National TB Control Program
National TB Reference Laboratory

Detection of *Mycobacterium tuberculosis* and Rifampicin Resistance by GeneXpert Test

(Standard Operating Procedure)

September 2019

Version 2.0

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1. Purpose

This SOP is written to describe the procedure for detection of *Mycobacterium tuberculosis* complex (MTBC) and Rifampicin (RIF) susceptibility using the GeneXpert MTB/RIF system.

2. Definitions and abbreviations

BSL	- Bio-safety level
DNA	- Deoxyribonucleic acid
Dx	- Diagnosis
GLI	- Global Laboratory Initiative
MDR-TB	- Multidrug-resistant TB
MTB	- *Mycobacterium tuberculosis*
MTBC	- *Mycobacterium tuberculosis* complex
PCC	- Probe Check Control
PCR	- Polymerase chain reaction
QC	- Quality control
RIF	- Rifampicin
rpoB	- gene encoding β-subunit of RNA polymerase and associated with RIF resistance
SOP	- Standard Operating Procedure
SPC	- Specimen Processing Control
TB	- Tuberculosis
v/v	- volume by volume
^{o}C	- degree Celsius

3. Responsibilities

Laboratory staff who perform GeneXpert MTB/RIF testing

4. Procedure

4.1. Principle

The GeneXpert MTB/RIF system is a fully automated nested real-time PCR system, which detects MTBC DNA in smear positive and negative sputum samples. It simultaneously identifies mutations in the *rpoB* gene, which is associated with rifampicin resistance.

The GeneXpert MTB/RIF system consists of the instrument, a computer, a barcode scanner and requires single-use disposable Xpert MTB/RIF cartridges that contain assay reagents. Following a 3-step sample preparation in the laboratory, the specimen is transferred into the MTB/RIF cartridge and entered into the GeneXpert instrument. By starting the test on the system software, the GeneXpert automates all following steps, including sample work-up, nucleic acid amplification, detection of the target sequence and result interpretation. The primers in the Xpert MTB/RIF assay amplify a portion of the *rpoB* gene containing the 81-base pair “core” region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with resistance to RIF.

The MTB/RIF assay as an entirely self-contained test with quality control of the various steps included. The assay includes a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. A Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. However, calibration of all the modules is required annually or after 2000 tests run by a module. It is important to monitor errors and invalid results to ensure timely corrective actions.

4.2. Samples

a) Sputum specimen (Natural or Induced)
b) CSF
c) Lymph node

Whenever possible, samples should be transported and stored at 2–8 °C prior to processing (the maximum time for storage and processing is 7 days).

4.3. Equipment and materials

1. GeneXpert instrument
2. MTB/RIF cartridges
3. Disposable graduated transfer pipettes
4. Sterile screw-capped specimen collection containers
5. Disposable gloves
6. Bio-hazard plastic bag for waste disposal
7. Timer
8. Indelible labeling marker
9. Sterile pipettes for sample processing (supplied with kit)
10. A jar for decontamination of pipettes
11. Rack for placing falcon tubes
12. Trays for placing cartridges
13. 50 mls Falcon tubes for sample processing
14. N95 Mask
15. Absorbent paper
16. Tissue grinder
17. Vortex mixer
18. Phosphate buffer saline
19. Centrifuge
20. Thermometer
21. Wash bottles
22. Towel
23. Lab coat
24. A4 Paper

4.4. Reagents and solutions

1. Sample reagent (supplied with kit)
2. 1% hypochlorite solution (freshly prepared)
3. 5% Phenol solution (freshly prepared)

4.5. Detailed instructions for use

4.5.1 Start-up of GeneXpert instrument

•Perform start-up of the instrument before start of specimens processing
•Turn on the computer, and then turn on the GeneXpert instrument.
•On the Windows desktop, double-click the GeneXpert Dx shortcut icon
•Log on to the GeneXpert Dx System software using your user name and password
•Click on "CHECK STATUS" and check if modules are available
•If modules are not available proceed to the "Troubleshooting" section of the User manual

4.5.2 Disinfect the working area

•Disinfect the working area using 1% hypochlorite solution

4.5.3 Labelling

•Label Xpert MTB/RIF cartridge with the sample ID.
(Do not put the label on the lid of the cartridge. Write on the sides of the cartridge or affix ID label).

4.5.4 Preparation of samples for testing

•Tissues must be processed within a biological safety cabinet, given the risk of producing aerosols while grinding and homogenizing samples.

