PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC72300 – Xpert MTB/RIF	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date:	Next Review Date:
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved:
Accreditation Canada Applicable Standard: NA	

Uncontrolled When Printed

GUIDING PRINCIPLE:

The Xpert MTB/RIF assay is a semi-quantitative, nested real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for the detection of *Mycobacterium tuberculosis* (MTB) complex DNA in unprocessed sputum samples. In specimens where *Mycobacterium tuberculosis* complex is detected, the Xpert MTB/RIF Assay can also detect rifampin-resistance. The Xpert MTB/RIF Assay is intended for use with specimens from patients for whom there is clinical suspicion of tuberculosis (TB) and who have received no antituberculosis therapy.

PURPOSE/RATIONALE:

This standard operating procedure describes the Xpert MTB/RIF test using the GeneXpert System.

SCOPE/APPLICABILITY:

This standard operating procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for MTB/RIF using the GeneXpert System.

SAMPLE INFORMATION:

Type	• Sputum • Aerosol induced sputum
Volume	• 1 mL
Collection Container	• Orange top, sterile container
Stability	• Room temperature up to 3 days • Refrigerated up to 10 days
Storage Requirements	Room temperature or refrigerated

Criteria for rejection	1. Unlabeled/mislabeled samples 2. Sample container label does not match patient identification on requisition 3. Sample not stored correctly 4. Insufficient amount of sample received

REAGENTS and/or MEDIA:

- Xpert MTB/RIF cartridge
- Sample reagent bottle

SUPPLIES:

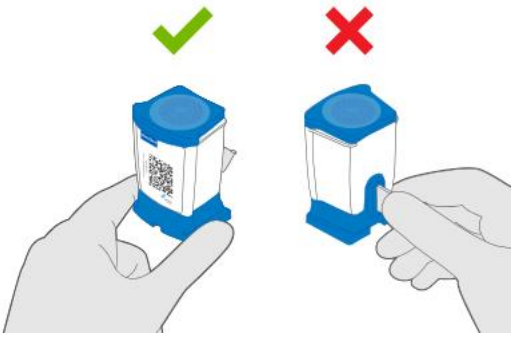
- Personal Protective Equipment
- Absorbent bench liner
- Orange autoclave bag
- Spray bottles
- Wet waste container
- Conical tube rack
- Transfer pipettes provided in kit
- Disposable, transfer pipettes
- Dry waste container
- Accel TB 1L bottle
- Accel TB wipes
- 70% isopropyl alcohol
- Mechanical pipette and tips
- 50 mL conical tubes

EQUIPMENT:

- GeneXpert System
- Printer
- Class II biosafety cabinet (BSC)
- Vortex mixer
- Refrigerator

ENVIRONMENTAL CONTROLS:

- Store Xpert MTB/RIF cartridges upright between 2°C to 28°C
- Do not use a cartridge that has been damaged or leaked, dropped, or shaken
- Open a cartridge only when ready to add sample. An open cartridge must be loaded onto the GeneXpert within 30 minutes
- Cartridges are single use. Do not attempt to open or re-use a cartridge
- Cartridges and test samples stored at 4°C must be brought to room temperature before running the assay
- Do not touch the Reaction Tube, always handle the cartridge by its Body:



SPECIAL SAFETY PRECAUTIONS:

- Patient samples should only be opened and prepared for testing in a contained environment (i.e., certified Class II BSC)
- Personal Protective Equipment (PPE) required when working with suspect MTB samples includes: lab gown, nitrile gloves and BSC
- The test operator and all personnel in the immediate vicinity should be wearing appropriate PPE at all times when working with suspect MTB samples, in the event of a spill outside of the BSC
- Used cartridges should not be opened
- All personnel handling potential MTB samples should be knowledgeable in their laboratory's biological spill clean-up protocol for infectious respiratory samples
- Handle all samples and waste materials as if they were capable of transmitting infectious agents
- A dropped cartridge is unlikely to open if it has been firmly re-closed after loading. In the event that a cartridge is dropped outside of the BSC (open or closed), follow the STHA Biological Spill Control procedure

QUALITY CONTROL:

- Refer to MIC60100-Xpert MTB-RIF Quality Control for quality control procedure
- Record all results on MIC60101-QC Results Record-Xpert MTB-RIF

PROCEDURE INSTRUCTIONS:

