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OBJECTIVE

To perform Xpert MTB/RIF Ultra (Ultra) assay on fresh stool sample using the sucrose flotation stool processing method

FIELD OF APPLICATION

This procedure applies to all laboratories participating to the TB Speed Stool processing study.

RESPONSIBILITIES

The international Laboratory Coordinator (iLC) is responsible for the dissemination of this SOP to the national CTUs. The national Laboratory Coordinators (nLC) are responsible for the dissemination of this SOP to the trial site. The laboratory manager is responsible for the distribution and the application of this SOP.
iLC and nLC are responsible for monitoring the correct implementation of this SOP in all laboratories participating to the trial.
The laboratory personnel are responsible for processing the stool samples using the sucrose flotation method, performing the Ultra test and recording the test results.

ABBREVIATIONS

CTU	Clinical trials unit
eCRF	electronic Case report form
iLC	International Lab Coordinator
nLC	National Lab Coordinator
SOP	Standard Operation Procedure
TB	Tuberculosis
Ultra	Xpert MTB/Rif Ultra

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SAFETY

The below steps are performed under a Biosafety Cabinet or wearing a N95 mask.

- Treat all samples as potentially infectious biological material.
- If any spillage occurs, clean affected area with 0.5% Sodium hypochlorite (bleach).
- Dispose of all waste in a biohazard medical waste bin.

Refer to the National Health Laboratory Safety and Waste Management Manual for further safety considerations.

EQUIPMENT AND MATERIALS

- Bio-safety cabinet class II
- 0.5% Sodium hypochlorite (bleach)
- 70% Alcohol (or Ethanol or EtOH)
- PPE (personal protective equipment)
- Toilet paper or plastic sheet
- Specimen rack
- Timer
- Marker
- Conical tube sterile 50 ml/15ml (Falcon tube)
- Pipet 1000 uL or Sterile graduated Pasteur pipettes (2 ml)
- Tongue depressor
- Electronic balance
- Sterile 50% Sheather's solution (cf. annexe 1)
- Gauze
- Centrifuge for 15 mL tubes
- Xpert MTB/RIF Ultra kit (sample reagent, cartridge, Sterile disposable transfer pipet)
- GeneXpert machine

BIOLOGICAL SAMPLES

Fresh stool sample.

STOOL HANDLING

- Transfer of stool from the inclusion ward to the GeneXpert laboratory is done within 24 hours after collection according to study-specific procedures (See ***SOP Sample Transportation***).
- Specimen reception time at the laboratory level and stool appearance must be reported in the "stool sample tracking form" provided with the samples by

the laboratory technician receiving the samples (See ***SOP Stool sampling***)and in the "Stool sample laboratory worksheet"

- Upon reception at the laboratory stool sample will be stored in the fridge at 4°C
- Stool should be processed within 48°C after collection
- Before handling allow the stool to warm up to room temperature

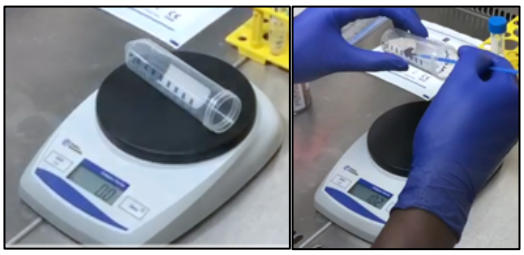
Materials

Solid to sticky stool		Liquid stool	
Falcon tube 50 mL + rack 	tongue depressor or plastic oese 	Stool container 	Pasteur pipet 

Marker	Electronic balance (precision 0.1 g)
	

Step by step procedure

1. Homogenize the stool using a tongue depressor or a wooden stick
2. Label a 50 mL Falcon tube or stool container (tube n°1) with sample ID and method abbreviation "SF"
3. <u>For solid to sticky stools:</u>
   Transfer 0.5 gram from the sample with a tongue depressor into a **50 ml Falcon tube**: weigh an empty container, tare the balance, and weigh 0.5 gram of stool (±0.1)

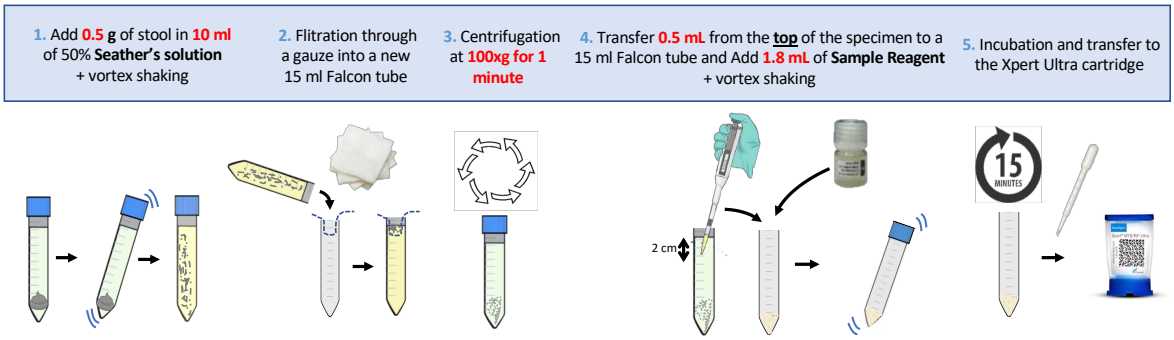


   <u>For liquid stools:</u>
   Transfer 0.5 gram from the sample with a Pasteur pipet into the **stool container**: weigh an empty container, tare the balance, and weigh 0.5 gram of stool (± 0.1)

