TRAINING UPDATE

Lab Location:	SGMC & WOMC	**Date Distributed:**	7/1/2020
Department:	Core Lab	**Due Date:**	7/31/2020
		Implementation:	**6/23/2020**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Clostridium difficile Toxin B PCR using Cepheid GeneXpert® SGMC.M1003 v2

Description of change(s):

Section	Reason
8.2	Deleted typing patient name and MRN into instrument
10.6	Added interfaced reporting
19	Added addendum A – Cepheid Interface info

This revised SOP was implemented on June 23, 2020

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	***Clostridium difficile* Toxin B PCR using Cepheid GeneXpert®**
Prepared by	Ron Master Date: 2/18/2019
Owner	Ron Master Date: 2/18/2019

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Cepheid GeneXpert *Clostridium difficile* PCR	Real-time Polymerase Chain Reaction (PCR) Assay / GeneXpert System	CDPCR

Synonyms/Abbreviations
Clostridium difficile PCR, Xpert *Clostridium difficile*

Department
Core Lab

2. ANALYTICAL PRINCIPLE

The GeneXpert Dx System automates and integrates sample purification/extraction, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR (qPCR) assay. Real-time RT-PCR is used for assays that detect RNA.

The Xpert *C. difficile/Epi* Assay uses real-time PCR to detect DNA. The Xpert *C. difficile/Epi* Assay (where *Epi* means epidemiological) includes reagents for the detection of toxigenic *C. difficile* and the presumptive detection of sequences found in 027/NAP1/BI strains. A Sample Processing Control (SPC) is also included. The SPC is present to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction. The assay detects the toxin B gene (*tcdB*), the binary toxin gene (CDT), and the single-base-pair deletion at nucleotide 117 within the gene encoding a negative regulator of toxin production (*tcdC*Δ117).

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Not applicable
Specimen Collection and/or Timing	Not applicable
Special Collection Procedures	Transfer liquid or soft stool (but not urine) into the container. Avoid mixing toilet paper, water, or soap with the sample.
Other	None

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Liquid or semi-formed stool None
Collection Container	Dry sterile leak-proof container
Volume - Optimum **- Minimum**	5 mL 1 mL
Transport Container & Temperature	Tightly sealed leak-proof container kept
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated: 5 days
	Frozen: Not applicable
Timing Considerations	Not applicable
Unacceptable Specimens & Actions to Take	• Specimen other than liquid or semi-formed stool • Specimen with less than 1 mL • Specimen past stability requirement • Stool in a wrong transport container • Stool in preservative or mixed with urine **Note**: Room temperature samples may be tested if received and refrigerated within 24 hours.
Compromising Physical Characteristics	Not applicable
Other Considerations	Refrigerated samples are to be kept at 2-8°C for up to 5 days.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits or reagents must be reviewed for any changes before the kit is used.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
Xpert® *C. difficile*/Epi, GX, IVD Kit	Cepheid, GXCDIFF/EPI-10 (SC#175562) or GXCDIFF/EPI-120 (SC#179367) or equivalent

4.2 Reagent Preparation and Storage

Assay Kit - Xpert® *C. difficile/Epi*, GXCDIFF/EPI-10 or GXCDIFF/EPI-120	
Xpert *C. difficile/Epi* Assay Cartridges with integrated reaction tubes	Cartridge: • Bead 1 (freeze-dried) • Bead 2 (freeze-dried) • Bead 3 (freeze-dried) • Reagent 1 (3.0 mL per cartridge) • Reagent 2 (3.0 mL per cartridge) – sodium hydroxide
Xpert *C. difficile/Epi* Assay Reagent Pouch	1 per kit
Sample (Elution) Reagent (Guanidinium thiocyanate)	GXCDIFF/EPI-10 x 2.0 mL per pouch GXCDIFF/EPI-120 – 125 x 2.0 mL per pouch
Storage/Stability	2-28°C / Manufacturer's expiration date Do not use a cartridge that has leaked Do not use a cartridge that has been dropped Do not use a cartridge that has a damaged reaction tube
Preparation	None required

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

GeneXpert® C. difficile/Epi PCR Assay	**Supplier and Catalog Number**
Sample Processing Control (SPC)	Cartridge component
Probe Check (PCC)	Cartridge component
ZeptoMetrix NATtrol™ *Clostridium sordellii* External Negative Control	Fisher Cat# 22-156-720; ZeptoMetrix Cat# NATCSO-6MC
ZeptoMetrix NATtrol™ *Clostridium difficile* NAP1 External Positive Control	Fisher Cat# 22-156-713; ZeptoMetrix Cat# NATCDI-6MC

6.2 Control Preparation and Storage

Sample processing control (SPC) - Included in the Cartridge	
Storage	Refer to section 4
Stability	Refer to section 4
Preparation	Ready to use

Probe Check Control (PCC) - Included in the Cartridge	
Container	Refer to section 4
Storage	Refer to section 4
Stability	Ready to use

ZeptoMetrix NATtrol™ *Clostridium difficile* NAP1 External Positive Control	
Container	6 x 0.5 mL vials per pack
Storage	Store at 2–8°C
Stability	Stable until expiration date.
Preparation	Control is supplied ready for use. No additional preparation is required. **Wearing clean gloves,** label 1 cartridge and 1 Elution Buffer appropriately. • Vortex NATtrol™ control for 5-10 seconds. • Add 20 uL NATtrol™ into Elution Buffer vial. • Mix well by vortexing for 10 seconds. • Using a sterile transfer pipette, remove all sample from elution buffer and transfer into the "S" chamber of the Assay cartridge. Close cartridge when complete. • Control is now ready to be loaded into instrument. **Change gloves**.