Step	Action
Preparing the run	
1	Order GeneXpert MTB testing in the LIS: • In SoftMic, accession the order using the test code **PCMTB**. Add the sputum number in the site area of order entry if on requisition • Collect, receive and plate the order • Label the requisition with the requisition label and scan into SoftMedia • Place the sample barcode label and media barcode labels in the pouch of the biohazard bag • Place sample in the GeneXpert bin in the microbiology specimen fridge
2	Ensure the daily maintenance for the GeneXpert has been completed and is documented on MIC72110-Maintenance Record-GeneXpert.
3	Turn on the BSC and set up for TB testing with the following: • Absorbent pad on working surface • Small beaker to hold conical tube • Wet waste container half full with Accel TB • Dry waste container containing an orange autoclave bag • Spray bottle with 70% isopropyl alcohol and spray bottle with Accel TB • Accel TB wipes • Xpert MTB/RIF cartridge and Sample reagent • Transfer pipettes provided in kit • Disposable pipettes • Timer

4	On the TB Workroom bench, place the number of conical tubes needed for the run in a conical tube rack. Using the mechanical pipette, add 2000 µL of sample reagent to each tube. This will give a 2:1 sample reagent to sputum ratio. Place the rack in the BSC once sample reagent is added.

Step	Action
Preparing the Cartridge	
1	In the BSC, open biohazard bag and discard in the dry waste container. Wipe or spray each sample with Accel TB and place on the absorbent pad. Once dry, label the sample with the sample label. Leave the media barcode labels on the right-hand side of the working area.
2	Label the conical tube with the MTBCT media barcode label.
3	Prepare work area for sample transfer: • Move the sample container to the front of the absorbent pad • Move the corresponding conical tube to the small beaker • Remove the lid of the conical tube • Add a folded Accel TB wipe to the front area of the absorbent pad
4	Open the sample container carefully and place lid upside down on the Accel TB wipe. Using the disposable, transfer pipette, carefully transfer approximately 1 mL of sputum sample to the conical tube. Place the pipette in the wet waste container after the sample is added. **NOTE:** If the sample is thick or stuck to the sides or top of the collection container, use the pipette to add some of the sample reagent from the conical tube to the sample container. Replace lid and vortex to help liquify sample **NOTE:** If the sample volume is <0.5 mL, write **NSQ** on the patient label for cancellation after the run is complete. Do NOT process the sample **NOTE:** If the sample volume is 0.5 to 1 mL, write **AFBFL** on the patient label for specimen quality comment to be added after the run is complete. The sample WILL be processed
5	Tightly secure the lid on the conical tube and vortex thoroughly for at least 10 seconds (avoid wetting inside of lid to prevent aerosols on opening).
6	Allow to stand for 10 minutes at room temperature and then vortex again for at least 10 seconds.
7	Allow to stand for an additional 5 minutes at room temperature before proceeding to inoculating the cartridge.
8	After the 5 minute incubation, move the first conical tube to the small beaker and apply the corresponding MTBGX media barcode label to the right-hand side of the GeneXpert cartridge, near the base. **NOTE:** Do not cover the barcode label on the front of the cartridge
9	Open the conical tube carefully to avoid touching droplets on the inner lid and place upright on the Accel TB wipe to avoid droplets falling on the work surface.
10	Pry open the cartridge lid and open wrapper of the transfer pipette provided in the kit.
11	Aspirate the liquefied sample to the marked line on the pipette.

12	Dispense the sample into the cartridge along the side of the loading chamber to avoid creating bubbles and minimize aerosols in the chamber: 
13	Rinse the pipette in the wet waste container with Accel TB and allow to soak for at least 30 minutes.
14	Recap the conical tube and move to the back of the rack.
15	Firmly snap close the lid to seal the cartridge and place on the left-hand side of the BSC.
16	Spray gloves thoroughly with isopropyl alcohol, rub together and allow to air dry. **NOTE:** Always disinfect gloves between the loading of each sample
17	Repeat cartridge loading procedure for up to 6 additional samples. **NOTE:** Loaded cartridges must be processed on the GeneXpert within 30 minutes

Step	Action
Creating a Test Run	
1	Once cartridge loading is complete, spray outer gloves with isopropyl alcohol and remove. Replace with new set of gloves.
2	Transfer the loaded cartridges to the GeneXpert bench.
3	Log into the GeneXpert software using the username **admin1** and the password **covid19**.
4	On the GeneXpert software, click **Create Test** at the top left.
5	Using the scanner, scan the sample ID barcode and the cartridge barcode. Select **Start Test**.
6	Locate the module with the blinking green light, open the module door and load the cartridge.
7	Close the module door firmly, it will latch closed.

Step	Action
Cleaning the BSC	
1	Remove gloves and don a new pair.
2	In the BSC, spray samples and conical tubes with Accel TB. After 5 minutes place sample containers in the orange autoclave bag.
3	Wipe the "clean" area of the BSC with an Accel TB wipe.
4	Remove gloves and don a new pair. Transfer conical tubes to the fridge.

