

# Xpert® C. difficile/Epi

For use with GeneXpert® System with Touchscreen



## Catalog Numbers

**REF** GXCDIFF/EPI-10

**REF** GXCDIFF/EPI-120

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In Vitro Diagnostic Medical Device

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See [Revision History](#) for a description of changes.

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# Getting Started

## Product Information

### Proprietary Name

Xpert® *C. difficile*/Epi

### Common or Usual Name

Xpert *C. difficile*/Epi test

## Intended Use, Summary, and Principle of Procedure

### Intended Use

The Xpert *C. difficile*/Epi test is a qualitative *in vitro* diagnostic test for rapid detection of toxin B gene sequences and for presumptive identification of 027/NAP1/BI strains of toxigenic *Clostridioides difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection (CDI). Presumptive identification of 027/NAP1/BI strains of *C. difficile* is by detection of binary toxin (CDT) gene sequences and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a negative regulator in *C. difficile* toxin production. The test is performed on the GeneXpert® Instrument Systems and utilizes automated real-time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Xpert *C. difficile*/Epi test is intended as an aid in the diagnosis of CDI. Detection of 027/NAP1/BI strains of *C. difficile* by the Xpert *C. difficile*/Epi test is presumptive and is solely for epidemiological purposes and is not intended to guide or monitor treatment for *C. difficile* infections. Concomitant culture is necessary only if further typing or organism recovery is required.

### Summary and Explanation

*Clostridioides difficile* (*C. difficile*) is a Gram-positive, spore-forming anaerobic bacillus that was first linked to disease in 1978.<sup>1</sup> *Clostridioides difficile* infection (CDI) ranges from mild diarrhea to severe life-threatening pseudomembranous colitis.<sup>2</sup> Mature colonic bacterial flora in a healthy adult is generally resistant to *C. difficile* colonization.<sup>3</sup> However, if the normal colonic flora is altered, resistance to colonization is lost. The



most common risk factor is exposure to antibiotics.<sup>4</sup> *C. difficile*'s primary virulence factor is cytotoxin B.<sup>5</sup> The genes coding for toxin A (*tcdA*; the enterotoxin) and toxin B (*tcdB*) are parts of the pathogenicity locus (PaLoc).<sup>6,7</sup> Most pathogenic strains are toxin A-positive, toxin B-positive (A+B+) although toxin A-negative, toxin B-positive (A-B+) variant isolates have been recognized as pathogenic.<sup>8</sup> 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.<sup>9-11</sup>

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 O27, PFGE type NAP1 and REA type BI.<sup>8,12</sup> Strains of O27/NAP1/BI exhibit increased toxin production, which is attributed to deletions in the regulatory gene *tcdC* and they are thought to produce more spores, leading to enhanced persistence in the environment.<sup>13,14</sup> The identification of a presumptive positive or negative O27/NAP1/BI result may aid in the identification of possible sources of an O27/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.<sup>15,16</sup> Several rapid enzyme immunoassays have been developed for detection of toxins A and B. However, these tests have reduced sensitivity and specificity compared to the cell cytotoxicity test. Recently, PCR methods for the detection of the genes encoding toxin A and/or toxin B have been developed with high sensitivity and specificity as compared to the cell cytotoxicity and immunoassays.<sup>17</sup>

## Principle of the Procedure

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and real-time reverse-transcriptase PCR (RT-PCR) tests. (Real-time RT-PCR is used for assays that detect RNA. The Xpert *C. difficile*/Epi test uses real-time PCR to detect DNA.) The system consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is very rare. For a full description of the system, see the relevant system operator manual.

The Xpert *C. difficile*/Epi test (where Epi means epidemiology) includes reagents for the detection of toxigenic *C. difficile* and the presumptive detection of sequences found in O27/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 Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Cepheid Xpert *C. difficile*/Epi test is a rapid, automated in vitro diagnostic test for qualitative detection of toxin-producing *Clostridiodes difficile* directly from unformed (liquid or soft) stool specimens of patients suspected of having *Clostridiodes difficile* infection (CDI). The test 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 (*tcdCΔ117*). The combined presence of the genes encoding toxin B and binary toxin and the *tcdCΔ117* deletion have been associated with a hypervirulent *C. difficile* strain known as O27/NAP1/BI, which has been associated with severe disease outbreaks in healthcare facilities worldwide.<sup>12,13,15</sup> The test is performed on the Cepheid GeneXpert Instrument Systems.