6.3 Number and Frequency

- Sample Processing Control (SPC) and a Probe Check Control (PCC; internal controls) are run within each test.
- External *C. difficile* Controls are run with each new kit lot number or shipment or every 31 days, whichever is more frequent. External controls must be treated in the same manner as a patient samples.
- Enter the QC name as QC CDIFF POS and QC CDIFF NEG or scan the QC name barcode

6.4 Tolerance Limits and Criteria for Acceptable QC

A. Tolerance Limits

Control Type	Instrument-Reported Assay Result	Interpretation of Result
External Positive Control	See Section 10.1	See Section 10.1
External Negative Control	See Section 10.1	See Section 10.1
SPC	Passes if Meets the Assigned Acceptance Criteria. Refer to Section 10.1	
PCC		

B. Criteria for Acceptable QC
- All controls must yield acceptable results.
- Controls and patient data must be reviewed for acceptability and for atypical or unexpected results or trends prior to reporting patient results.
- DO NOT release results from runs with unacceptable controls or with unusual patterns, trends or distribution in patient values.

C. Corrective Action
- Report problem to supervisor or designee.
- All rejected runs must be effectively addressed and include the following documentation:
  - Control(s) that failed (e.g., positive control with negative result) and/or atypical or unexpected patient results
  - Actions taken
  - Statement of what was done with the patient samples from the affected run/batch,
  - Date and initials of the person recording the information.
- Patient samples in failed analytical runs must be reanalyzed.

NOTE: The laboratory director or designee may override rejection of partial or complete runs. Justification for the override must be documented in detail.

6.5 Documentation

- Record all Quality Control results (failed and successful) manually or electronically.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.
- Refer to Quest Diagnostics Records Management Program for Quality Control record retention requirements.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

- Cepheid GeneXpert System

7.2 Equipment

- Computer, monitor, printer, and required application software
- Biological Safety Cabinet
- Timer
- Refrigerator, 2-8°C
- Vortex

- Pipettor – 20uL (for control preparation)

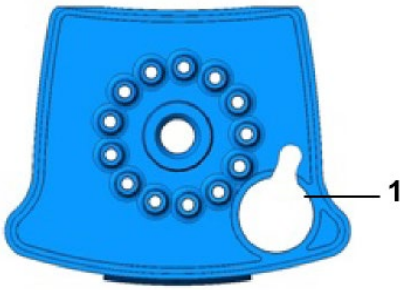
7.3 Supplies

- Dry sterile swab
- ~~Sterile loop~~
- Sterile transfer pipette
- Aerosol-filter Pipettor tips (for control preparation)
- Plastic-backed absorbent pads (Blood Bloc or equivalent)
- ~~Scissors (optional)~~
- Personal protective equipment (lab coat, powder-free gloves, face shields, and etc)
- Disposable biohazard waste containers (sharps, etc.)
- 10% bleach
- 70% ethanol

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Preparation of Cartridge
Notes: • All work must be performed in an appropriate Class 2 BSC. • Before testing, clean the work area with a solution of 1:10 dilution of household chlorine bleach and then repeat the cleaning of the work area with 70% ethanol. Wipe work surfaces dry completely before proceeding. • Change gloves if they become visually contaminated. • Do not open a cartridge until you are ready to perform testing. • Use the cartridge within 30 minutes after sample inoculation. • Do not use any reagents that have become discolored. • Do not touch the integrated reaction tube that is attached to the cartridge.	
1.	Remove a test cartridge and Sample Reagent vial from the package and label each with patient specimen number or external control information.
2.	Label the Sample Reagent vial and the Test Cartridge with the accession number.
3.	Briefly place a swab in the liquid/unformed stool sample. The swab does not need to be completely saturated.
4.	Insert the swab into the vial containing the Sample Reagent.
5.	Hold the swab by the stem near the rim of the vial, lift the swab a few millimeters from the bottom of the tube and push the stem against the edge of the vial to break it. Make sure the swab is short enough to allow the cap to close tightly. Note: Use clean gauze or plastic-backed absorbent pads for each sample when breaking off swab to minimize risks of contamination.
6.	Replace cap on Sample Reagent and vortex at high speed for 10 seconds.

8.1	Preparation of Cartridge
7.	Open the cartridge lid. Using a clean transfer pipette, transfer the entire contents of the Sample Reagent to the "S" chamber (labeled 1 below) of the Xpert Assay cartridge. 
8.	Close the cartridge lid and proceed to Section 8.2.

8.2	GeneXpert Analysis
1.	Turn on the computer, and then turn on the GeneXpert Instrument System.
2.	On the desktop, double-click the GeneXpert software icon.
3.	Log on to the GeneXpert Instrument System software using user name and password.
4.	In the GeneXpert Dx Systems window, click Create Test.
5.	In the Sample ID box, scan or type the accession number (e.g, F1234). Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the View Results window and all the reports.
6.	Scan the barcode on the Xpert Assay cartridge.
	~~Type the Patient's name and MRN in the Notes section. This will add another patient identifier to the system / report. Type in your tech code.~~
7.	In the GeneXpert Dx Systems, click Start Test.
8.	Open the instrument module door with the blinking green light and load the cartridge.
9.	Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
10.	Wait until the system releases the door lock before opening the module door and removing the cartridge. Dispose of the used cartridges in a biohazard waste container.
11.	A report is printed for each sample at the completion of testing.

