TRAINING UPDATE

Lab Location:	SGMC & WAH	**Date Distributed:**	8/16/2018
Department:	Core Lab	**Due Date:**	9/4/2018
		Implementation:	**9/4/2018**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Clostridium difficile Toxin B PCR using Cepheid GeneXpert® SGAHQDMD734 v1.2

Description of change(s):

Note changes in section 8 about labeling and entering patient identifiers into instrument

Section	Reason
6.3	Added QC names
8.1	Added vial & cartridge labeling
8.2	Clarified Sample ID, added identifier to Notes
10.6	Changed PCR result to match report, updated messages to match LIS

This revised SOP will be implemented on September 4, 2018

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	Microbiology/Molecular BPTs **Date: 09/18/2014**

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

Review		
Print Name and Title	**Signature**	**Date**

Corporate Approval	**Corporate Issue Date:** 11/3/2014	
Print Name and Title	**Signature**	**Date**
Paul Starolis, MT(ASCP) **National Laboratory Operations Director**	*On file*	**10/30/14**
Cathy Morris, MT(ASCP),CQA(ASQ) **CQA Manager (QC/ FDA Review)**	*On file*	**10/30/14**
Andrew N. Young, M.D., PhD **BPT Medical Advisor**		**10/30/14**
William M Miller, MD **Chief Laboratory Officer/Designee**		**11/3/14**

Retirement Date:	*Refer to the SmartSolve EDCS.*
Reason for retirement/replacement:	

Form ID: QDNQA303 v1 issued 8/05/13

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Cepheid GeneXpert *Clostridium difficile* PCR
Method	Real-time Polymerase Chain Reaction (PCR) Assay
Instrument	GeneXpert System
Synonyms	*Clostridium difficile* PCR, Xpert *Clostridium difficile*
Department	Core Lab

Order Code	**Test Name**
CDPCR	*Clostridium difficile* toxin B,QL real time PCR

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	Liquid or semi-formed stool	
-Other Acceptable	None	
Collection Container	Dry sterile leak-proof container	
Volume - Optimum	5 mL	
- Minimum	1 mL	
Transport Container & Temperature	Tightly sealed leak-proof container kept Frozen	
Stability & Storage Requirements	Room Temperature:	24 hours
	Refrigerated:	5 days
	Frozen:	Not applicable
Timing Considerations	Not applicable	

Form ID: QDNQA303 v1 issued 8/05/13

Criteria	
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	Thawed 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

Form ID: QDNQA303 v1 issued 8/05/13

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

- SPC and PCC are run within each test
- External *C. difficile* Controls are run once per day and should be included with the assay run. 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

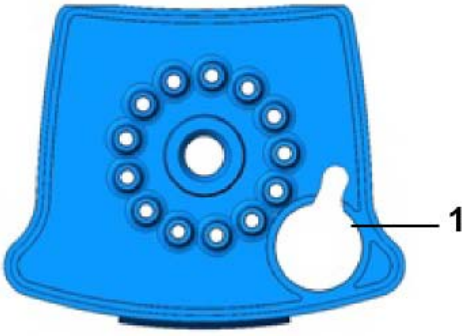
- Computer, monitor, printer, and required application software
- Biological Safety Cabinet
- Timer
- Refrigerator, 2-8°C
- Vortex
- Pipettor – 20uL (for control preparation)

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.	Note: Use clean gauze or plastic-backed absorbent pads for each sample when breaking off swab to minimize risks of contamination. 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.
6.	Replace cap on Sample Reagent and vortex at high speed for 10 seconds.
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.
7.	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.
8.	In the GeneXpert Dx Systems, click Start Test.
9.	Open the instrument module door with the blinking green light and load the cartridge.
10.	Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
11.	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.
12.	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.
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
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

Assay Result Reported	Interpretation of Result
	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. • 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 toxin production and antimicrobial resistance.	PHPV
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.

Form ID: QDNQA303 v1 issued 8/05/13

Enter Shift (1, 2, or 3)
Worksheet: Use WIM2 for WAH 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

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

Form ID: QDNQA303 v1 issued 8/05/13

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

Form ID: QDNQA303 v1 issued 8/05/13

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.

Form ID: QDNQA303 v1 issued 8/05/13

- 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 Program for Record Retention Requirements SOP.
- GeneXpert Dx System Operator Manual
- Cepheid GeneXpert® Dx System Maintenance, Micro procedure
- *Clostridium difficile* PCR Quality Control Chart (AG.F410)

17. REFERENCES

1. Xpert® MRSA Assay current package insert (11/2012).
2. American Society for Microbiology. 2010. A Practical Guidance Document for the Laboratory Detection of Toxigenic *Clostridium difficile.*
3. Larson HE, Price AB, Honour P, Borriello SP. *Clostridium difficile* and the aetiology of pseudomembranous colitis. Lancet1978;1:1063-1066.
4. Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. N Engl J Med 2002; 31:334-339.
5. Borriello SP. The influence of the normal flora on *Clostridium difficile* colonization of the gut. Ann Med 1990;22:61-67.
6. Bignardi GE. Risk factors for *Clostridium difficile* infection. J Hosp Infect. 1998; 40:1-15.

