Standard Operating Procedure (SOP)

on

Xpert MTB/RIF(Ultra) cartridge using GeneXpert testing System

Document Control: SOP-PR-011

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Standard Operating Procedure			
Title: SOP on Xpert MTB/RIF(ultra) cartridge using GeneXpert testing System			SOP No.: SOP-PR-011 Effective date:
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Date:			
Signature:			

REVISION RECORD		
Revision No.	**Reason for Revision**	**Revision Date**

2. Signature Page

Document control number	**SOP-PR-011**
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By signing this page, members of staff and management confirm they have read this SOP and promise to implement the procedures contained within.

Name	Title	Date	Signature

3. 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 (Ultra) cartridges. The procedure outlines the preparation of samples for Xpert MTB/RIF (Ultra) testing.

4. 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
ºC	- degree Celsius

5. Responsibilities

Laboratory staff who perform Gene Xpert MTB/RIF (Ultra) testing

6. Procedure

6.1. Principle

The GeneXpert MTB/RIF system is a fully automated semi quantitative nested real-time PCR system followed by high resolution melt technology, 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 (Ultra) cartridges that contain assay reagents. Following a 3-step sample preparation in the laboratory, the specimen is transferred into the MTB/RIF (Ultra) cartridge and entered 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. Two multicopy amplification targets (IS6110 and IS1081) and a larger PCR chamber were used to increase the sensitivity. The primers in the Xpert MTB/RIF (Ultra) assay amplify a portion of the *rpoB* gene containing the 81-base pair “core” region. The probes can differentiate between the conserved wild-type sequence and mutations in the core region that are associated with resistance to RIF.

The MTB/RIF (Ultra) 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.

6.2. Samples

a) Sputum specimen (Natural or Induced)
b) CSF
c) Lymph node aspirate
d) Gastric aspirate
e) Stool

6.3. Equipment and materials

Sputum specimen (Natural or Induced), CSF, Lymph node Aspirate, Gastric Aspirate

1. GeneXpert instrument Computer installed with the GeneXpert Dx software version 4.7b or higher MTB/RIF (Ultra) cartridges
2. Sterile disposable graduated transfer pipettes
3. Sterile screw-capped specimen collection containers
4. Disposable gloves
5. Bio-hazard plastic bag for waste disposal
6. Timer
7. Indelible labeling marker
8. Sterile pipettes for sample processing
9. A jar for decontamination of pipettes
10. Rack for placing falcon tubes
11. Trays for placing cartridges
12. 50 mls Falcon tubes for sample processing
13. N95 Mask
14. Absorbent paper

Stool

1. Disposable, screw-capped stool containers with a spoon (or, alternatively, screw-capped universal sputum or urine containers/cups)
2. Toilet paper or plastic sheet for stool collection (in case of onsite collection of the stool)

3. Plastic bag with absorbent material
4. Disposable gloves - Laboratory coat
5. Protective eyewear, Wooden sticks, Timer, Permanent marker pen
6. 0.5% sodium hypochlorite solution and 70% alcohol or other tuberculocidal disinfectant
7. Xpert MTB/RIF (Ultra) kit, including: - single-use, disposable, Xpert MTB/RIF (Ultra) cartridges
8. Sterile disposable transfer pipettes
9. Bottles with sample reagent (SR) - Spare sterile transfer pipettes with 2 mL marking (in case of many liquid stool samples) - GeneXpert instrument with appropriate infrastructure, equipped with a computer, GX 4.7b software and barcode reader (Cepheid Inc. Sunnyvale, USA) - Printer, if a standard Xpert test report should be issued

6.4. Reagents and solutions

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

6.5. Detailed instructions for use

6.5.1. 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 machine
- On the Windows desktop, double-click the GeneXpert Dx shortcut icon
- Log on to the GeneXpert Dx System software using your username and password
- Click on “CHECK STATUS” and check if modules are available
- If modules are not available proceed to the “Troubleshooting” section of the

6.5.2. Preparation of samples for testing

(a) Sputum

1. Disinfect the working area using 1% hypochlorite solution
2. Label each Xpert MTB/RIF (Ultra) cartridge with the sample ID. (Do not put the label on the lid of the cartridge or obstruct the existing 2D barcode on the cartridge. Write on the sides of the cartridge or affix ID label).
3. Transfer specimen from leak-proof sputum collection container to 50 ml falcon tube.
4. Add Sample Reagent 2:1 (v/v) to sample and close the lid