8.3	Retest Procedures
1.	If any of the test results mentioned below occur, repeat the test according to the instructions in the Retest Procedures section below. • An INVALID result indicates that the SPC failed. The sample was not properly processed or PCR was inhibited. • An ERROR result indicates that the Probe Check control failed and the assay was aborted. Possible causes include: the reaction tube being filled improperly; a reagent probe integrity problem was detected; or the maximum pressure limits were exceeded. • A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

8.3	Retest Procedures
2.	**Retest Procedure** For retest within 3 hours of an indeterminate result, use a new cartridge (do not re-use the cartridge) and new reagents. a. Transfer the remaining contents from the Sample Chamber to a new Sample Reagent vial using a disposable transfer pipette. b. Vortex and add the entire contents of the Sample Reagent to the Sample Chamber of the new Xpert *C. difficile*/*Epi* Assay cartridge. c. Close the lid and start new test For retest after 3 hours of an indeterminate result, repeat the test with a new swab sample.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

Not applicable

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window.
Possible results are:

Result				Interpretation
Toxin B	**Binary Toxin**	***tcd*C**	**SPC**	
+	+	+	+/-	Toxigenic *C. diff* POSITIVE 027-NAP1-BI PRESUMPTIVE POSITIVE
+	+	-	+/-	Toxigenic *C. diff* POSITIVE 027-NAP1-BI PRESUMPTIVE NEGATIVE
	-	+	+/-	
	-	-	+/-	
-	+	+	+	Toxigenic *C. diff* NEGATIVE 027-NAP1-BI PRESUMPTIVE NEGATIVE
	+	-	+	
	-	+	+	
	-	-	+	

Assay Result Reported	Interpretation of Result

Assay Result Reported	Interpretation of Result
Toxigenic *C. diff* POSITIVE; 027 PRESUMPTIVE POSITIVE	Toxin producing *C. difficile*, presumptive 027/NAP1/BI target DNA sequences are detected. • The toxigenic *C. difficile* target (Toxin B) AND both presumptive 027/NAP1/BI targets (Binary Toxin and *tcd*CΔ117) have Cts within the valid range and endpoints above the minimum setting. • SPC – N/A; SPC is ignored since *C. difficile* target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Toxigenic *C. diff* POSITIVE; 027 PRESUMPTIVE NEGATIVE	Toxin producing *C. difficile* target DNA sequences are detected. • The toxigenic *C. difficile* target (Toxin B) AND only one or none of the presumptive 027/NAP1/BI targets (Binary Toxin and *tcd*CΔ117) have Cts within the valid range and endpoints above the minimum setting. • SPC – N/A; SPC is ignored since *C. difficile* target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Toxigenic *C. diff* NEGATIVE; 027 PRESUMPTIVE NEGATIVE	Toxin producing *C. difficile* target DNA sequences are not detected. • Toxigenic *C. difficile* target (Toxin B) is not detected (regardless of whether Binary Toxin and/or *tcd*CΔ117 is 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.
INVALID	Presence or absence of *C. difficile* target DNA cannot be determined. Repeat 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* target DNA cannot be determined. Repeat test. • Toxin producing *C. difficile* targets — NO RESULT. • Binary Toxin (CDT) — NO RESULT. • *tcd*CΔ117 — NO RESULT. • Probe Check — FAIL*; one or more of the probe check results fail. *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* target DNA cannot be determined. Repeat test. • Toxin producing *C. difficile* targets — NO RESULT.

Assay Result Reported	Interpretation of Result
	• Binary Toxin (CDT) — NO RESULT. • *tcd*CΔ117 — NO RESULT. • Probe Check — N/A

10.2 Rounding

Not applicable

10.3 Units of Measure

Not applicable

10.4 Analytical Measurement Range (AMR)

Not applicable

10.5 Review Patient Data

- Review patient results for unusual patterns, trends or distribution.
- Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.

10.6 Repeat Criteria and Resulting

Repeat Criteria and Resulting	
IF the PCR result is …	**THEN…**
Error/No Result/Invalid	Repeat testing
Toxigenic *C. diff* POSITIVE and 027 presumptive POSITIVE.	Report CDBG as "Detected"; Add comment PHPV
Toxigenic *C. diff* POSITIVE and 027 presumptive NEGATIVE.	Report CDBG as "Detected"; Add comment NHPV
Toxigenic *C. diff* NEGATIVE	Report CDBG as "Not Detected"
Remains unresolved following repeat testing	Report as INVLD; Add comment MPSP

Message	Code
Detected	DET
Not Detected	NTD
In addition, the toxigenic *C. difficile* is PRESUMPTIVELY POSITIVE for a genetic marker of the hypervirulent 027 NAP1 BI strain, which has been associated with increased	PHPV

Message	Code
toxin production and antimicrobial resistance.	
Simultaneous testing does not identify a genetic marker of the hypervirulent 027 NAP1 BI strain for toxigenic C. difficile	NHPV
After repeat analysis, non-amplification of the internal control suggests the presence of PCR inhibitors in the patient sample. An additional sample should be submitted for testing if clinically warranted.	MPSP
The stool sample is POSITIVE for toxigenic *C. difficile*. This result is consistent with *C. difficile* infection (CDI) if accompanied by appropriate clinical symptoms.	*Comment added automatically if *C. difficile* Toxin B PCR is Detected

Use function **MEM** to enter results.
Enter Shift (1, 2, or 3)
Worksheet: Use WIM2 for WOMC or SIM2 for SGMC.
Test: <Enter>
Enter "A" (Accept)
Enter Accession number
Press <Enter> until Result screen displayed
Key in result using appropriate code from above

If instrument is interfaced with Sunquest, use function **OEM** to view and release results.