4. Discard the tongue depressor or the Pasteur pipet
5. The sample is ready for processing for Ultra

STOOL PROCESSING PROCEDURE

Procedure overview



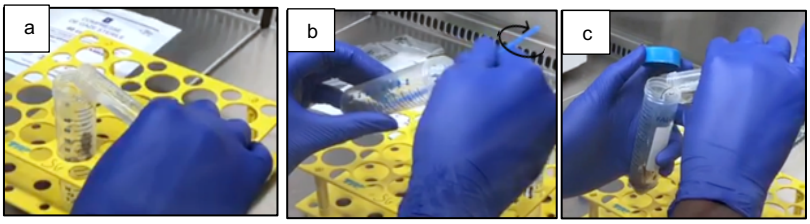
Materials and equipment

Equipment	
Vortex	Centrifuge
	

consumable and small equipment			
0.5g of stool + rack	Sheather's solution (10 mL) (tube n°2)	Falcon tube 15 mL (tube n°3)	Sterile Gauze
			
Timer	Marker	Pasteur pipet (2 mL)	Sample Reagent + Cartridge + Pipet (Included in the MTB/Rif Ultra kit)
			

Step by step procedure

1. Mix 0.5 g of stool specimen with 10 mL of Sheather's solution (cf Appendix 1 for Sheather solution preparation):
   a. add 5 mL of the solution into the initial stool container or 50 mL Falcon tube (tube n°1)
   b. mix vigorously using a wooden stick or plastic loop
   c. add the 5-remaining ml of Sheather's solution
   d. mix again



2. Vortex 30s to break up the stool and visually check for particles. If stool is not broken up, vortex another 30s

3. Put several layers of sterile gauze directly into the 15ml sheather's solution tube (tube n°2) and pour the stool solution slowly on the sterile gauze.

4. Centrifuge at 100 x g for 1 minute (no brake)
   Carefully, remove tube from centrifuge without disturbing the suspension

5. Label a new falcon tube 15 mL (tube N°3) with sample ID and add 1.8 mL of Sample Reagent.

6. Carefully retrieve 0.5 mL from the top of the tube (first 2 cm) and transfer into the 15 mL falcon containing the sample reagent using a 2 mL graduated Pasteur pipet.

   Note: Some particles might remain on the top of the tube and you might not see a clear upper layer. Pipet by avoiding solid particles as much as possible. Place the pipet tip just under the floating particles and slowly aspirate the 0.5 mL of stool solution. If particles are trapped, slowly pipet the solution back into the bottle and pipet again immediately.
   If you disturbed the solution by pipetting back too quickly centrifuge again the solution.

7. Screw the cap, and shake vigorously 20 times.

8. Incubate for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation shake again the specimen vigorously 20 times. Samples should be liquefied.
   If the sample is not liquefied and there are still solid parts visible shake the sample again and leave for another 10 minutes.

7. Using the sterile transfer pipette included in the Ultra kit, aspirate the **liquefied sample** into the transfer pipette until the meniscus is above the minimum mark.

8. Open the cartridge lid. Transfer sample into the open port of the Ultra cartridge. Dispense slowly to minimize the risk of aerosol formation.

9. Close the cartridge lid. Make sure the lid snaps firmly into place.

Appendix 1: Preparation of Sheather's solution

Equipment, materials and regents

- Distilled water
- Sucrose (Difco)
- Electronically balance
- Heating Magnetic stirrer and bar magnet
- Screw cap glass bottle, 1000 ml capacity
- Graduated cylinder
- Autoclave
- Falcon tube 15 mL

Stool Processing consumable and small equipment			
Sucrose (density of 1.29cm^3)	Distilled water	Electronic balance (precision 0.1 g)	Heating Magnetic stirrer and bar Magnet
			
Screw cap glass bottle (1000 mL)	Graduated Cylinder	Falcon tube 15 mL (x50)	Autoclave + tape
			

Procedure

1. Dissolve gently 454 g of sucrose in 355 mL of distilled water over low-heat on a stove or a heating magnetic stirrer using a bar magnet.
2. Autoclave the solution 15 min at 110 ° C
3. Aliquot 10 mL of this solution in sterile 15 ml Falcon tubes under a sterile BSC.
4. Store the aliquot at 4° C to prevent mold contamination (the tubes can be stored up to 1 months)