Standard Operating Procedure (SOP)

on

Xpert MTB/XDR cartridge using GeneXpert testing System

National TB Programme

Department of Public Health

Ministry of Health

The Republic of the Union of Myanmar

Table of Contents

List of Figures

1. Preparation, Review and Approval

Standard Operating Procedure			
Title: SOP Xpert MTB/XDR cartridge using GeneXpert testing System			SOP No.: SOP-PR-011
			Effective date:
Area: Quality Management, Version 1			Review period:
	Prepared by	**Reviewed by**	**Approved by**
Name:			Dr. Wint Wint Nyunt
Designation:			Head/Sr.Consultant Microbiologist, NTRL
Date:			
Signature:			

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

2. Signature Page

Document control number	**SOP-PR-011**
Document control title	**Standard Operation Procedure (SOP) Xpert MTB/XDR cartridge using GeneXpert testing System**

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

Xpert MTB/XDR sets new standards by detecting mutations associated with resistance towards Isoniazid (INH), Fluoroquinolones (FLQ), second-line injectable drug (SLID) (Amikacin, Kanamycin, Capreomycin) and Ethionamide (ETH) in a single test.

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
INH	Isoniazid
FLQ	Fluoroquinolone
ETH	Ethionamide
SLID	second line injectable drug
QRDR	Quinolone resistance determining regions
XDR	extensively drug resistance tuberculosis
rpoB	gene encoding β-subunit of RNA polymerase and associated with RIF resistance
katG	gene
fabG1	gene
oxyR	*ahpC intergenic region*
inhA	promoter
gyrA	gene
gyrB	gene
Rrs	gene
eis	promoter
SOP	Standard Operating Procedure
SPC	Specimen Processing Control
TB	Tuberculosis
v/v	volume by volume

5. Responsibility

Laboratory staff who perform GeneXpert MTB/XDR testing

6. Procedure

1. 6.1. Principle

The Xpert MTB/XDR Assay is an automated in vitro diagnostic test for detection of MTB complex DNA and resistance associated mutations. The assay is performed on Cepheid GeneXpert Instrument Systems equipped with GeneXpert 10 color modules.

The GeneXpert Instrument System integrate and automate sample processing, nucleic acid amplification, and detection of the target sequences in samples using nested real-time PCR and melt peak detection. The GeneXpert Instrument Systems consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing the results. The system requires UPS and the use of single-use disposable Xpert cartridges that contain target specific polymerase chain reaction (PCR) reagents and hosts the PCR process and melt peak detection. Because the Xpert cartridges are self-contained, risk of cross-contamination between samples is minimized.

The Xpert MTB/XDR Assay cartridge includes reagents for the detection of MTB profile and 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. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The Xpert MTB/XDR Assay cartridge has all reagents on board, except sample reagent (SR) which requires the user to add the SR to the specimen prior loading the treated specimen into the cartridge. The results are interpreted by the GeneXpert software from measured fluorescent signals and embedded calculation algorithms and are shown in the View Results window in tabular and graphic formats. It also reports if the test is invalid, has encountered an error or produces no result. The Xpert MTB/XDR detects XDR MTB with resistance to INH, ETH, FLQs, and SLIDs directly from unprocessed sputum or from concentrated sediment from sputum in less than 90 minutes.

The Xpert MTB/XDR assay is intended for use as a reflex test for a specimen (unprocessed sputum, concentrated sputum sediments, or MGIT culture) that is determined to be MTB positive. In specimens where MTB is detected, the Xpert MTB/XDR assay can also detect isoniazid (INH) resistance associated mutations in the *katG* and *fabG1* genes, *oxyR-ahpC* intergenic region and *inhA* promoter; ethionamide (ETH) resistance associated with *inhA* promoter mutations only; fluoroquinolone (FLQ) resistance associated mutations in the *gyrA* and *gyrB* quinolone resistance determining regions (QRDR); and second line injectable drug (SLID) associated mutations in the *rrs* gene and the *eis* promoter region.

2. 6.2. Sample

(a) Sputum specimen (Natural/Expectorated or Induced)

- Collect high quality sputum specimens as per the available SOPs. Reject specimens with obvious food particles or other solid particles.
- Proper specimen collection, storage and transport are essential for correct results
- Whenever possible, samples (unprocessed sputum) should be transported and stored at 2–35°C prior to processing (the maximum time for storage and processing is 7 days). The Xpert MTB/XDR assay can be used to test left-over SR treated specimen from Xpert MTB/RIF or Xpert MTB/RIF Ultra assays. However, in such cases, the volume of the leftover SR treated specimen must be ≥ 2mL and the mix should be stored at 2–8 °C for no longer than 4 hours or at 35°C for no longer than 2.5 hours.