Shift: Press Enter
Device: Type in **WOCE** (White Oak) **or SGCE** (Shady Grove)
Refer to addendum A for additional information on interfaced results.

11. EXPECTED VALUES

11.1 Reference Ranges

Not detected

11.2 Critical Value

Detected

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Clostridium difficile (*C. difficile*) is a Gram-positive, spore-forming anaerobic bacillus that was first linked to disease in 1978. *C. difficile* infection (CDI) ranges from diarrhea to severe life-threatening pseudomembranous colitis. *C. difficile's* primary virulence factor is cytotoxin B. The genes coding for toxin A (*tcdA*; the enterotoxin) and toxin B (*tcdB*) are parts of the pathogenicity locus (PaLoc). Most pathogenic strains are toxin A-positive, toxin B-positive (A+B+) strains although toxin A-negative, toxin B-positive (A-B+) variant isolates have been recognized as pathogenic. Some strains of *C. difficile* also produce an actin-specific ADP-ribosyltransferase called CDT or binary toxin. The binary toxin locus contains two genes (*cdtA* and *cdtB*) and is located outside the PaLoc.

In the last several years, there have been outbreaks of CDI attributed to a number of emerging "hypervirulent" strains that include fluoroquinolone resistant strains belonging to PCR ribotype 027, PFGE type NAP1 and REA type BI. Strains of 027/NAP1/BI exhibit increased toxin production, which is being attributed to deletions in the regulatory gene *tcdC* and they are thought to produce more spores, leading to enhanced persistence in the environment. The identification of a presumptive positive or negative 027/ NAP1/BI result may aid in the identification of possible sources of an 027/NAP1/BI outbreak.

C. difficile diagnosis has been traditionally based on the detection of toxin A or B. Both the labor intensive culture procedure, followed by cell cytotoxicity testing on the isolates, and cytotoxicity cell assay on stool specimens are still considered to be the "gold standard" because of high specificity. Several rapid enzyme immunoassays have been developed for detection of toxin A and B. However, these tests have reduced sensitivity and specificity compared to the cell cytotoxicity assay. Recently, PCR methods for the detection of toxin A and/or toxin B have been developed with high sensitivity and specificity as compared to the cell cytotoxicity and immunoassays.

13. PROCEDURE NOTES

- **FDA Status: FDA Exempt/Cleared or Approved**

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a solution of 1:10 dilution of household chlorine bleach and then repeat the cleaning of the work area with 70% ethanol. Wipe work surfaces dry completely before proceeding.
- Results from Xpert *C. difficile/Epi* Assays are NOT intended to guide treatment of *C. difficile* infections.
- Performance characteristics were not established for patients < 2 years of age.

- The Xpert *C. difficile/Epi* Assay does not provide susceptibility results. A separate specimen aliquot and additional time are required to culture and perform susceptibility testing.
- Do not substitute Xpert *C. difficile/Epi* Assay reagents with other reagents.
- Do not open the Xpert *C. difficile/Epi* Assay cartridge lid except when adding sample and reagents or performing a retest.
- Do not use a cartridge that has been dropped.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert *C. difficile/Epi* Assay cartridge is used to process one test. Do not reuse spent cartridges.

14. LIMITATIONS OF METHOD

14.1 Precision

Not applicable

14.2 Interfering Substances

As indicated in the package insert, twenty-one (21) biological and chemical substances occasionally used or found in stool specimens were tested for interference with the Xpert *C. difficile/Epi* Assay. Potentially interfering substances include, but are not limited to Vagisil cream and zinc oxide paste (see "Assay Limitations"). The 19 substances listed below showed no detectable interference with the Xpert *C. difficile/Epi* Assay.

Substance	Substance
Whole Blood	K-Y Jelly/Gelée
Mucin (porcine)	Vaseline
Kaopectate	Dulcolax
Imodium	Preparation H Portable Wipes
Pepto-Bismol	Vaginal Contraceptive Film (VCF)
Preparation H	Vancomycin
Fleet	Metronidazole
Fecal fats	Anusol Plus
Monistat	E-Z-HDTM High Density Barium Sulfate for suspension
Hydrocortisone Cream Longs Drugs	

14.3 Clinical Sensitivity/Specificity/Predictive Values

As indicated in the Package Insert, the Xpert *C. difficile/Epi* assay had overall sensitivity, specificity, positive predicative value, and negative predicative value of 88.7%, 90.9%, 55.4%, and 99.8% respectively when compared to direct culture with strain typing.

- Non-027/NAP1/BI isolates representing toxinotype XIV will be reported "Toxigenic *C. diff* POSITIVE; 027 PRESUMPTIVE POSITIVE" using the Xpert *C. difficile/Epi* Assay.
- Occasionally, non-027/NAP1/BI isolates representing toxinotypes IV, V and X will be reported "Toxigenic *C. diff* POSITIVE; 027 PRESUMPTIVE POSITIVE" using the Xpert *C. difficile/Epi* Assay.
- The performance of the Xpert *C. difficile/Epi* Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Results from the Xpert *C. difficile/Epi* Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- 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. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Because of the dilution factor associated with the retest procedure, it is possible that *C. difficile* positive specimens, very near or at the limit of detection (LoD) of the *C. difficile/Epi* Assay, may result in a false negative result upon retest.
- Inhibition of the Xpert *C. difficile/Epi* Assay has been observed in the presence of the following substances: Zinc oxide paste and Vagisil® cream.
- Outbreaks of CDI may be caused by strains other than 027/NAP1/BI.
- 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.

