tuberculosis; TST: tuberculin skin test; ZN: Ziehl–Neelsen

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Annex 2. List of activities for the implementation of stool testing

No.	Activity	Condition (yes/ no/in progress)
1	Adapt national policies and guidance to include stool as a primary sample for the diagnosis of TB in children with signs and symptoms of TB	
2	Train health care providers on the new national guidance including the diagnostic algorithms incorporating stool	
3	Train health care providers on treatment initiation using stool Xpert results	
4	Train health care providers on the collection of stools	
5	Train laboratory staff on the stool processing method(s)	
6	Assign and train the focal point (site coordinator)	
7	Adapt the reporting and registration tools to include a provision for stool	
8	Adapt the digital data collection and connectivity tools to include a provision for stool	
9	Add indicators specific for stool testing to the standard monitoring and supervision list for Xpert MTB/RIF and Xpert Ultra testing	
10	Ensure availability of supplies needed for stool collection (e.g. stool container and stool collection flyer)	
11	Ensure availability of supplies to transport stool samples (e.g plastic bag and cooler box) and arrange that stool can be transported through the routine sample referral network	
12	Ensure availability of the additional supplies for the stool processing method using Xpert MTB/RIF or Xpert Ultra	
13	Ensure availability of the SOPs and bench aids for stool testing	
14	Ensure availability of enough Xpert MTB/RIF or Xpert Ultra cartridges for stool testing	

SOP: standard operating procedure; TB: tuberculosis.

Annex 3. Laboratory tool for assessing stool testing by Xpert MTB/RIF and Xpert MTB/RIF Ultra

Laboratory stool testing assessment

The laboratory indicators listed in this tool should be integrated into the standard monitoring and supervision tool for Xpert MTB/RIF and Xpert Ultra testing already used by the country *(1).*

Name of institution		
District		
Date of visit		
Name and contact details of staff met	Name: Designation:	Tel/email:
	Name: Designation:	Tel/email:
	Name: Designation:	Tel/email:
Names of assessors		

	Number or Yes/no/partial	Comments
1. Stool samples obtained		
Number of children with signs and symptoms of TB for whom a stool sample has been collected per month		
2. Quality of the collected stool sample		
Are stool samples kept between 2 °C and 8 °C during transport?		
Are stool samples kept between 2 °C and 8 °C before testing?		
Are correct collection pots used?		
Are lids properly closed?		
Number of stool pots with stool found on the outside of the pot		
Are pots properly filled (minimum bottom of container covered, maximum ½ of pot)?		
Number of stool samples rejected by the laboratory		Indicate rejection criteria:
3. Quality of sample processing		
Number of stool samples processed per month		
Indicate the type of stool received:		
Formed		
Semi formed		
Liquid		
Number of samples processed within 3 days of stool submission		
Number of samples processed 3 or more days after stool submission		Maximum number of days
Is the correct amount of stool used for stool testing?		
Is the processing performed according to the SOP for stool testing by Xpert MTB/RIF or Xpert Ultra?		
Number of stool samples with an Xpert MTB positive result (total per month):		
MTB detected high		
MTB detected medium		
MTB detected low		
MTB detected very low		
MTB trace detected		
Number of stool samples with MTB not detected		
Number of stool samples with an error code		Error codes: Code x no. of tests

	Number or Yes/no/partial	Comments
Number of stool samples with "Invalid"		
Number of stool samples with "No result"		
Number of stool samples not tested		Reason for not testing:
Number of samples with rifampicin resistance		
Number of samples with rifampicin indeterminate		
	Yes/no/partial	Comments
4. Supply management		
Are Xpert Ultra cartridges always available for stool testing?		Reason if not:
Does the monthly consumption of Xpert Ultra cartridge consider stool testing?		Number of cartridges/ months:
Any stockout of containers for stool collection in the last quarter?		
Any stockout of applicator to support transfer of stool to SR buffer in the last quarter?		
Any stock out of supplies required for stool processing in the last quarter?		
Any stock out of reagents for stool processing in the last quarter?		
	Yes/no/partial	Comments
5. Registration and reporting tools		
Does request form have a provision to request stool testing?		
Does laboratory register have a provision to add stool as sample type?		
Is "stool" indicated within the sample type field of the GeneXpert?		
Are stool testing results entered into the laboratory information system?		

MTB: *Mycobacterium tuberculosis;* SOP: standard operating procedure; TB: tuberculosis.

Additional comments

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Reference for Annex 3

1. Tuberculosis technical scorecard Xpert MTB/RIF. Geneva: Stop TB Partnership; 2020 (https://stoptb.org/wg/gli/assets/documents/5%20Find-TB-Scorecard-Xpert-Low-Res.pdf, accessed January 2022).

World Health Organization

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