PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC10300 – Xpert *C.difficile*	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

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 *Clostridium 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 Dx System.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) and Medical Laboratory Assistants (MLAs) processing specimens for *C.difficile* using the GeneXpert Dx 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. Repeat testing on positive samples will not be performed within 7 days (Cancellation code: |XCDP) 6. Repeat testing on negative samples will not be performed within 7 days (Cancellation code: IXCDN) 7. Testing will not be performed on patients <12months old (Cancellation code: |XCD1) 8. Testing for *C. difficile* Toxin is not performed on formed stools (Cancellation code: IXCDT)

REAGENTS and/or MEDIA:

- Xpert *C.difficile* cartridge
- Sample reagent

SUPPLIES:

- Personal protective equipment
- Dry waste container
- Sterile, dry swabs
- Disposable transfer pipettes

EQUIPMENT:

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

ENVIRONMENTAL CONTROLS:

- Store Xpert *C.difficile* assay cartridges upright between 2°C to 28°C
- Do not open a cartridge lid until you are ready to perform testing
- 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 potential infectious materials or cultures.

- Ensure that appropriate hang 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:

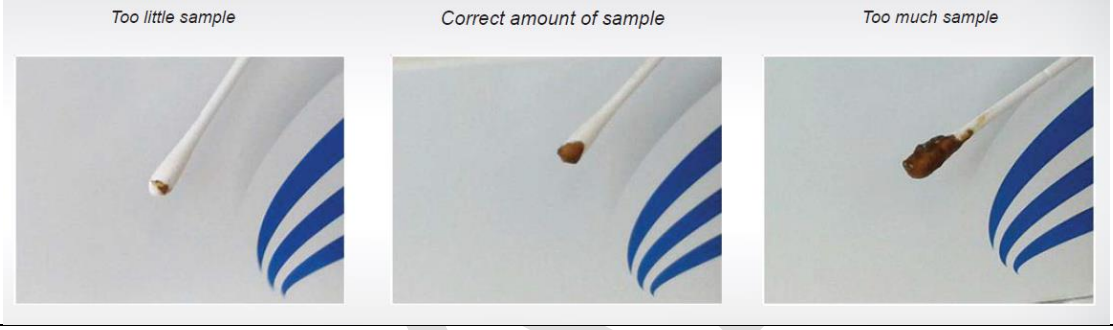
- ZeptoMetrix NATrol positive and negative controls need to be performed on every new lot number and/or shipment of cartridges
- Refer to instructions below to perform quality control testing
- Record all results on MIC10610-Xpert *C.difficile* QC Results Record

QUALITY CONTROL INSTRUCTIONS:

Step	Action
Performing Quality Control	
1	Shake the NATrol control vigorously for 5 seconds.
2	Remove the cartridge and reagent from the package.
3	On the right hand side of the cartridge, write with a marker which control is being run; positive or negative.
4	Briefly place a swab in the control solution.
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 to the Sample Chamber of the cartridge.
10	Firmly snap close the lid to seal the cartridge.
11	Follow "Creating a Test Run" section below to load the cartridge on the GeneXpert. **NOTE:** Select Manual Entry to enter specimen ID. Use Positive Control or Negative Control as the name
12	Document results on MIC81610-Xpert *C.difficile* QC Results Record and place in the GeneXpert PCR Testing Quality Control Results binder.

PROCEDURE INSTRUCTIONS:

Step	Action
Preparing the Run	
1	Order GeneXpert SARS-CoV-2 testing in the LIS: • Medipatient the order if required • In SoftMic, accession the order using the test code **PCCDI** • Add any "copies to" if required • 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 • Deliver samples to the blue bin labelled Microbiology Samples
2	Ensure the daily maintenance for the GeneXpert has been completed and is documented on MIC81110-Maintenance Record-GeneXpert.

Step	Action
Preparing the Cartridge	
1	Remove the cartridge and reagent from the package. Acquire a swab and a pipette for each sample being tested.
2	Apply the 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	The test must be started within 30 minutes of adding reagents to the cartridge.
4	Vortex the sample for 10 seconds to ensure it is evenly mixed.
5	Briefly place a swab in the unformed stool sample. The swab does not need to be completely saturated: 
6	Insert the swab into the vial containing the Sample Reagent.
7	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
8	Close the lid and vortex at high speed for 10 seconds.
9	Pry open the cartridge lid and open wrapper of the transfer pipette.
10	Transfer the entire contents of the Sample Reagent to the Sample Chamber of the cartridge: 
11	Firmly snap close the lid to seal the cartridge and place in the cartridge tray.

Step	Action
Creating a Test Run	
1	Transfer the loaded cartridges in the cartridge tray 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
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
Toxigenic *C.difficile* NEGATIVE	• *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
Toxigenic *C.difficile* POSITIVE	• Toxin producing *C.difficile* target DNA sequences are detected • The toxin producing *C.difficile* target(s) have Cts within the valid range and endpoint above the minimum setting • SPC: NA (not applicable); SPC is ignored since *C.difficile* target amplification may compete with this control • Probe Check: PASS; all probe check results pass
NO RESULT	• Presence or absence of *C.difficile* target DNA cannot be determined • 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/ INVALID	• Presence or absence of *C.difficile* cannot be determined • Toxin producing *C.difficile* targets-NO RESULT • Probe Check: FAIL; one or more probe checks have failed • Repeat testing as per testing procedure

REPORTING INSTRUCTIONS:

Toxigenic *C.difficile* **NEGATIVE**	• Report: **NEGATIVE - C.difficile toxin B gene NOT DETECTED**

Toxigenic *C.difficile* **POSITIVE**	• Report: **POSITIVE - C difficile toxin B gene DETECTED** • Phone results to patient location ➢ Document call in the Call Box in SoftMic • Report will automatically print to OCPHO (HPU1) • Copy report to Stanton IPAC (SIPAC) if ER or inpatient

Toxigenic *C.difficile* **NO RESULT**	• Retest the sample with a new cartridge ➢ Add comment in TCOMM that testing was repeated • If repeat testing is the same: ➢ Phone the ordering location and request a new sample be collected ➢ Report: **NO RESULT - Presence of C.diff toxin cannot be determined** ➢ From the keypad add key R to add repeat sample collection comment

LIMITATIONS:

1. This test detects but does not differentiate the NAP1 (Ribotype 027) strain from other toxigenic strains of *C.difficile*.
2. 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.
3. Positive results observed with immunocompromised pediatric patients may reflect asymptomatic carriage of *C.difficile*.
4. Detection of *C.difficile* nucleic acid in stools confirms the presence of these organisms in diarrheal patients but may not indicate that *C.difficile* are the etiologic agents of the diarrhea.
5. Results from the Xpert *C.difficile* Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
6. 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.
7. Inhibition of the Xpert *C.difficile* Assay has been observed in the presence of the following substances: Zinc oxide paste and Vagisil cream.
8. False negative results may occur when the infecting organism has genomic mutations, insertions, deletions or rearrangements or when performed very early in the course of illness.

CROSS-REFERENCES:

- MIC81110-Maintenance Record-GeneXpert
- MIC10610-Xpert *C.difficile* QC Results Record

REFERENCES:

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

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	30 Jan 22	Initial Release	L. Steven