3. 6.3. Equipment and materials

1. GeneXpert instrument 10 colour modules
2. Xpert MTB/XDR 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 labelling marker (Permanent marker)
9. Sterile pipettes for sample processing (supplied along with the kit)
10. A jar for decontamination of pipettes

11. Rack for placing falcon tubes
12. Trays for placing cartridges
13. Sterile 50 ml Falcon screw-capped tubes for sample processing
14. N95 Mask
15. Absorbent paper
16. Vortex mixer
17. Thermometer
18. Wash bottles
19. Towel
20. Lab coat
21. A4 Paper

4. 6.4. Reagents and solutions

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

5. 6.5. Detailed instructions for use

6. 6.5.1. Start-up of GeneXpert instrument

- Perform start-up of the instrument before start of specimens processing
- Turn on the GeneXpert Dx instrument, and then turn on the computer.
- 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 User manual

- 6.5.2. Disinfect the working area

- Disinfect the working area using 1% hypochlorite solution

6.5.3. Labelling

Remove a cartridge from the package. Inspect the cartridge for damage. If damaged, do not use it.Label Xpert MTB/XDR 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).

Figure 1. Write on side of cartridge

- 6.5.4. Preparation of samples for testing

1. 6.5.4.1. Sputum

1. Transfer specimen from leak-proof sputum collection container to sterile 50 ml falcon tube.
2. Add Sample Reagent 2:1 (v/v) to sample and close the lid
3. Shake vigorously 10 to 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 10 to 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 for maximum of 4 hrs.)

Figure 2. Add processed specimen to Xpert MTB/XDR assay cartridge

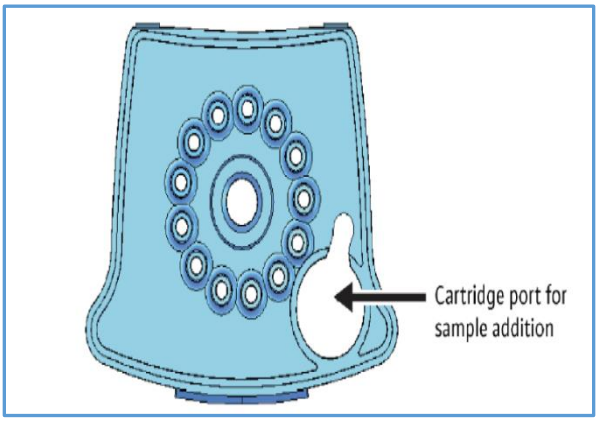

Figure 3. Xpert MTB/XDR cartridge (top view)

- ## 6.5.5. Starting the test (same format for ultra)

7. Start the test within 30 minutes of adding the sample to the cartridge
8. In the GeneXpert Dx System window, click “CREATE TEST”. The Scan Cartridge Barcode dialog box appears
9. Scan the barcode on the Xpert MTB/XDR cartridge. The Create Test window appears
10. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

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 the Note field. The sample ID is associated with the test results and is shown in the "View Results" window and all the reports.