# Reagents, Instruments, and Materials

## Reagents

### Materials Provided

The Xpert *C. difficile/Epi* test kit (GXCDIFF/EPI-10) contains sufficient reagents to process 10 specimens or quality control samples. The Xpert *C. difficile/Epi* test kit (GXCDIFF/EPI-120) contains sufficient reagents to process 120 specimens or quality control samples.

The kits contain the following:

<b>Xpert <i>C. difficile/Epi</i> test Cartridges with Integrated reaction tubes</b>	<b>10</b>	<b>120</b>
<ul style="list-style-type: none"><li>Bead 1, Bead 2, and Bead 3 (freeze-dried)</li></ul>	<ul style="list-style-type: none"><li>1 of each per cartridge</li></ul>	<ul style="list-style-type: none"><li>1 of each per cartridge</li></ul>
<ul style="list-style-type: none"><li>Reagent 1</li></ul>	<ul style="list-style-type: none"><li>3.0 mL per cartridge</li></ul>	<ul style="list-style-type: none"><li>3.0 mL per cartridge</li></ul>
<ul style="list-style-type: none"><li>Reagent 2 (Sodium Hydroxide)</li></ul>	<ul style="list-style-type: none"><li>3.0 mL per cartridge</li></ul>	<ul style="list-style-type: none"><li>3.0 mL per cartridge</li></ul>
<b>Xpert <i>C. difficile/Epi</i> test Reagent pouch</b>	<b>1 per kit</b>	<b>1 per kit</b>
<ul style="list-style-type: none"><li>Sample Reagent (Guanidinium thiocyanate)</li></ul>	<ul style="list-style-type: none"><li>10 x 2.0 mL per vial</li></ul>	<ul style="list-style-type: none"><li>120 x 2.0 mL per vial</li></ul>
<b>CD</b>	<b>1 per kit</b>	<b>1 per kit</b>
<ul style="list-style-type: none"><li>Assay Definition File (ADF)</li><li>Instructions to import ADF into GX software</li><li>Instructions for Use (Package Insert)</li></ul>		

**Note** Safety Data Sheets (SDS) are available at [www.cepheid.com](http://www.cepheid.com) or [www.cepheidinternational.com](http://www.cepheidinternational.com) under the **SUPPORT** tab.

**Note** The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.



## Materials Required but Not Provided

- GeneXpert system with touchscreen: GeneXpert instrument, touchscreen unit with built-in scanner, Cepheid OS software version 2.0 or higher, and operator manual.
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Vortex mixer
- Dry swab for transfer of the stool specimen, Cepheid Sample Collection Device catalog number 900-0370 (Copan Venturi Transystem<sup>®</sup> Culture, 139CFA) or Cepheid Single-Use Disposable Swab catalog number SDPS-120 (Copan 138CS01.PH)
- Disposable transfer pipettes


## Materials Available but Not Provided

KWIK-STIKS<sup>™</sup> from MicroBioLogics catalog #0329 (toxigenic *C. difficile*) as positive control, and catalog #0527 (non-toxigenic *C. difficile*) and catalog #0331 (*C. sordelli*) as negative controls.

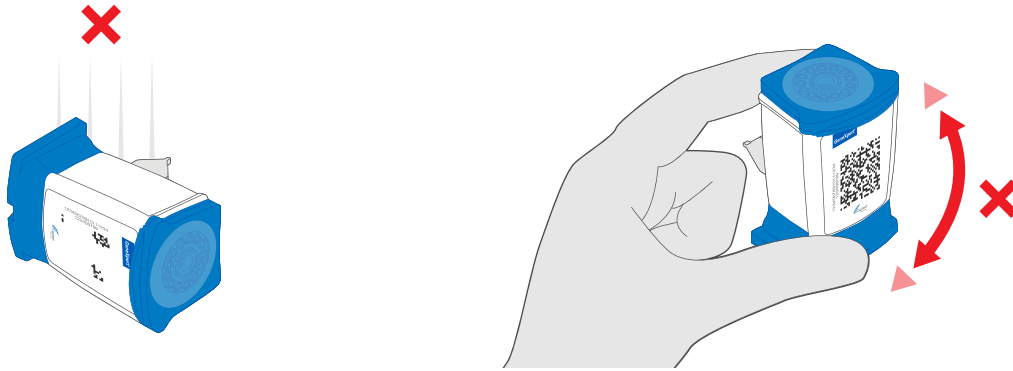
*C. difficile* strains from ATCC #9689 (wild type toxigenic *C. difficile*) and #BAA-1870 (NAP1/027 *C. difficile*) as positive controls, and #700057 (non-toxigenic *C. difficile*) as negative control are also available.