(a) Sputum

1. Transfer specimen from leak-proof sputum collection container to 50 ml falcon tube.
2. Add Sample Reagent 2:1 (v/v) to sample and close the lid
3. Shake vigorously 20 times or vortex till sample liquefy well (at least 10 sec).
4. Incubate for 10 minutes at room temperature
5. Shake the specimen again vigorously 20 times or vortex till solution dissolve well (at least 10 sec).
6. Incubate for another 5 minutes at room temperature
7. If there are still clumps of sputum, shake again vigorously and incubate for another 3-5 minutes

8. Using the sterile transfer pipette, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark (= 2ml)
9. Open the cartridge lid
10. Transfer sample into the open port of the Xpert MTB/RIF cartridge (Fig. 1)
11. Make sure that no bubbles are created when transferring the specimen into the cartridge as this can lead to an error (no result)
12. Dispense slowly to minimize the risk of aerosol formation
13. Close the cartridge lid
14. Make sure the lid snaps firmly into place
15. Keep the remaining liquefied sample at 2-8ºC for repeat testing when be required (it can be kept at 2-8ºC till 12 hrs.)

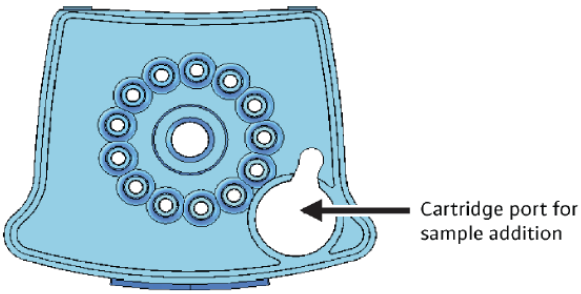

Figure 1. Xpert MTB/RIF cartridge (top view).

(b) CSF

1. According to the volume of CSF, perform the following steps:
   a. If there is more than 5 ml of CSF: Transfer all of the specimens to a conical centrifuge tube, and concentrate the specimen at 3000 g for 15 minutes. Discard the supernatant. Resuspend the deposit to a final volume of 2 ml by adding the Xpert MTB/RIF sample reagent. Then, add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge.
   b. If there is 1-5 ml of CSF: Add an equal volume of sample reagent to the CSF. Add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge.
   c. If there is 0.1-1 ml of CSF: Resuspend the CSF to a final volume of 2 ml by adding the Xpert MTB/RIF sample reagent. Then, add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge.
   d. If there is less than 0.1 ml: This is an insufficient sample for testing using the Xpert MTB/RIF assay. Request another sample.
2. Follow the step no. 9-15 as mentioned in (a) sputum

(c) Lymph nodes / Lymph node aspirate (for Xpert MTB/RIF test)

Tissues must be processed within a biological safety cabinet, given the risk of producing aerosols while grinding and homogenizing samples.

1. Cut the tissue specimen into small pieces with sterile pair of forceps and scissors in a sterile mortar (or homogenizer or tissue grinder).
2. Add 2 ml of sterile phosphate buffer (PBS).
3. Grind the solution of tissue and PBS using a mortar and pestle (or homogenizer or tissue grinder) until a homogeneous suspension has been obtained.
4. Transfer 0.7 ml of the homogenized tissue specimen by transfer pipette to a sterile, conical screw-capped tube. (Avoid transferring any clumps of tissue that have not been properly homogenized.)
5. Add a double volume of Sample Reagent (1.4 ml) to 0.7 ml of homogenized tissue by transfer pipette.
6. Follow the step no. 3-15 as mentioned in (a) sputum.

4.5.5 Starting the test

1. Start the test within 30 minutes of adding the sample to the cartridge
2. In the GeneXpert Dx System window, click "CREATE TEST". The Scan Cartridge Barcode dialog box appears
3. Scan the barcode on the Xpert MTB/RIF cartridge. The Create Test window appears
4. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
5. In the Sample ID box, type the sample laboratory serial number (ID). Make sure you type the correct sample ID, patient ID and write in Note field. The sample ID is associated with the test results and is shown in the "View Results" window and all the reports.

