PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC72400 – Xpert *C. difficile*	Policy Number: 15-148-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 18/03/2024	Next Review Date: 18/03/2026
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 18/03/2024
Accreditation Canada Applicable Standard: NA	

Uncontrolled When Printed

GUIDING PRINCIPLE:
The Xpert *C. difficile* assay is a qualitative *in vitro* diagnostic test for rapid detection of toxin B gene sequences from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridiodes difficile* infection (CDI). The test utilizes automated real time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*.

PURPOSE/RATIONALE:
This standard operating procedure describes the Xpert *C. difficile* test using the GeneXpert System.

SCOPE/APPLICABILITY:
This standard operating procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for *C. difficile* using the GeneXpert System.

SAMPLE INFORMATION:

Type	• Stool (Unformed)
Collection Container	• Orange top, sterile container
Stability	• Room temperature up to 24 hours • Refrigerated up to 5 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 in sterile container

	4. Sample not stored correctly 5. Testing performed within the last 7 days 6. Testing will not be performed on patients <12months old 7. Testing for *C. difficile* is not performed on formed stools

REAGENTS and/or MEDIA:

- Xpert *C. difficile* cartridge
- Sample reagent

SUPPLIES:

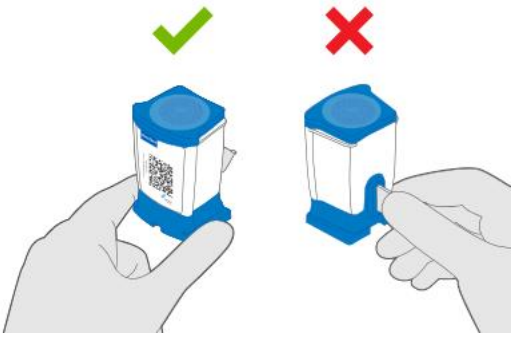
- Personal protective equipment
- Absorbent bench liner
- Orange autoclave bag
- Wet waste container
- Dry waste container
- Accel TB 1L bottle
- Accel TB wipes
- Sterile, breakpoint swabs
- Disposable transfer pipettes

EQUIPMENT:

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

ENVIRONMENTAL CONTROLS:

- Store Xpert *C. difficile* 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:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to MIC60080-Xpert *C. difficile* Quality Control for quality control procedure
- Record all results on MIC60081-QC Results Record-Xpert *C. difficile*

PROCEDURE INSTRUCTIONS:

Step	Action
Preparing the Run	
1	Order GeneXpert *C. difficile* testing in the LIS: • In SoftMic, accession the order using the test code **PCCDI** • 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 label in the pouch of the biohazard bag • Place samples 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 *C. difficile* testing with the following: • Absorbent pad on working surface • Wet waste container half full with Accel TB • Dry waste container containing an orange autoclave bag • Accel TB wipes • Xpert *C. difficile* cartridge • Sample reagent • Sterile, breakpoint swab • Sterile, transfer pipette

Step	Action
Preparing the Cartridge	
1	Remove the cartridge and Sample Reagent from the package. Acquire a swab and a pipette for each sample being tested.
2	Apply the CDIFF media barcode label to the right hand side of the cartridge, near the base. **NOTE:** Do not cover the barcode label on the front of the cartridge
3	Vortex the sample for 10 seconds to ensure it is evenly mixed.
4	Briefly place the swab in the unformed stool sample. The swab does not need to be completely saturated: 
5	Insert the swab into the vial containing the Sample Reagent.
6	Hold the swab by the stem near the rim of the vial and push the stem against the edge of the vial to break it. **NOTE:** Make sure the swab is short enough to allow the cap to close
7	Close the lid and vortex at high speed for 10 seconds.
8	Pry open the cartridge lid and open wrapper of the transfer pipette.
9	Transfer the entire contents of the Sample Reagent into the cartridge along the side of the loading chamber to avoid creating bubbles and minimize aerosols in the chamber: 
10	Firmly snap close the lid to seal the cartridge and place on the left hand side of the BSC.
11	Repeat cartridge loading procedure for up to 16 additional samples. **NOTE:** Loaded cartridges must be processed on the GeneXpert within 30 minutes

Step	Action
Creating a Test Run	
1	Transfer the loaded cartridges to the GeneXpert bench.
2	Log into the GeneXpert software using the username **admin1** and the password **covid19**.
3	Confirm that all modules are detected by the software and ready for testing.
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	Wipe the "clean" area of the BSC with an Accel TB wipe.
3	Remove gloves and don a new pair. Transfer samples to a biohazard bag and place in the refrigerator.