15. SAFETY

- Reagent 1 contains sodium hydroxide (pH > 12.5); (R34 EU Risk) which is corrosive to eyes and skin requiring eye and skin protection.
- Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

- Biological Safety Cabinet, Micro procedure
- Laboratory Quality Control Program
- Laboratory Safety Manual
- Safety Data Sheets (SDS)
- Quest Diagnostics Incorporated Records Management Procedure
- *Clostridium difficile* Toxin B PCR using Cepheid® GeneXpert (QDMD734)
- GeneXpert Dx System Operator Manual
- Cepheid GeneXpert® Dx System Maintenance, Micro procedure
- *Clostridium difficile* PCR Quality Control Log (AG.F410)

- Cepheid GeneXpert® C. difficile Toxin B PCR Individual Quality Control Plans (SGAH.VC371, WAH.VC253)

17. REFERENCES

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18. DOCUMENT HISTORY

Version	Date	Section	Revision	Revised By	Approved By
			Supersedes SGAHQDMD734v1.3		
1	6/8/20	Header	Changed WAH to WOMC	L Barrett	R Master
		8.2	Deleted typing patient name and MRN into instrument		
		10.6	Added interfaced reporting		
		19	Added addendum A		

19. ADDENDA

A. Cepheid Testing and Running via Sunquest Interface

Addendum A

Cepheid Testing and Running via Sunquest Interface

A. General Information:

1. This interface does NOT go through DI-Instrument Manager. Cepheid is interfaced directly to Sunquest. The Sunquest interface is set up for Autoverification.
2. All tests will auto-file except for those that must be called.
3. If the test is positive for *C. difficile*, then the results will be held in Sunquest. These results must be called and documented per routine process.
4. Use function OEM on Sunquest SmarTerm to review results.
    a. Access OEM
        - At DEVICE: prompt, type in Method code **WOCE** (WOMC) or **SGCE** (SGMC).
        - Results will display cup by cup.
            - Those that were auto-filed require no action, proceed to next cup.
            - For positive results that were held, continue with steps b and c below.
        - Refer to *OEM - On Line Result Entry Method* procedure (LIS SOP) for additional information about review and release of results.

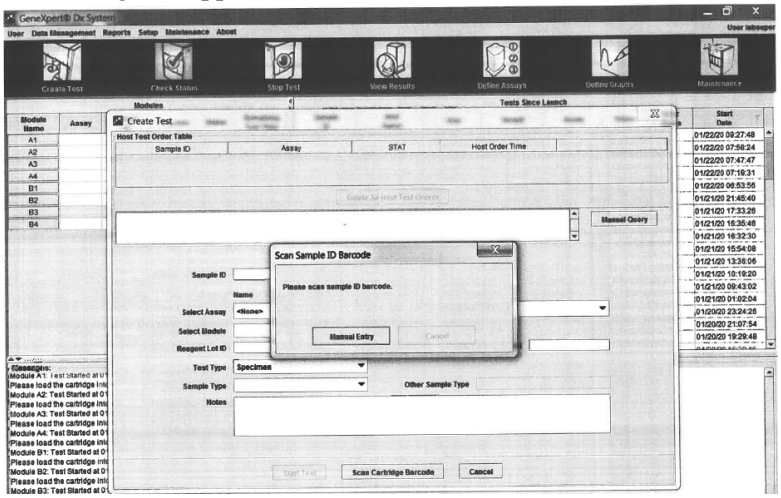
    b. Call results. Append CBACK documentation to results including who you called, date, time and tech code. Required format is:

        -CBACK-;full name of person called DATE TIME Tech code
        Example -CBACK-;Sue Smith 032420 1420 4568

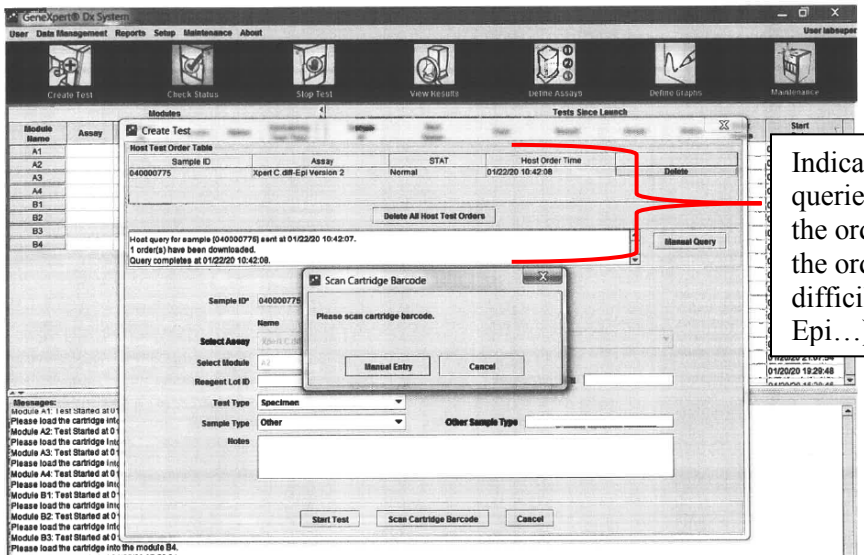
    c. Click on Accept to release results.
5. Perform an OFC (Online File Cleanup) at least once per shift. This process cleans up the online data that was sent to Sunquest.
    a. In Sunquest (SmarTerm) access function OFC
    b. Type in the method code (WOCE or SGCE).
    c. At the Start at Cup Number prompt, type in 1 and then press ENTER.
    d. At the Stop at Cup Number prompt, press ENTER.

