

Institution <b>Laboratory name</b> Location Head/Responsible person	<b>Standard Operating Procedure (SOP)</b> <b>Specimen processing of CSF, lymph nodes  and other tissues for Xpert MTB/RIF</b>	Code: Version: no. Date: of release Page: <b>1</b> of <b>7</b>
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## 1. Scope

This SOP describes methods of specimen processing CSF, lymph nodes and tissues for testing in the Xpert MTB/RIF assay and for purposes of culturing *Mycobacterium tuberculosis* culture on solid and / or liquid media.

## 2. Definitions and abbreviations

BSC : biological safety cabinet  
CSF: cerebrospinal fluid  
ID: patient's specimen identification, usually laboratory number  
LJ: Löwenstein–Jensen  
NTP: national tuberculosis programme  
PBS: Phosphate buffer 0.067 mol/ litre, pH 6.8  
RCF: relative centrifugal force

## 3. Procedure

### 3.1 Principle

WHO has issued policy recommendations for the use of Xpert MTB/RIF in the diagnosis of extrapulmonary TB and rifampicin resistance detection

- Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test in testing **cerebrospinal fluid specimens** from patients presumed to have TB meningitis (strong recommendation given the urgency of rapid diagnosis, very low quality of evidence);
- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, and/or histopathology) for testing of specific non-respiratory specimens (**lymph nodes and other tissues**) from patients presumed to have extrapulmonary TB (conditional recommendation, very low quality of evidence).

For CSF specimens, Xpert MTB/RIF should be preferentially used over culture if the sample volume is low or additional specimens cannot be obtained, in order to reach quick diagnosis. If sufficient volume of material is available, concentration methods should be used to increase yield;

Individuals presumed to have extrapulmonary TB but with a single Xpert MTB/RIF - negative result should undergo further diagnostic testing and hence processing of tissue samples (lymph nodes and other tissues) for Xpert MTB/RIF should include a decontamination step to enable samples to be concurrently cultures

Pleural fluid is a suboptimal sample for the bacterial confirmation of pleural TB, using any method. A pleural biopsy is the preferred sample.

**These recommendations do not apply to stool, urine or blood, given the lack of data on the utility of Xpert MTB/RIF on these specimens.**

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### **3.2 General considerations**

#### *Important points about specimen processing procedures*

- Process all specimens as soon as possible, for an optimal culture recovery of MTB. Longer transport should not affect Xpert positivity
- Ensure that the Xpert MTB/RIF cartridge and any culture media to be inoculated are labelled correctly and clearly.
- **Tissues must be processed within a BSC** given the risk of aerosol production while grinding and homogenizing samples.
- **CSF samples are paucibacillary** and can be processed using the same precautions as for sputum EXCEPT when concentrated by centrifugation
- It is important to use Safe Working Practices to avoid contamination by bacteria other than tubercle bacilli and especially cross-contamination by tubercle bacilli from other specimens.
- When sufficient sample is available, culture should be performed concurrently
- Samples requiring decontamination must have the exposure time to decontamination reagents strictly controlled.
- **Decontaminate samples** for culture using either 4% NaOH or NaOH-NALC **depending on usual practice** in the laboratory. The example below uses 4% NaOH.

### **3.3 Specimen processing**

The Xpert MTB/RIF assay can be used directly for CSF specimens and homogenised extrapulmonary samples (lymph node biopsies and other tissues) or on decontaminated specimens if culture is performed concurrently.

Whenever possible, specimens should be transported and stored at 2 to 8°C prior to processing (a maximum of 7 days).

#### **3.3.1 Lymph nodes and other tissues (for Xpert MTB/RIF only)**

1. Cut the tissue sample into small pieces in a sterile mortar (or homogenizer / tissue grinder) using a clean, sterile pair of forceps and scissors
2. Add approximately 2ml of sterile phosphate buffer (PBS)
3. Grind tissue/PBS-solution with a mortar and pestle (or homogenizer / tissue grinder) until a homogeneous suspension is obtained
4. Transfer approximately 0.7 ml of homogenized tissue sample to a sterile conical, screw-capped tube using a transfer pipette

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**NOTE: Avoid transferring any clumps of tissue which have not been properly homogenized.**

5. Add a double volume of Xpert MTB/RIF Sample Reagent (1.4 ml) to 0.7 ml of homogenized tissue using a transfer pipette
6. Vigorously shake 10 to 20 times or vortex for at least 10 seconds
7. Incubate for 10 minutes at room temperature, and again shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds
8. Incubate the sample at room temperature for an additional 5 minutes
9. Using a fresh transfer pipette, transfer 2ml of the processed sample to the Xpert MTB/RIF cartridge
10. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