Data Entry Format for Gene Xpert Test

Patient ID

Patient's Name, Patient Home Town

(eg:Chaw Su Myat,Hlaingtharyar)

Sample ID

GeneXpert Site -Year-Lab registration no-GeneXpert registration no-Treatment unit

(eg:LTA-19-2539-1305,Hlaing TBC)

Note

Age,Sex, Previously treated for TB,HIV Status, Reason for Examination, Microscopy Result

M, F,	Yes, No, Unk,	HIV+, HIV-, HIV?,	Dx, Fu, MDR, DM, Na,	Pos, Neg, Nd,

(eg: 34,M,Yes, HIV+,Dx, Pos,)

6. Click “Start Test”
7. In the dialog box that appears, type your user name and password
8. Open the instrument module door with the blinking green light and load the cartridge
9. Close the door
10. The test starts and the green light stops blinking
11. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge

4.6. Reading, interpretation, recording and reporting

- In the GeneXpert Dx System window, click “VIEW RESULTS” on the menu bar. The View Results window appears
- If the software reports “Error”, Invalid”, or “No result”, repeat the test using the already prepared specimen and a new cartridge
- If the repeated test shows “Error”, “Invalid” or “No result” again, should proceed according to troubleshooting manual to exclude technical problems before requesting a new specimen
- Record the results in an GeneXpert register for TB laboratory examination
- Use red pen to record positive results
- Report the results as soon as possible
- Report “MTB not detected” or “MTB detected”
- For rifampicin resistance results, report “Rif resistance not detected” or “Rif resistance detected”
- Report “Please submit a new specimen” if the system repeatedly did not produce a result and you have excluded and/or fixed a technical problem

4.7. Quality control

- Maintain the instrument according to SOP
- Validate results prior reporting
- Monitor the errors and invalid results
- Participate in EQA program periodically

5. Waste Management and other safety precautions

- Dispose of used cartridges in the appropriate specimen waste containers according to your institution's standard practices
- At the end of each day, the used sputum containers, pipettes and cartridges must be sealed in a bag and incinerated as soon as possible
- Keep the bag in a safe, closed bin or large bucket until it can be incinerated in a bag
- In intermediate or central laboratories where there is an autoclave, infectious waste should be collected in an autoclavable bag and should be autoclaved before incineration
- Make sure the tubes are tightly closed before shaking
- Prepare samples for testing in a well-ventilated area
- Clean-up spill immediately according to the procedure for management of specimen's spills

6. References

- Cepheid GeneXpert GXMTB/RIF-10. Package inserts. 300-6252 Rev. D, September 2010 2015;
- Cepheid GeneXpert Dx System. Operator Manual. Software version 4.0. 300–7607, Rev.C.1
- Procedure for use and maintenance of personal protective equipment
- Procedure for spill management
- Procedure for disinfection and decontamination
- Procedure for waste management
- The laboratory safety manual
- The WHO TB laboratory safety manual, 2013
- http://apps.who.int/iris/bitstream/10665/77949/1/9789241504638_eng.pdf
- MSDS for the reagents and solutions used in the procedure

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GeneXpert Maintenance Checklist

Installation date	
Serial Number	

Month	
Year	

Please write your initials in the boxes when done

Daily Maintance	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean work area																															
Discard used cartridges																															
Keep module doors upright																															

Please write date and your initials in the boxes when done

Weekly Maintance	Week 1	Week 2	Week 3	Week 4	Week 5
Power down GeneXpert insturment and computer					
Monthly Maintance					
Archive and delete tests- Save on CD					
Clean fan filters*					
Clean module PCR tube slots					
Clean cartidge bays and plunger rods					
Clean instrument surface					
Yearly Maintance					
Perform module calibration with XpertCheck					

*Time of clean fan filter vary from weekly to monthly depending on weather condition and loation of Xpert room.

Quarterly Report on laboratory supplies and equipment stock for Township Level

Name of township -
Name of Technician -

Store -

Quarter of	Date of completion of this form:

Table 2

Item	Gene Xpert Cartridge (No. ofCartridges)	Pasture Pipette (No. of pippettedges)	Falcon tube (50 ml) (No of Falcon tube)	Absrorbent paper (Pcs)	Sodium Hypochlorite solution (----------/bot)	Glove 100 pcs/box	Ethonal (1L/bot)	Auto clavable Bag (100 pcs/pkt)
Opening Balance								
Received during quarter								
Issued during quarter								
Closing Balance								
Expire Date								
1 month need								
Month in Hand								

Countersigned by Township Medical Officer

Signature .....

Name .....

Designation .....

Prepared By Laboratory Technician

Signature .....

Name .....

Designation .....

Annex (2)

TB (05)