Step	Action
Generating a Test Report	
1	A report is generated automatically upon completion of a run.
2	To view runs or reprint: Select **View Results** on the menu bar. Click **Report** → Check **Patient ID** → Click **Preview PDF** → Click **Print**

INTERPRETATION OF RESULTS:

RESULT	INTERPRETATION
MTB NOT DETECTED	• The MTB target is not detected within the sample • SPC: PASS. The SPC met the acceptance criteria • Probe Check: PASS. All probe check results pass
MTB DETECTED RIF Resistance DETECTED	• The MTB target is present within the sample • SPC: NA. An SPC signal not required • Probe Check: PASS. All probe check results pass
MTB DETECTED RIF Resistance NOT DETECTED	• The MTB target is present within the sample • SPC: NA. An SPC signal not required • Probe Check: PASS. All probe check results pass
MTB DETECTED RIF Resistance INDETERMINANT	• The MTB target is present within the sample • RIF resistance could not be determined due to insufficient signal detection • SPC: NA. An SPC signal not required • Probe Check: PASS. All probe check results pass
INVALID	• The presence or absence of MTB cannot be determined. **Repeat the test** • The SPC does not meet the acceptance criteria, the sample was not properly processed, or PCR was inhibited. Repeat the test • SPC: FAIL. The MTB target result is negative, and the SPC Ct is not within valid range • Probe Check: PASS. All probe check results pass
ERROR	• The presence or absence of MTB cannot be determined. **Repeat the test** • MTB: NO RESULT • SPC: NO RESULT • Probe Check: FAIL. All or one of the probe check results failed **NOTE:** If the probe check passed, the error is caused by a system component failure.
NO RESULT	• The presence or absence of MTB cannot be determined. **Repeat the test** • A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress • MTB: NO RESULT • SPC: NO RESULT • Probe Check: NA

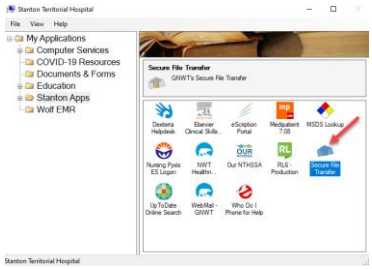
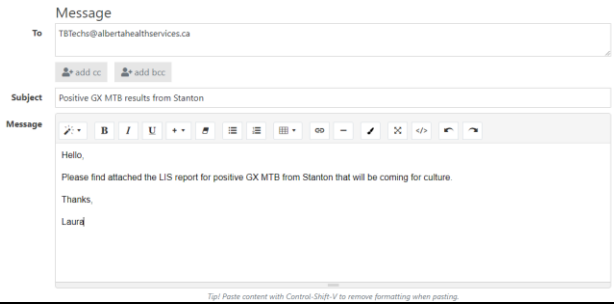
REPORTING INSTRUCTIONS:

GX Result: MTB **NOT DETECTED**	• Report: **NEGATIVE**
GX Result: MTB **DETECTED**	• Report: **POSITIVE** • Report RIF resistance: ➢ From the PCMTB Keypad o Select Key 5 for Rifampin resistant o Select Key 6 for Rifampin sensitive o Select Key 7 for Rifampin indeterminant ➢ Phone result to OCPHO (HPU1) at (867) 920 8646: o Document call in the call log ➢ Phone result to ordering location: o Document call in the call log ➢ Report will automatically print to OCPHO (HPU1) ➢ Report will automatically print to Stanton IPAC (SIPAC) or Inuvik IPAC (IIPAC) if ER or inpatient ➢ Check patients home address. If from Nunavut: o Phone result to the applicable Nunavut CPHO o Copy result to the applicable Nunavut CPHO
GX Result: MTB **TRACE**	• Report: **POSITIVE** ➢ From the PCMTB Keypad o Select Key + • Report RIF resistance: ➢ From the PCMTB keypad o Select Key 5 for Rifampin resistant o Select Key 6 for Rifampin sensitive o Select Key 7 for Rifampin indeterminant ➢ Phone result to OCPHO (HPU1) at (867) 920 8646: o Document call in the call log ➢ Phone result to ordering location: o Document call in the call log ➢ Report will automatically print to OCPHO (HPU1) ➢ Report will automatically print to Stanton IPAC (SIPAC) or Inuvik IPAC (IIPAC) if ER or inpatient ➢ Check patients home address. If from Nunavut: o Phone result to the applicable Nunavut CPHO o Copy result to the applicable Nunavut CPHO
GX Result: MTB **INVALID or NO RESULT**	• Retest the sample with a new cartridge: ➢ Add comment in TCOMM that testing was repeated • If repeat testing is the same: ➢ Report: **NO RESULT** ➢ From the keypad add key 4 to add repeat sample collection comment