In addition, strains may be obtained from the Centers for Disease Control and Prevention, Division of Healthcare Quality Promotion.

## Warnings and Precautions

- For *in vitro* diagnostic use. 
- Results from Xpert *C. difficile*/Epi tests are NOT intended to guide treatment of *C. difficile* infections.<sup>21</sup>
- Treat all biological specimens, including used cartridges and reagents, 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. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention<sup>19</sup> and the Clinical and Laboratory Standards Institute.<sup>20</sup>
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Performing the Xpert *C. difficile*/Epi test outside the recommended storage temperature ranges and time may produce erroneous or invalid results.
- Performance characteristics were not established for patients < 2 years of age.
- The Xpert *C. difficile*/Epi test 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 test reagents with other reagents.
- Do not open the Xpert *C. difficile*/Epi test cartridge lid except when adding sample and reagents or performing a retest.
- Do not use a cartridge that has been dropped after removing it from the packaging.

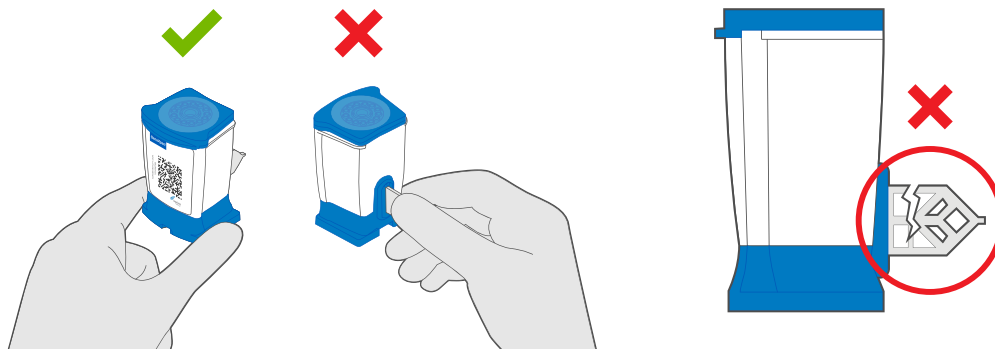




- Do not place the sample ID label on the cartridge lid or on the barcode label.



- Hold the cartridge by the base. Do not touch the reaction tube at the rear of cartridge, as this could cause damage that would interfere with light passing through it during the test. Do not use a cartridge with a damaged reaction tube.



- Each single-use Xpert C. *difficile*/Epi test cartridge is used to process one test. Do not reuse spent cartridges.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

## Chemical Hazards, Storage and Handling



## Chemical Hazards<sup>23,24</sup>

- UN GHS Hazard Pictogram:
- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed.
  - Causes skin irritation.
  - Causes serious eye irritation.
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash thoroughly after handling.
    - Do not eat, drink, or smoke when using this product.
    - Avoid release to the environment.
    - Wear protective gloves/protective clothing/eye protection/face protection.
  - **Response**
    - IF ON SKIN: Wash with plenty of soap and water.
    - Take off contaminated clothing and wash before reuse.
    - Specific treatment, see supplemental first aid information.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.
    - IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician if you feel unwell.
    - Rinse mouth.
  - **Storage/Disposal**
    - Dispose of contents and/or container in accordance with local, regional, national, and/or international regulations.

## Storage and Handling

- Store the Xpert *C. difficile*/Epi test kit at 2–28 °C.
- Do not use reagents or cartridges that have passed the expiration date.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use any reagents that have become cloudy or discolored.

# Specimen Collection, Testing, and Results

## Specimen Collection

### Specimen Collection, Transport and Storage

1. Collect the unformed stool specimen in a clean container. Follow your institution's guidelines for collecting samples for *C. difficile* testing.
2. Label with the Sample ID and send to the laboratory.
3. Store specimen at 2–8 °C. The specimen is stable for up to 5 days when stored at 2–8 °C. Alternatively, specimens can be kept at room temperature (20–30 °C) for up to 24 hours.