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
NOT DETECTED	• *C. difficile* target DNA sequences are not detected • Toxins producing *C. difficile* targets not detected • SPC: PASS; SPC has a Ct within the valid range and endpoint above the endpoint minimum setting • Probe Check: PASS; all probe check results pass
DETECTED	• Toxin producing *C. difficile* target DNA sequences are detected • The toxin producing *C. difficile* target(s) have a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored since *C. difficile* target amplification may compete with this control • Probe Check: PASS; all probe check results pass

INVALID	• Presence or absence of *C. difficile* cannot be determined. **Repeat the test** • SPC: FAIL; SPC target result is negative and the SPC Ct is not within valid range and endpoint below minimum setting • Probe Check: PASS; all probe check results pass
ERROR	• Presence or absence of *C. difficile* cannot be determined. **Repeat the test** • Toxin producing *C. difficile* targets-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 the maximum pressure limit exceeding the acceptable range
NO RESULT	• Presence or absence of *C. difficile* cannot be determined. **Repeat the test** • Toxin producing *C. difficile* targets: NO RESULT • Probe Check: NA

REPORTING INSTRUCTIONS:

GX Result: Toxigenic *C. difficile* **NOT DETECTED**	• Report: **NEGATIVE**
GX Result: Toxigenic *C. difficile* **DETECTED**	• Report: **POSITIVE** • Phone result to ordering location: ➢ Document call in the call log • Report will automatically print to OCPHO (HPU1) • Report will automatically print to Stanton IPAC (SIPAC) and Inuvik IPAC (IIPAC) if ER or inpatient
GX Result: Toxigenic *C. difficile* **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 R to add repeat sample collection comment
GX Result: Toxigenic *C. difficile* **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

Step	Action
Completing the Run	
1	Check the Resulting Worklist-GeneXpert.

2	In the BSC, remove the used pipettes from the wet waste container and place into the dry waste container.
3	Remove the autoclave bag from the dry waste container. Tie and wipe outside with an Accel TB wipe. Place in the biohazard waste.
4	Wipe the BSC with an Accel TB wipe. Turn off the blower and lower the sash. Remove gloves and don a fresh pair.
5	Ensure all used cartridges from the GeneXpert are discarded in the biohazard waste.
6	When all testing of patient samples and disinfection of surfaces is complete, remove PPE and place in the biohazard waste. Retrieve samples from the refrigerator and place in the micro specimen fridge in the daily sample bucket.

LIMITATIONS:

1. This test targets the *tcdB* gene for Toxin B production. This test will not detect strains of *C. difficile* that do not contain the *tcdB* gene.
2. Positive results observed with immunocompromised pediatric patients may reflect asymptomatic carriage of *C. difficile*.
3. Results from the Xpert *C. difficile* Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
4. Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test.
5. Inhibition of the Xpert *C. difficile* Assay has been observed in the presence of the following substances: Zinc oxide paste and Vagisil cream.
6. False negative results may occur when the infecting organism has genomic mutations, insertions, deletions or rearrangements or when performed early in the course of illness.

CROSS-REFERENCES:

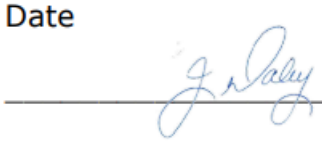
- MIC60080-Xpert *C. difficile* Quality Control
- MIC60081-QC Results Record-Xpert *C. difficile* QC
- MIC72110-Maintenance Record-GeneXpert

REFERENCES:

1. Cepheid GeneXpert. Xpert *C. difficile* Assay Instructions for Use. 300-9680 Rev. F, March 2016
2. Cepheid GeneXpert. *GeneXpert System User Manual*. 301-0045, Rev.C, June 2012

APPROVAL:

March 18, 2024
Date



Director, Laboratory and Diagnostic Imaging Services

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	30 Jan 22	Initial Release	L. Steven
2.0	14 Feb 24	Procedure reviewed	L. Steven