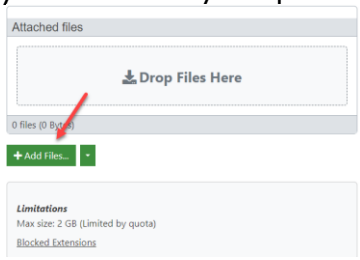
GX Result: MTB **ERROR**	• Follow the instructions on the printout using the instrument manual • Retest the sample with a new cartridge ➢ Add comment in TCOMM that testing was repeated

Insufficient quantity of sample for GX testing	• Cancel the specimen using the NSQ cancellation comment **|XIQ**

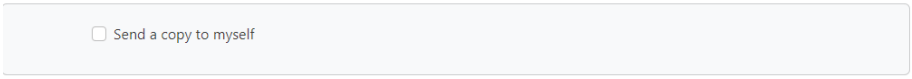
Low volume sample received	• In Order Entry, add the specimen quality comment **AFBFL**

Step	Action
Notification of Positive Results to APL	
1	If MTB is detected and other sputum samples are being sent to APL for culture, APL needs to be notified of the positive result.
2	After the sample is final in the LIS and notifications have been made, print a copy of the report to the lab printer.
3	Email a copy of the report to yourself and save to your personal drive on your computer.
4	Send a SFT (secure file transfer) to the APL TBTECH email address: TBTechs@albertahealthservices.ca i. Select Secure File Transfer on the X1A links:  ii. Login using your GNWT email login information iii. Enter the TBTechs email in the To line iv. In the Subject line add the subject "Positive GX MTB results from Stanton" v. In the Message box, type a short message that you are sending the LIS report for positive GX MTB sample that will be coming to APL for culture: 

vi. Add the saved LIS report to the email. Select +Add Files and select the LIS report you saved to your personal drive:



vii. After the LIS report is added, select the "Send a copy to myself box":



viii. Select "Send" to send the email. You will get a copy of the SFT to your email if you selected the "Send a copy to myself box"

Step	Action
Completing the Run	
1	Check the Resulting Worklist-GeneXpert.
2	Retrieve the conical tube rack from the fridge and place in the BSC.
3	Retrieve the used GeneXpert cartridges from the GeneXpert. If all the tests were negative, discard cartridges in the biohazard waste. If any of the tests were positive, place the cartridges in the BSC.
4	In the BSC, remove the used pipettes from the wet waste container and place into the dry waste container.
5	Add the conical tubes and the absorbent orange pad to the dry waste container.
6	If all the samples in the run were negative, remove the autoclave bag from the dry waste container. Tie and wipe outside with an Accel TB wipe and discard.
7	If any of the samples were positive for MTB, add the positive GeneXpert cartridges to the dry waste container. Remove the autoclave bag from the dry waste container and wipe the outside with an Accel TB wipe. Remove from the BSC. Tape the bag closed using autoclave tape and place into the autoclave. Refer to MIC75000-Steris AMSCO 250LS Autoclave for autoclave instructions. Change gloves and return to the BSC.
8	Wipe the BSC with an Accel TB wipe. Turn off the blower and lower the sash. Remove gloves and don a fresh pair.
9	Ensure all used cartridges from the GeneXpert are discarded in the biohazard waste if negative or autoclaved if positive.
10	When all testing of patient samples and disinfection of surfaces is complete, remove PPE and place in the biohazard waste.

LIMITATIONS:

1. Because the detection of MTB is dependent on the number of organisms present in the sample, reliable results are dependant on proper specimen collection, handling, and storage.
2. A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of MTB and Rifampin resistance.
3. The performance of the Xpert MTB/RIF Assay is dependent on operator proficiency and adherence to assay procedures. Assay procedural errors may cause false positive or false negative results.

CROSS-REFERENCES:

- MIC60100-Xpert MTB-RIF Quality Control
- MIC60101-QC Results Record-Xpert MTB-RIF
- MIC72110-Maintenance Record-GeneXpert
- MIC75000-Autoclave

REFERENCES:

1. Cepheid GeneXpert. *Xpert MTB/RIF Ultra* package insert. 301-5987, Rev J, August 2020
2. Cepheid GeneXpert. *GeneXpert System User Manual*. 301-0045, Rev.C, June 2012
3. Biosafety Advisory Committee. *STHA Biosafety Program Manual*. January 2016

APPROVAL:

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Date

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Director, Laboratory and Diagnostic Imaging Services

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	15 Oct 22	Initial Release	L. Steven
2.0	01 Oct 24	Procedure reviewed	L. Steven