5. Shake vigorously 20 times or vortex till sample liquify well.
6. Incubate for 10 minutes at room temperature
7. Shake the specimen again vigorously 20 times or vortex till solution dissolve well.
8. Continue incubation for another 5 minutes
9. Observe if samples liquefied completely and no clumps of sputum are visible
10. If there are still clumps of sputum, shake again vigorously, and incubate for another 3-5 min
11. Using the sterile transfer pipette, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark (= 2ml)
12. Open the cartridge lid
13. Transfer sample into the open port of the Xpert MTB/RIF cartridge (Fig. 1)
14. Make sure that no bubbles are created when transferring the specimen into the cartridge as this can lead to an error (no result)
15. Dispense slowly to minimize the risk of aerosol formation
16. Close the cartridge lid
17. Make sure the lid snaps firmly into place
18. Keep the remaining liquefied sample at 2-8ºC for repeat testing when be required (it can be kept at 2-8ºC till 12 hrs.)

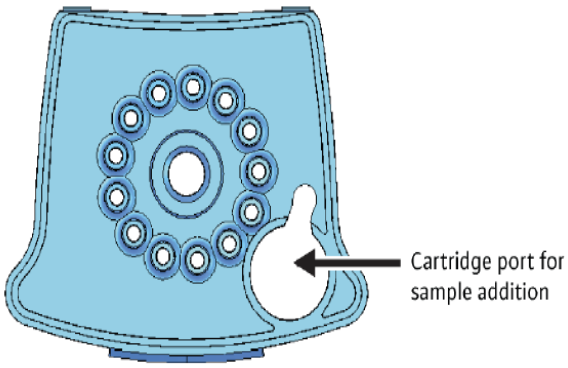

Figure 1. Xpert MTB/RIF cartridge (top view).

(b) CSF

1. Disinfect the working area using 1% hypochlorite solution
2. Label each Xpert MTB/RIF cartridge with the sample ID.
3. According to the volume of CSF, perform the following steps:
   a. If there is more than 5 ml of CSF: Transfer all of the specimen 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.

4. Follow the step no. 12-18 as mentioned in (a) sputum.

(c) Lymph node aspirate

1. Disinfect the working area using 1% hypochlorite solution
2. Label each Xpert MTB/RIF cartridge with the sample ID.
3. Resuspend the lymph nodes 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.
4. Follow the step no. 12-18 as mentioned in (a) sputum.

(d) Gastric aspirate

1. Disinfect the working area using 1% hypochlorite solution
2. Label each Xpert MTB/RIF cartridge with the sample ID.
3. Add an equal volume of sample reagent to the Gastric Aspirate. Add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge.
4. Follow the step no. 12-18 as mentioned in (a) sputum.

(e) Stool

Take a bottle containing 8 mL of SR bottle from the Xpert MTB/RIF Ultra kit and label it with the unique ID

If the stool is solid, transfer about 0.8 gm of stool (similar size to a peanut or the tip of little finger) into SR bottle.

If the stool is liquid or watery, use a transfer pipette to remove 2 mL of SR from the SR bottle and dispose it. Subsequently, use the same pipette to transfer 2 mL of the stool sample into the SR bottle.

The stool specimen processing procedure is adopted by a Simplified One-Step (SOS) specimen processing method developed by KNCV foundation.

Close the lid of the SR bottle tightly and shake the bottle vigorously for 30 seconds. *(Do not vortex as this may lead to the formation of a stable suspension of fine particles which may not sediment well.)*

Incubate the bottle for 10 minutes at room temperature.

Shake the bottle vigorously again for 30 seconds (do not vortex). Slightly untighten the screw cap of the SR bottle and put bottle in such position that the supernatant can easily be aspirated.

Let the bottle stand for 10 minutes at room temperature to allow the solid particles and debris to settle.

If the stool debris has not fully sedimented, the incubation time can be prolonged with an additional 10 min.

If there are still solid parts visible in the supernatant (upper layer) after the prolonged incubation time, then repeat steps 4 and 5.