B. Running Tests on Cepheid:

1. Create Test
   a. In the GeneXpert Dx System window, click **Create Test** on the menu bar. The Scan Sample ID Barcode dialog box appears.



   b. Scan the Sunquest barcode label.



Indicates the Cepheid queried Sunquest and found the order. In this example the order was for a C difficile PCR (Xpert C diff-Epi…)

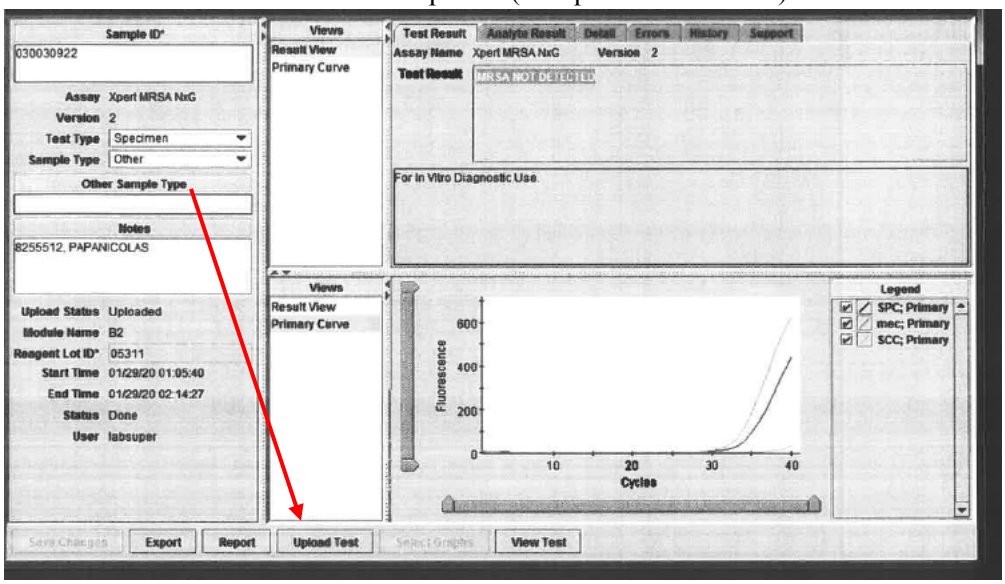
   c. Scan the cartridge barcode.

2. Click **OK**
3. Click **Create Test**
4. Load cartridge
5. Verify that the test has started before walking away
6. When testing is completed results will print to Cepheid printer.

C. Manually uploading results to Sunquest (Example Sunquest downtime)

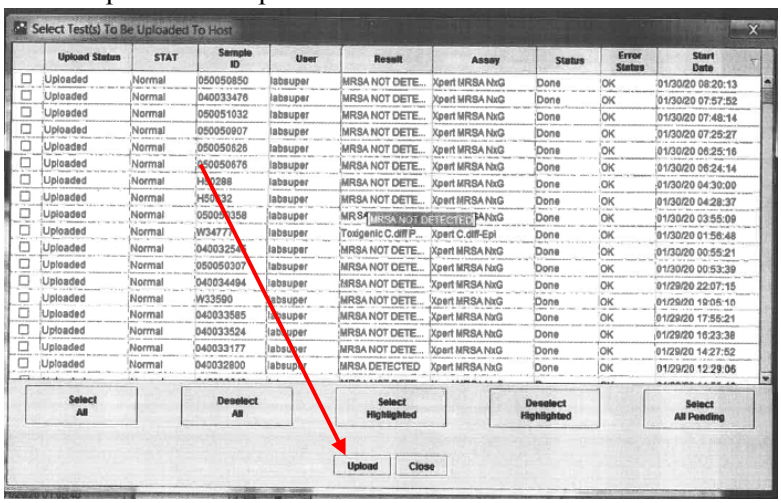
1. From the Cepheid, go to VIEW RESULTS

   a. Click on **UPLOAD TEST** and find the Sample ID (Sunquest Accession #).



   b. Check off the one that you want to upload (located to the left of the Update Status column). Note: You can check off one or more accession numbers at the same time.

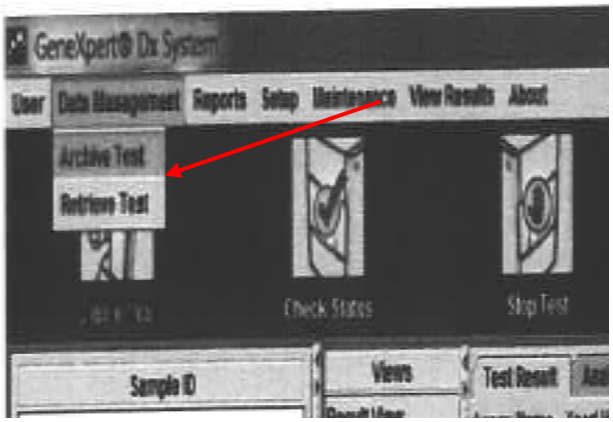
   c. Click on **UPLOAD** to resend to Sunquest. Results will now upload into Sunquest. It make take a little time for upload to complete.