***3.3.2 Lymph nodes and other tissues (Non-sterile collections – Xpert MTB/RIF and culture)***

1. Cut the tissue sample into small pieces in a sterile mortar (or homogenizer / tissue grinder) using a clean, sterile pair of forceps and scissors
2. Add approximately 2ml of sterile phosphate buffer (PBS)
3. Grind tissue/PBS-solution with a mortar and pestle (or homogenizer / tissue grinder) until a homogeneous suspension is obtained
4. Use a sterile transfer pipette to add the suspension into a 50ml conical tube
5. Add an equal volume of 4% NaOH and tighten the screw-cap
6. Vortex thoroughly to homogenise the suspension
7. Stand for 15 minutes at room temperature.
8. Fill the tube to within 2 cm of the top (e.g. to the 50-ml mark on the tube) with PBS
9. Centrifuge at 3000g for 15 minutes
10. Carefully pour off the supernatant through a funnel into a discard can containing 5% phenol or other mycobacterial disinfectant
11. Re-suspend the deposit in approximately 1-2 ml PBS
12. Use another sterile transfer pipette to inoculate deposit into liquid media and/or onto two slopes of egg-based medium labelled with the sample ID number.
13. Label a Xpert/MTB/RIF cartridge with the sample ID

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14. Using a transfer pipette, transfer approximately 0.7 ml of homogenized tissue sample to a conical, screw-capped tube for the Xpert MTB/RIF.

**NOTE: Avoid transferring any clumps of tissue which have not been properly homogenized.**

15. Using another transfer pipette, add a double volume of Xpert MTB/RIF Sample Reagent (1.4 ml) to 0.7 ml of homogenized tissue.

16. Vigorously shake 10 to 20 times or vortex for at least 10 seconds

17. Incubate for 10 minutes at room temperature, and again shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds

18. Incubate the sample at room temperature for an additional 5 minutes

19. Using a fresh transfer pipette, transfer 2ml of the processed sample to the Xpert MTB/RIF cartridge

20. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

### ***3.3.3 Lymph nodes and other tissues (Sterile collection – Xpert MTB/RIF and culture)***

1. Cut the tissue sample into small pieces in a sterile mortar (or homogenizer / tissue grinder) using a clean, sterile pair of forceps and scissors.

2. Add approximately 2ml of sterile phosphate buffer (PBS)

3. Grind tissue/PBS-solution with a mortar and pestle (or homogenizer / tissue grinder) until a homogeneous suspension is obtained and adjust to a final volume of approximately 2ml with PBS

4. Transfer the suspension with a sterile transfer pipette to a 50ml conical tube

5. Use a another transfer pipette to inoculate suspension into liquid media and/or onto two slopes of egg-based medium labelled with the sample ID number

6. Label an Xpert/MTB/RIF cartridge with the sample ID

7. Transfer approximately 0.7 ml of homogenized tissue sample to a conical, screw-capped tube for the Xpert MTB/RIF using a transfer pipette

**NOTE: Avoid transferring any clumps of tissue which have not been properly homogenized.**

8. Transfer a double volume of Xpert MTB/RIF Sample Reagent (1.4 ml) to 0.7 ml of homogenized tissue using a transfer pipette

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9. Vigorously shake 10 to 20 times or vortex for at least 10 seconds
10. Incubate for 10 minutes at room temperature, and again shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds
11. Incubate the sample at room temperature for an additional 5 minutes.
12. Using a fresh transfer pipette, transfer 2ml ml of the processed sample to the Xpert MTB/RIF cartridge
13. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

### 3.3.4 CSF

The preferred processing method for CSF in Xpert MTB/RIF depends on the volume of sample available for testing.

***NOTE. Blood stained and xanthochromic CSF samples may cause false negative Xpert MTB/RIF results***

#### **More than 5 ml of CSF**

1. Transfer all of the sample to a conical centrifuge tube and concentrate sample at 3000g for 15 minutes
2. Carefully pour off the supernatant through a funnel into a discard can containing 5% phenol or other mycobacterial disinfectant

**NOTE: Decanting concentrated CSF should be performed within a BSC**

3. Re-suspend the deposit to a final volume of 2ml with Xpert MTB/RIF sample reagent.
4. Label an Xpert/MTB/RIF cartridge with the sample ID
5. Using a fresh transfer pipette, transfer 2ml ml of the concentrated CSF sample to the Xpert MTB/RIF cartridge
6. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

#### **1-5 ml of CSF (including blood-stained or xanthochromic samples)**

1. Add an equal volume of the CSF to the sample reagent
2. Add 2ml of the sample mixture directly to the Xpert MTB/RIF cartridge
3. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

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#### **0.1-1ml of CSF**

1. Re-suspend the CSF to a final volume of 2 ml with Xpert MTB/RIF sample reagent.
2. Add 2ml of the sample mixture directly to the Xpert MTB/RIF cartridge
3. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

#### **Less than 0.1ml**

1. Insufficient sample for testing in the Xpert MTB/RIF assay

#### **4. Related documents**

1. Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children. A pre-publication version of the policy guidance may be accessed at:  
<http://www.stoptb.org/wg/gli/assets/documents/WHO Policy Statement on Xpert MTB-RIF 2013 pre publication 22102013.pdf>
2. The full Expert Group meeting report is available at:  
<http://www.stoptb.org/wg/gli/assets/documents/Xpert%20Meeting%20Report%2024102013%20%20Pre%20publication%20FINAL.pdf>