Proceed with the next steps as instructed by the manufacturer:

Like sputum specimens, after being processed (disinfection for contamination/centrifugation), the supernatant or pellets can be stored for up to 3 days at 2-8ºC.

Specimens processed with SR can be stored for up to 12 hours at 2-8°C or up to 5 hours at room temperature.

Cartridge added with sample can be stored for up to 4 hours at room temperature *(Do not store the cartridge added with sample in the refrigerator*).

6.5.3. 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.

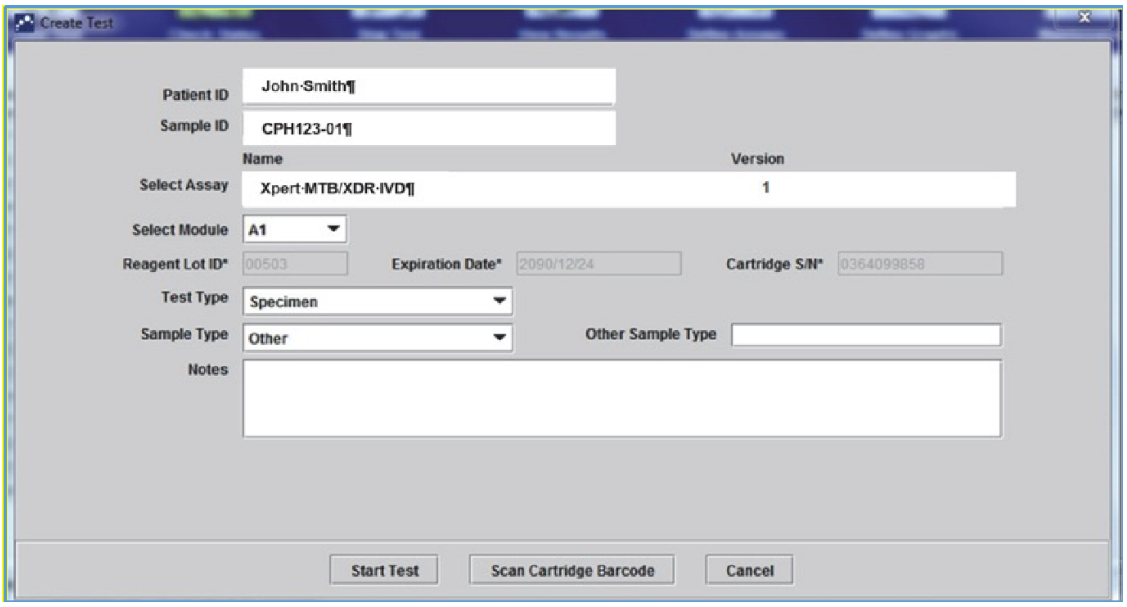

Figure 2. GX Dx Create Test Window

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. When the test is finished, the green light turns off.
11. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.