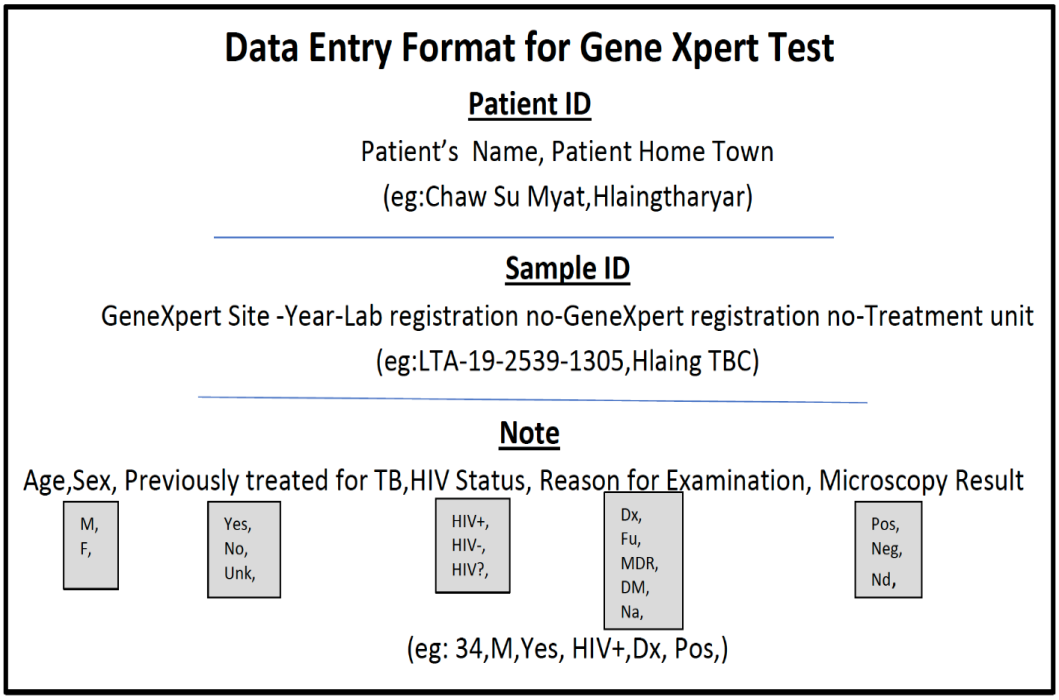

Figure 4. GX Dx Create Test Window

11. Click "Start Test"
12. In the dialog box that appears, type your username and password
13. Open the instrument module door with the blinking green light and load the cartridge
14. Close the door
15. The test starts and the green light stops blinking. When the test is finished, the green light turns off.
16. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge

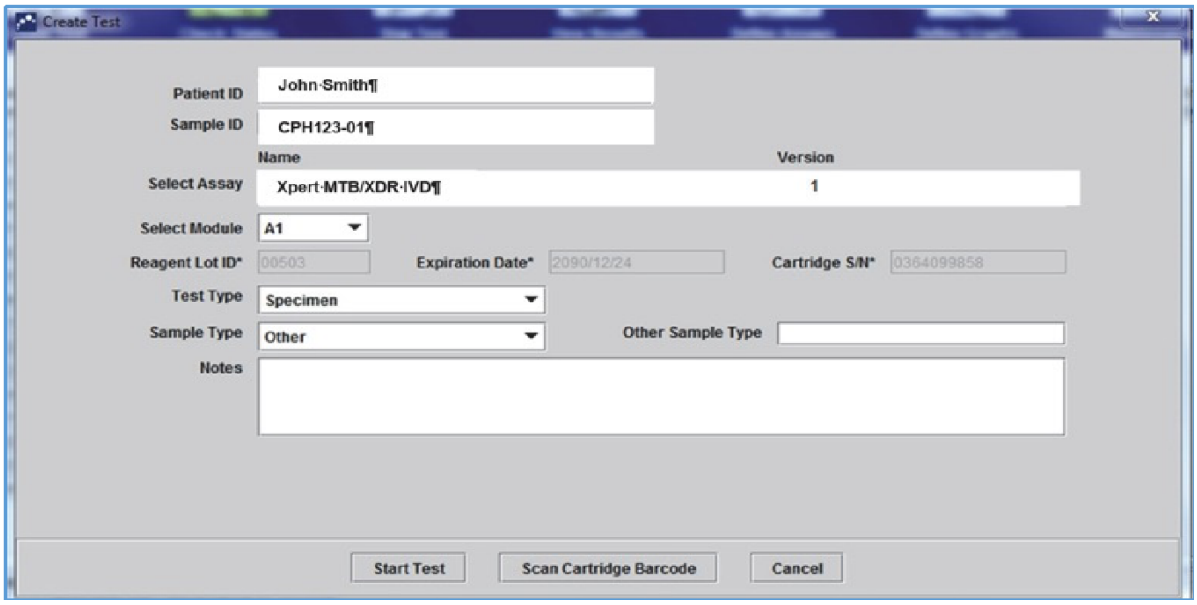

Figure 5. Example of information to be entered in the create test form of GX DX system

- 6.5.6. 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 all "Resistance detected" results
- Report the results as soon as possible
- 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

Drug Class	Result Call
N/A	INVALID/ERROR/NO RESULT MTB DETECTED MTB NOT DETECTED
Isoniazid	Low INH Resistance DETECTED INH Resistance DETECTED INH Resistance NOT DETECTED INH Resistance INDETERMINATE
Fluoroquinolone	Low FLQ Resistance DETECTED FLQ Resistance DETECTED FLQ Resistance NOT DETECTED FLQ Resistance INDETERMINATE
Amikacin	AMK Resistance DETECTED AMK Resistance NOT DETECTED AMK Resistance INDETERMINATE
Kanamycin	KAN Resistance DETECTED KAN Resistance NOT DETECTED KAN Resistance INDETERMINATE
Capreomycin	CAP Resistance DETECTED CAP Resistance NOT DETECTED CAP Resistance INDETERMINATE
Ethionamide	ETH Resistance DETECTED ETH Resistance NOT DETECTED

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 are 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 may be 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

1. Cepheid GXMTB/XDR-10. Package inserts. 302-3514, Rev. C, April 2021
2. Cepheid GeneXpert Dx System. Operator Manual. Software version 6.2 or higher
3. Procedure for use and maintenance of personal protective equipment
4. Procedure for spill management
5. Procedure for disinfection and decontamination
6. Procedure for waste management
7. The laboratory safety manual
8. The WHO TB laboratory safety manual, 2013
9. MSDS for the reagents and solutions used in the procedure

11. Annexures:

Annex 1: 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: _____