## Procedure

### Preparing the Cartridge

 **Important** Start the test within 30 minutes of adding the sample to the cartridge.

To add the sample into the cartridge:

1. Remove the cartridge and Sample Reagent from the package.
2. Briefly place a swab in the unformed stool sample. The swab does not need to be completely saturated.
3. Insert the swab into the vial containing the Sample Reagent.  
**Note** Use sterile gauze to minimize risks of contamination.
4. 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.
5. Close the lid and vortex at high speed for 10 seconds.
6. Open the cartridge lid. Using a clean transfer pipette (not supplied), transfer the entire contents of the Sample Reagent to the Sample Chamber of the Xpert *C. difficile*/Epi test cartridge.
7. Close the cartridge lid.



Figure 1 Xpert C. difficile/Epi test cartridge (top view)

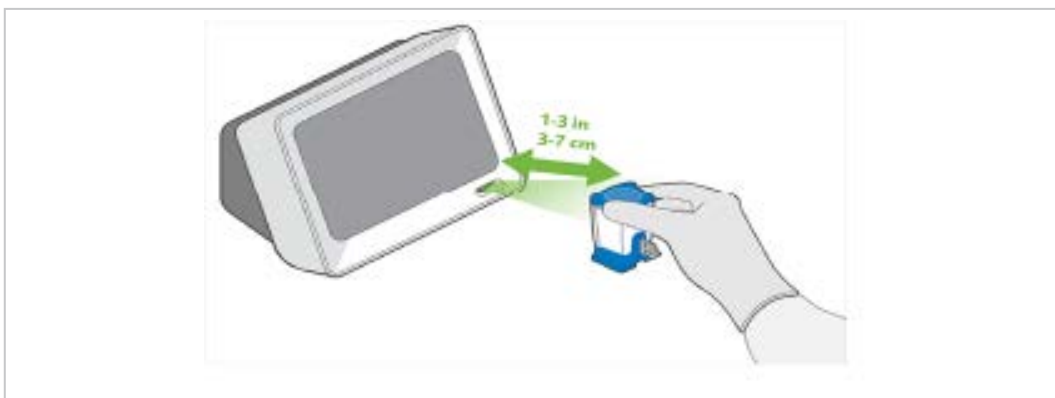
## Starting the Test: GeneXpert System with Touchscreen

**i Important** Before you start the test, make sure that:

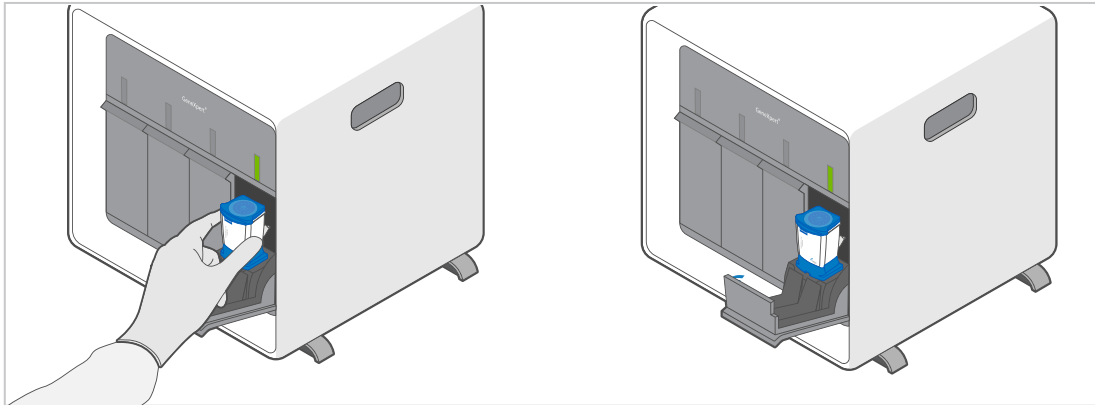
- The system is running the correct Cepheid OS software version shown in section - **Materials Required but Not Provided.**
- The correct assay definition file is imported into the software.