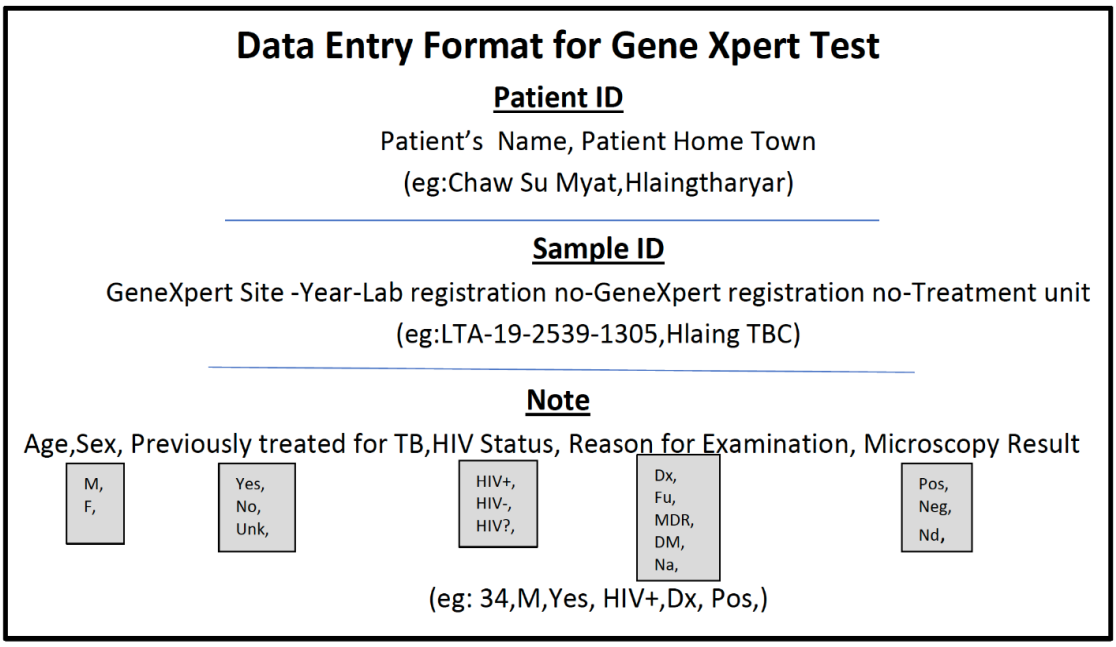

Figure 3: Example of information to be entered in the create test form of GX DX system

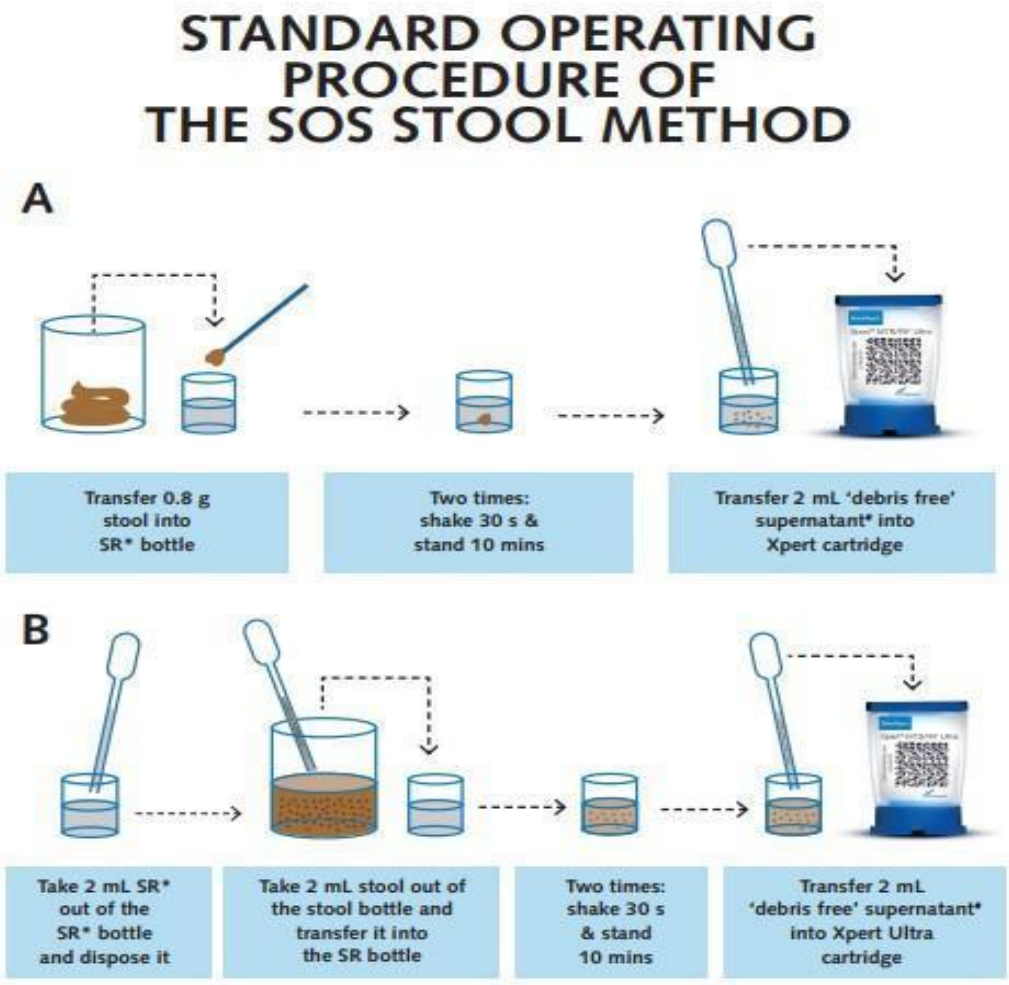

Figure 4: Standard Operating Procedure of the SOS Stool Method

Figure 5: GXP (Ultra) Workflow

6.6. Reading, interpretation, recording and reporting

6.6.1. Viewing results on the GeneXpert software (Basic user settings)

- In the GeneXpert Dx System window, click “VIEW RESULTS” on the menu bar. The View Results window appears
- If the software reports “Invalid”, ask the new sputum samples again and repeat the test.
- If the software reports “Error”, 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