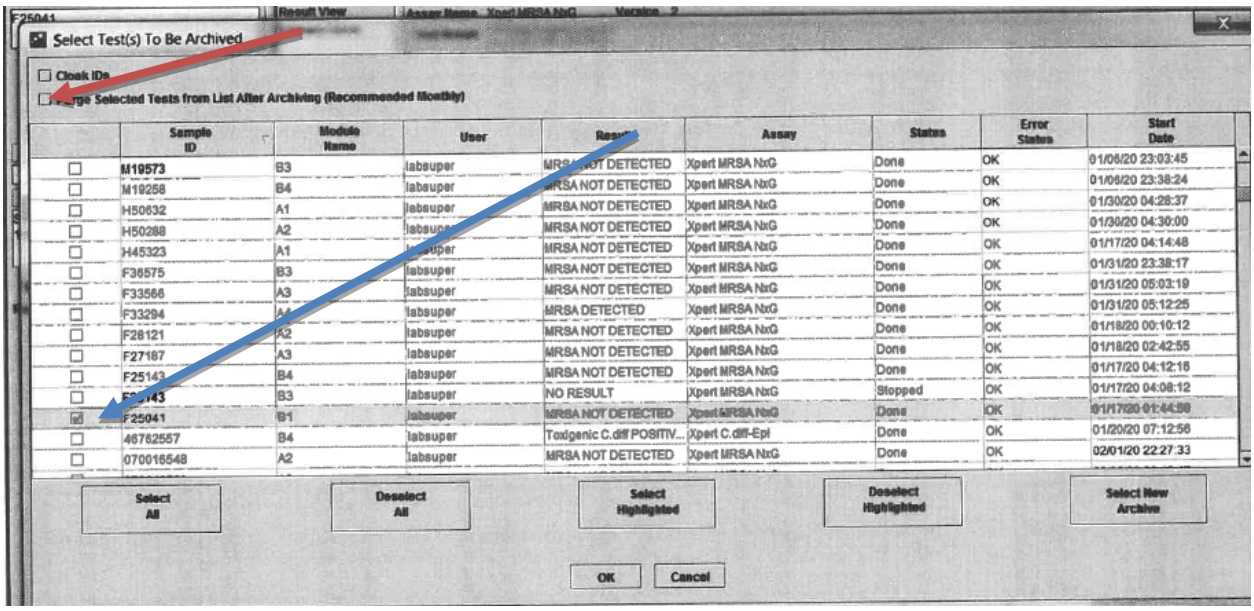
   d. Review in Sunquest OEM to document any positive result call notification.

D. Editing Sample ID (SQ Accession #)

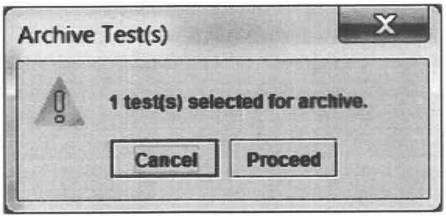
1. From the main screen -→Data Management-→ Click on Archive Test



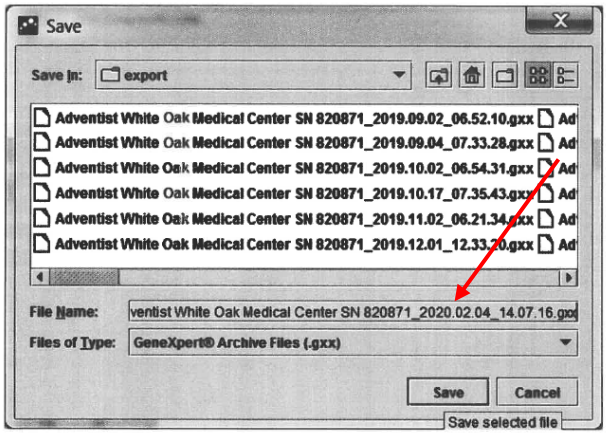
2. In the upper left corner click on **Purge Selected Tests from the LIS after Archiving** (red arrow). Then locate the Sample ID (SQ Accession#) that you want and select it by clicking on box to the left of the Sample ID (blue arrow). Then click on OK.



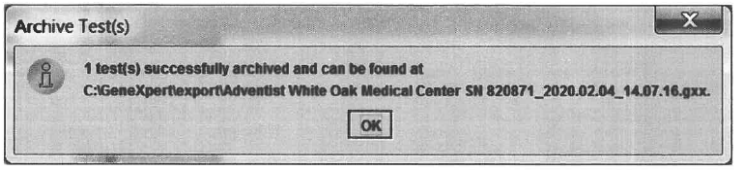
3. At the Archive Test prompt, click on **Proceed**.



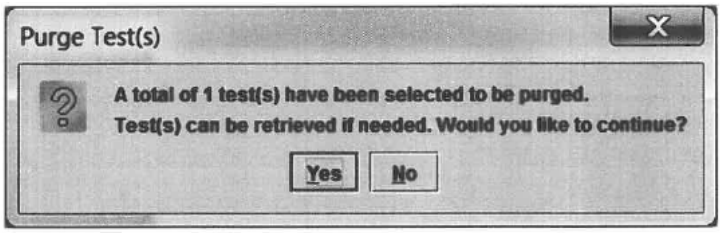
4. Archive file is generated (File name is system generated) and click on **SAVE**. Note that the File Name has the date and time as part of the file name. In the example below "2020.02.04_1407" is the date of 2/4/20 and time of 1407.