**Note** The default workflow is shown. Your system administrator may alter the workflow.

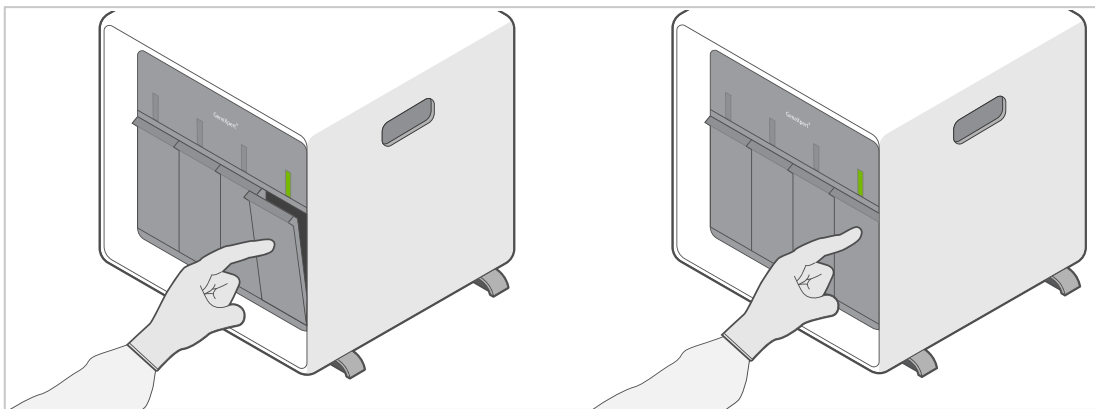
1. Turn on GeneXpert system with touchscreen.
2. Log on to system software using your username and password.
3. On the Modules tab, touch **Start Test.**
4. Follow onscreen prompts to create new test and enter patient and sample information.
5. Scan or manually input the cartridge serial number. If scanning, hold the cartridge about 1-3 inches (3-7 cm) away from the scanner. The scanner projects a green crosshair, which you center on the barcode. Scanning is complete when you hear an audible beep. Touch **Continue.**



6. Select the desired test and touch **Continue.**
7. Watch the cartridge preparation video, if needed.
8. On the Confirm screen, review all data and touch **Confirm.**
9. Open the module door under flashing green light and insert the cartridge.



10. Close cartridge module door completely by pressing until it latches. The test starts.



11. When the test completes, the **Results Summary** screen appears. Open the module door and remove cartridge.
12. Dispose of used cartridge in appropriate waste container according to your institution's standard practices.

## Viewing Results: GeneXpert System with Touchscreen

The GeneXpert System with Touchscreen results screen will automatically interpret test results for you and clearly show them in the **View Results** window.

1. Tap **Results**.
2. Tap the test to be viewed in the Results screen.
3. Click **OK**.
4. To generate a PDF report file, touch **View Report**. More detailed instructions for viewing and uploading results are available in your system operator manual.

## Quality Control

Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

**Sample Processing Control (SPC)** — Ensures the sample was correctly processed. The SPC that is included in each cartridge verifies adequate processing of the sample bacteria. The SPC verifies that lysis of *C. difficile* bacteria and spores have occurred, if the organisms are present, and verifies that specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the real-time PCR test. The SPC



should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

**Probe Check Control (PCC)** — Before the start of the PCR reaction, the GeneXpert Instrument Systems measure the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

**External Controls** — Following good laboratory practice, external controls may be used in accordance with local, state, federal accrediting organizations, as applicable.

## Results

The results are interpreted by the GeneXpert Instrument Systems from measured fluorescent signals and embedded calculation algorithms and will be shown in the **View Results** window. Possible results are summarized in [Table 1](#); additional details are provided following the table.

**Table 1. Xpert C. difficile/Epi test Possible Results**

Results				Interpretations
Toxin B	Binary Toxin	tcdC	SPC	
+	+	+	+/-	Toxigenic <i>C. diff</i> POSITIVE 027-NAP1-BI PRESUMPTIVE POSITIVE
+	+	-	+/-	Toxigenic <i>C. diff</i> POSITIVE 027-NAP1-BI PRESUMPTIVE NEGATIVE
	-	+	+/-	
	-	-	+/-	
-	+	+	+	Toxigenic <i>C. diff</i> NEGATIVE 027-NAP1-BI PRESUMPTIVE NEGATIVE
	+	-	+	
	-	+	+	
	-	-	+	