6.6.2. Viewing results on the GeneXpert software (Basic user settings)

- 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

6.6.3. Result reporting

Result	Abbreviation
MTB not detected	N
MTB detected; RIF resistance not detected	T
MTB detected; RIF resistance detected	RR
MTB detected; RIF resistance indeterminate	TI
MTB detected trace; RIF resistance indeterminate	TT
Error / Invalid / No results	I

6.6.4. GxAlert system use for standardized report

GxAlert, based on the Aspect™ software platform is designed to work with Cepheid's GeneXpert®.

7. Quality control

- Maintain the instrument according to SOP
- Validate results prior reporting
- Monitor the errors and invalid results
- Participate in EQA program periodically with CDC, Atlanta (JICA fund) in 2017, Vietnam in 2018 – 2019

8. 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

9. Biosafety measures in laboratory performing Sputum Microscopy and GXP test

Classification of TB laboratories

3 main levels of procedural risk, based on the activities being performed and their associated risks

- low TB risk
- moderate TB risk
- high TB risk (such as a TB containment laboratory)

Low-risk TB laboratories

Low-risk laboratories that follow the minimum biosafety requirements as the viscous nature of sputum is not prone to generating aerosols when good laboratory techniques are followed.

Low risk laboratories can:

- Manipulate sputum specimens for direct sputum-smear microscopy
- Manipulate sputum specimens for the Xpert MTB/RIF® assay (Cepheid, Sunnyvale Ca., USA)

Factors that increase the risk of infection in low -risk TB laboratories

- improper use of bench spaces
- leak specimen containers

· Careless manipulation of specimens which may lead to subsequent aerosolization

- specimens may be shaken vigorously
- ventilation or illumination may be poor

The following biosafety requirements should be established

1. Use of bench space:

The bench used to process specimens for direct sputum-smear microscopy, or Xpert MTB/RIF assay should be separate from areas used to receive specimens and from administrative areas.

2. Ventilation:

Smears performed directly on sputum samples, and processing specimens for the Xpert MTB/RIF assay, may both be carried out on an open bench in an adequately ventilated area when appropriate microbiological techniques are used.

Adequate ventilation for TB laboratories

TB laboratories is typically described as directional airflow with 6–12 air exchanges per hour (ACH) . Directional airflow refers to air flowing from clean areas towards areas where aerosols maybe generated; this air should be safely discharged from the room. “Air exchanges per hour” refers to the number of room volumes of air expelled per hour and replaced with clean air. When mechanical ventilation is used, air exchanges per hour can be readily calculated

For low-risk procedures, natural ventilation should be sufficient providing that air flows away from the technician and across the work area along with potentially infectious materials, then away from occupied areas of the room and outside the laboratory; this flow should provide protection from aerosols that might be generated in the work area. In order to have directional control of contaminants in the air, air should move at least 0.5 m/s.

Ventilation can be ensured by opening windows if the local climate allows. When the climate prevents windows from being opened, consideration should be given to using mechanical ventilation systems that provide an inward flow of air without recirculation in the room. Air conditioners should be placed only after the direction of airflow has been considered. It is important to ensure that air in the laboratory flows away from the technicians.

Minimizing the generation of aerosols:

Care should be taken when opening specimen containers, which may have been shaken during transportation to the laboratory. The risk of infectious material spattering in an open Bunsen burner flame should be avoided when drying smears. It is preferable to air-dry smears and use a flame to fix the smears only when they are completely dry. Disposable wooden applicator sticks, or transfer loops are preferred for making smears.

3. Handling leaking specimen containers:

The integrity of specimen containers delivered to the laboratory needs to be checked upon arrival to the laboratory. Leaking containers may need to be discarded and a fresh sample requested. If an adequate specimen remains in a leaking container, the container may be decontaminated with a suitable disinfectant before processing. Samples should be transported to the laboratory in an upright position to minimize leakage.