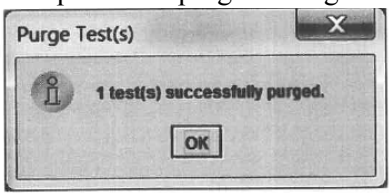
5. Archive message displays, click on **OK**



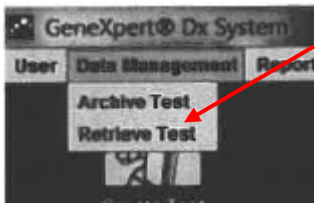
6. Purge message displays, click on **OK**



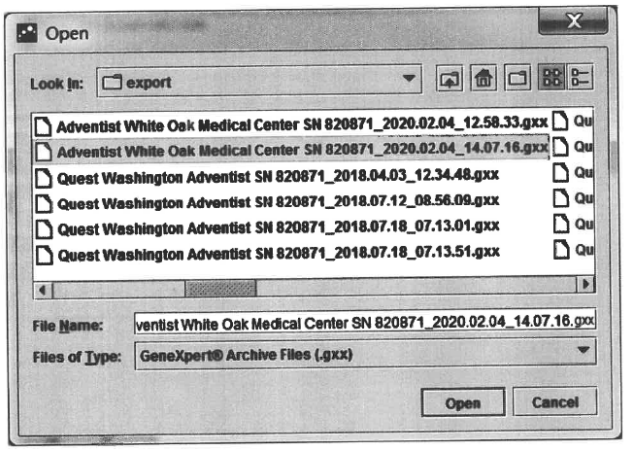
7. Completion of purge message displays



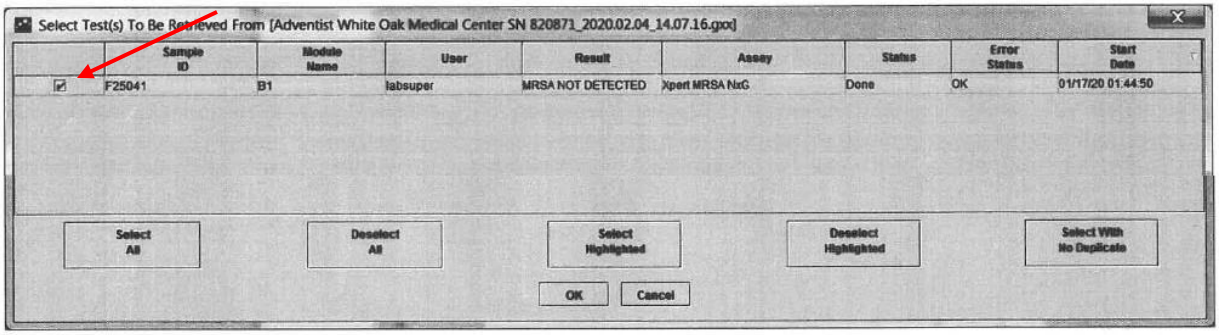
8. Retrieve test by going to Main screen -→ Data Management-→ Retrieve Test



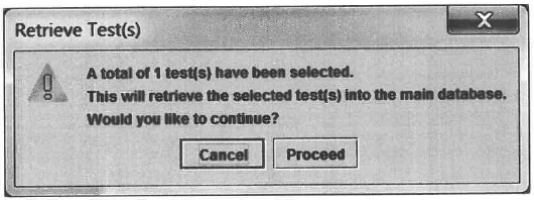
9. Locate file that you exported (Note, part of the file name consists of the date and time file was created.). Highlight the file and click on Open.

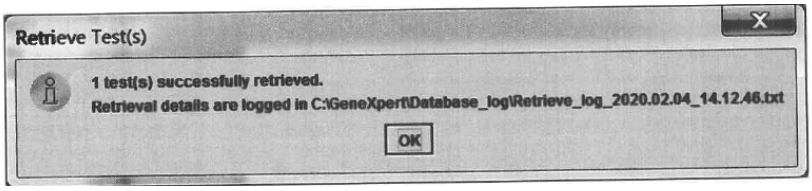


10. To the left of the Sample ID, check off the Sample ID (SQ acc #) that you want to retrieve to edit. Then click on OK.

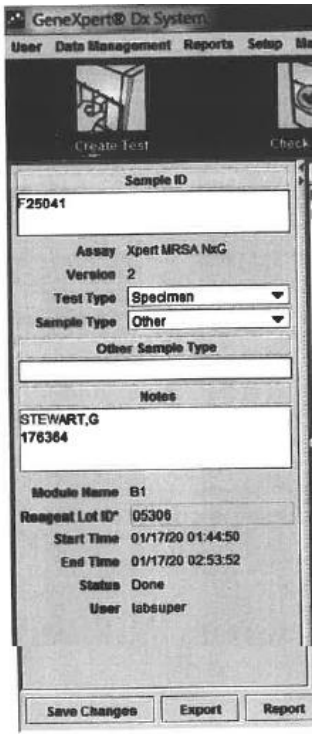


11. Retrieve prompt displays. Click on **Proceed**. Retrieve Test(s) confirm displays. Click on **OK**.

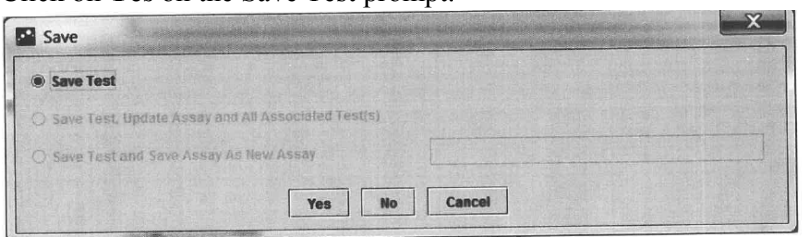




12. Proceed to edit Sample ID (SQ Accession #). Click on Save when you are done.



13. Click on **Yes** on the Save Test prompt.



14. Follow the steps in part C above to upload the results to Sunquest.