Form ID: QDNQA303 v1 issued 8/05/13

7. Kelly CP, Pothoulakis C, Lamont JT. *Clostridium difficile* colitis. N Engl J Med 1994; 330:257-262.
8. Braun V, Hundsberger T, Leukel P, et al. Definition of the single integration site of the pathogenicity locus of *Clostridium difficile*.1996; Gene. 181:29-38.
9. Hammond GA, Johnson JL. The toxigenic element of *Clostridium difficile* strain VPI 10463. Microb Pathog. 1995;19:203-213.
10. Sambol SP, Merrigan MM, Lyerly D, et al. Toxin gene analysis of a variant strain of *Clostridium difficile* that causes human clinical disease. Infect. Immun. 2000;68:5480-5487.
11. Gonçalves C, Decré D, Barbut F, et al. Prevalence and characterization of a binary toxin (actin-specific ADP-ribosyl-transferase) from *Clostridium difficile*. J Clin Microbiol. 2004;42:1933-1939.
12. Stubbs S, Rupnik M, Gibert M, et al. Production of actin-specific ADP-ribosyltransferase (binary toxin) by strains of *Clostridium difficile*. FEMS Microbiol Lett. 2000;186:307-312.
13. Popoff MR, Rubin EJ, Gill DM, Boquet P. Action-specific ADP-ribotransferase produced by a *Clostridium difficile* strain. Infect Immun. 1988;56:2299-2306.
14. Kuijper EJ, Coignard B, Tull P. ESCMID Study Group for *Clostridium difficile*; EU Member States; European Centre for Disease Prevention and Control. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clin Microbiol Infect. 2006; 12 Suppl 6:2-18.
15. Curry SR, Marsh JW, Muto CA, *et al. tcdC* genotypes associated with severe *TcdC* truncation in an epidemic clone and other strains of *Clostridium difficile*. J Clin Microbiol. 2007 Jan;45:215-221. Erratum in: J Clin Microbiol. 2007 Jun;45(6):2103.
16. Weiss K, Boisvert A, Chagnon M, et al. Multipronged Intervention Strategy to Control an Outbreak of *Clostridium difficile* Infection (CDI) and Its Impact on the Rates of CDI from 2002 to 2007. Infect Control Hosp Epidemiol. 2009;30(2):156-162.
17. MacCannell DR, Louie TJ, Gregson DB, et al. Molecular analysis of *Clostridium difficile* PCR ribotype 027 isolates from Eastern and Western Canada. J Clin Microbiol. 2006;44:2147-2152.
18. Wilkins TD, Lyerly DM. *Clostridium difficile* testing: after 20 years, still challenging. Clin Microbiol. 2003 Feb;41:531-534.
19. Delmee M. Laboratory diagnosis of *Clostridium difficile* disease. Clin Microbiol Infect. 2001;7:411-416.
20. Poutanen SM, Simor AE. *Clostridium difficile*-associated diarrhea in adults. CMAJ. 2004;171:51-58.
21. Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories. Richmond JY and McKinney RW (eds) (1993). HHS Publication number (CDC) 93-8395.

Form ID: QDNQA303 v1 issued 8/05/13

22. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to latest edition).

23. Cohen SH, Gerding D, Johnson S, et al. SHEA-IDSA Guideline: Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol 2010;31:431–455.

24. Killgore G, Thompson A, Johnson S, et al. Comparison of seven techniques for typing international epidemic strains of *Clostridium difficile*: restriction endonuclease analysis, pulsed-field gel electrophoresis, PCR-ribotyping, multilocus sequence typing, multilocus variable-number tandem-repeat analysis, amplified fragment length polymorphism, and surface layer protein A gene sequence typing. J Clin Microbiol 2008;46:431–437.

18. DOCUMENT HISTORY

Version	Date	Section	Revision	Revised By	Approved By
1.0	4/20/18	Header, Footer	Added Site and updated version #	R. Master	R. Master, N. Cacciabeve
1.0	4/20/18	1	Added Dept name & local order code	R. Master	
1.0	4/20/18	3.2	Removed frozen storage	L Barrett	
1.0	4/20/18	4	Deleted saline	R. Master	
1.0	4/20/18	6.1, 6.2	Deleted preparation of controls from stock cultures. Changed Zeptometrix volume to 20uL	R. Master	
1.0	4/20/18	7.2	Add 20uL pipettor	R. Master	
1.0	4/20/18	7.3	Added bleach and ethanol to supplies	R. Master	
1.0	4/20/18	8.1, 13	Added work area cleaning procedure	R. Master	
1.0	4/20/18	8.3	Added Retest Procedure from Product Insert	R. Master	
1.0	4/20/18	10.6	Added Local LIS Result codes, Deleted comment for patient <1YO Clarified Reporting	R. Master, M. Sabonis	
1.0	4/20/18	11.2	Added local priority information	R. Master	
1.0	4/20/18	11.3	Deleted QLS Standard Message Codes	R. Master	
1.0	4/20/18	13	Removed “with Modifications” to FDA Status	R. Master	
1.0	4/20/18	16	Added Local Related Documents	R. Master	

Version	Date	Section	Revision	Revised By	Approved By
1.1	8/13/18	6.3	Added QC names	R Master	R Master
1.1	8/13/18	8.1	Added vial & cartridge labeling	R Master	
1.1	8/13/18	8.2	Clarified Sample ID, added identifier to Notes	L Barrett	
1.1	8/13/18	10.6	Changed PCR result to match report, updated messages to match LIS	L Barrett	

19. ADDENDA

None