4. Personal protective equipment:

Protective laboratory coats should be always worn in the laboratory. Gloves must be worn for all procedures that involve direct contact, or may involve accidental contact, with sputum, blood, body fluids and other potentially infectious material. Gloves must be changed regularly and should not be reused. Staff should always wash their hands before leaving the laboratory. Respirators are not required for use during the preparation of sputum smears.

10 .References

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11. Annexes

Annex1: Request for examination of biological specimen for TB (TB-O5)

Referred/ Treatment unit: _____ Date of request: _____

Patient's name: _____ Nationality: ☐ N ☐ NN

Age (years): _____ Date of birth: _____ Sex: ☐ Male ☐ Female

Patient's address: _____

_____ Telephone: _____

Previously treated for TB	☐ Yes	☐ No	☐ Unknown
if Yes: Took full course	☐ Yes	☐ No	☐ Unknown
DM status:	☐ Yes	☐ No	☐ Unknown
HIV Status:	☐ Positive	☐ Negative	☐ Unknown
Contact of DR-TB	☐ Yes	☐ No	

Reason for examination: _____

☐ Diagnosis Presumptive TB Reg./OPD No._____

☐ Follow-up Township DS TB / DR-TB No._____ Month of treatment_____

Specimen type: ☐ Sputum ☐ Gastric aspirate ☐ CSF ☐ Other (specify):_____

Test(s) requested: ☐ Microscopy ☐ Xpert MTB/RIF ☐ Xpert MTB /RIF Ultra ☐ Xpert MTB/XDR

☐ Line probe assay (FL) ☐ Line probe assay (SL)

☐ Other molecular tests…….

☐ Culture ☐ DST ……

Date of Specimen Collected ……

Requested by Signature: _____

Name: _____

Designation: _____

Contact phone no. : _____

Microscopy results (to be completed in laboratory) ☐ FM ☐ ZN

Date of specimen received	Laboratory serial number(s)	Specimen type	Visual appearance (blood-stained, mucopurulent or saliva)	Result (tick one)				
				Negative	Scanty	+	++	+++

Examined by Signature: _____

Name: _____

Designation: _____

Date of result: _____

☐ **X-pert MTB/RIF test result** ☐ **Xpert MTB/RIF Ultra test result** (to be completed in the laboratory)

Date of specimen collected: _____ X-pert Lab No : _____

N	I	T	RR	TI	TT

N = No MTB, **I** = Invalid / No result, **T** = MTB detected, **RR** = Rif resistant, **TI** = MTB (+) Rif resistance indeterminate, **TT** = MTB detected (trace), RIF resistance indeterminate

X-pert MTB/XDR test result (to be completed in the laboratory if test is performed)

Date of specimen collected: _____ X-pert Lab No: _____

Results						
MTB	☐ MTB detected		☐ MTB not detected		☐ Invalid/Error	
DST [a]	**H**	**FQ**	**Am**	**Km**	**Cm**	**Eth**

[a] Results codes: **S** = Resistance not detected; **R** = Resistance detected; **LR**= Low resistance detected; **TI** = MTB (+) & Resistance indeterminate;

Examined by Signature: _____

Name: _____

Designation: _____ Date of result: _____

Culture results (to *be completed in the laboratory*)

Media Used	Date of Specimen Received	Laboratory Serial no	Results*(tick one)*						
			Negative	scanty	+	++	+++	NTM	Contaminated
L-J									
MGIT									

Examined by Signature: _____

Name: _____

Designation: _____ Date of result: _____

Drug susceptibility test (DST) and line probe assay (LPA) results (to be completed in the laboratory)

Date sample received	Method [b]	Laboratory serial number(s)	Results [c] (mark for each drug)													
			H	R	Z	E	S	Am	Km	Cm	FQ	Bdq	Lzd	Cfz	Pa	

[b] Specify: solid media DST; liquid media DST; direct LPA; indirect LPA

[c] Results codes: **R** = Resistant; **S** = Susceptible; **C** = Contaminated; **—** = Not done

Signature of Microbiologist: _____

Name: _____

Designation: _____ Date of result: _____