Overview

This document details instructions for use of the sucrose flotation method (SF). The SF is intended to be used with the Xpert MTB/RIF Ultra cartridge, performed on the Cepheid GeneXpert® System. The SF enables extraction of Mycobacterium Tuberculosis Complex (MTB) from stool samples in a form suitable for input into a Xpert® cartridge. All users should have appropriate training with the Xpert MTB/RIF Ultra Assay and the SF.

Materials required

- Disposable gloves, face mask and other appropriate protective clothing
- Timer and permanent pen
- Electronic balance
- 50 mL Falcon tube (x1)(Tube 1)
- 10 ml of Sheather's solution (Tube 2)
- 15 mL Falcon tube (x1) (Tube 3)
- Tubes rack
- Plastic loop or tongue depressor/ Pasteur pipet
- Sterile Gauze
- Centrifuge for 15 mL Falcon tube
- Vortex
- Graduated 2 mL Pasteur pipet (x2)
- Xpert MTB/Rif Ultra Kit

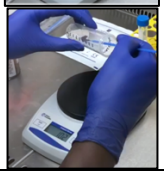
Warnings and precautions

- The SF is for initial performance evaluation use only. It is not suitable for clinical diagnostic use, so the results must not be relied on to direct the path of patient care.
- Treat all biological specimens, including used Cartridges & consumables, as if capable of transmitting infectious agents. Because it is often impossible to know which specimens might be infectious, all biological specimens should be treated with your institution's standard safe handling & disposal precautions.
- Wear protective disposable gloves, clothing, face mask when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Follow your institution's safety procedures for working with chemicals when handling the Sample Reagent. Read the Sample Reagent MSDS documents.
- Follow your institution's biosafety procedures for handling and processing infectious bodily samples (including for infectious TB samples)
- When processing more than one sample at a time, open only one Cartridge, add the treated sample and close the Cartridge before processing the next sample. It is recommended to change gloves between samples.
- Do not substitute assay reagents with other reagents.
- Do not use any components if they are past their expiry date or appear to be broken.
- Follow your institution's waste procedures regarding proper disposal of used components and unused reagents. Institutions should check their local hazardous waste disposal requirements.

Sucrose flotation stool processing method Use Instructions


1 Put on protective disposable gloves, face mask and appropriate protective clothing

solid to sticky stools

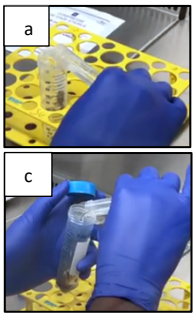



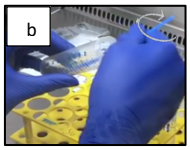
liquid stools

2 **Stool sampling**

For solid to sticky stools:
Label a **50 mL falcon tube** (tube N°1) with the sample ID and "SF", weigh the empty tube (without the screw cap), tare the balance, and add **0.5 gram** (± 0.1) of stool using a tongue depressor or a plastic loop.

For liquid stools:
Label a stool container and weigh the empty stool container, tare the balance, and add 0.5 gram of stool using a **Pasteur pipet**.

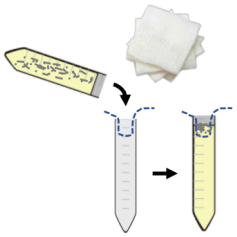




3 **Mix with Sheather's solution**

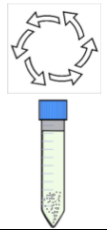
Mix 0.5 g of stool specimen with 10 mL of Sheather's solution:

a. add 5 mL of the solution into the initial stool container or 50 mL Falcon tube
b. mix vigorously,
c. add the 5-remaining ml and vortex 30s



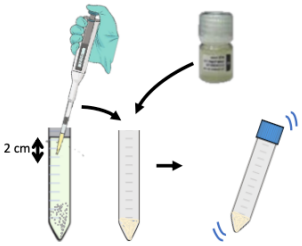
4 **Filtration**

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.



5 **Centrifugation**

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



6 **Mix with sample reagent**

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

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 or a 1000 uL Micropipet.

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.

Screw the cap, and shake vigorously 20 times or vortex.

7 **<u>Incubation and transfer to the Ultra cartridge</u>**

Incubate for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation again shake 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.

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

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

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

Safely dispose of used falcon tube and continue as per the GeneXpert system instructions