Table 2. Xpert C. difficile/Epi test Results and Interpretations

Result	Interpretation
<b>Toxigenic C.diff POSITIVE; 027 PRESUMPTIVE POSITIVE</b>	<p>Toxin producing <i>C. difficile</i>, presumptive 027/NAP1/BI target DNA sequences are detected.</p> <ul style="list-style-type: none"> <li>The toxigenic <i>C. difficile</i> target (Toxin B) AND both presumptive 027/NAP1/BI targets (Binary Toxin and <i>tcdCA117</i>) have Cts within the valid range and endpoints above the minimum setting.</li> <li>SPC – NA (not applicable); SPC is ignored since <i>C. difficile</i> target amplification may compete with this control.</li> <li>Probe Check – PASS; all probe check results pass.</li> </ul> <p><b>Note:</b> Non-027/NAP1/BI isolates representing toxinotype XIV and occasionally toxinotypes IV, V and X will be reported “<b>Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE POSITIVE</b>” (Figure 2) using the Xpert <i>C. difficile</i>/Epi test.</p>
<b>Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE</b>	<p>Toxin producing <i>C. difficile</i> target DNA sequences are detected.</p> <ul style="list-style-type: none"> <li>The toxigenic <i>C. difficile</i> target (Toxin B) AND only one or none of the presumptive 027/NAP1/BI targets (Binary Toxin and <i>tcdCA117</i>) have Cts within the valid range and endpoints above the minimum setting.</li> <li>SPC – NA (not applicable); SPC is ignored since <i>C. difficile</i> target amplification may compete with this control</li> <li>Probe Check – PASS; all probe check results pass.</li> </ul>
<b>Toxigenic C. diff NEGATIVE; 027 PRESUMPTIVE NEGATIVE</b>	<p>Toxin producing <i>C. difficile</i> target DNA sequences are not detected.</p> <ul style="list-style-type: none"> <li>Toxigenic <i>C. difficile</i> target (Toxin B) is not detected (regardless of whether Binary Toxin and/or <i>tcdCA117</i> is detected).</li> <li>SPC – PASS; SPC has a Ct within the valid range and endpoint above the endpoint minimum setting.</li> <li>Probe Check – PASS; all probe check results pass.</li> </ul>
<b>INVALID</b>	<p>Presence or absence of <i>C. difficile</i> target DNA cannot be determined. Repeat test according to the instructions in the <a href="#">Retest Procedure</a> section below.</p> <ul style="list-style-type: none"> <li>SPC – FAIL; SPC target result is negative and the SPC Ct is not within valid range and endpoint below minimum setting.</li> <li>Probe Check – PASS; all probe check results pass.</li> </ul>
<b>ERROR</b>	<p>Presence or absence of <i>C. difficile</i> target DNA cannot be determined. Repeat test according to instructions in the section below.</p> <ul style="list-style-type: none"> <li>Toxin producing <i>C. difficile</i> targets — NO RESULT</li> <li>Binary Toxin (CDT) — NO RESULT</li> <li><i>tcdCA117</i> — NO RESULT</li> <li>Probe Check — FAIL*; one or more of the probe check results fail.</li> </ul> <p>*If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range.</p>
<b>NO RESULT</b>	<p>Presence or absence of <i>C. difficile</i> target DNA cannot be determined. Repeat test according to instructions in the section below.</p> <ul style="list-style-type: none"> <li>Toxin producing <i>C. difficile</i> targets — NO RESULT</li> <li>Binary Toxin (CDT) — NO RESULT</li> <li><i>tcdCA117</i> — NO RESULT</li> <li>Probe Check — NA (not applicable)</li> </ul>



## Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test according to the instructions in the Retest Procedure 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 test 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.

## Retest Procedure

For retest within 3 hours of an indeterminate result, use a new cartridge (do not re-use the cartridge) and new reagents.

1. Transfer remaining contents from the Sample Chamber to a new Sample Reagent vial using a disposable transfer pipette.
2. Vortex and add the entire contents of the Sample Reagent to the Sample Chamber of the new Xpert *C. difficile*/Epi test cartridge.
3. Close the lid and start new test.  
For retest after 3 hours of an indeterminate result, repeat the test with a new swab sample.

## Limitations

### Limitations of the Procedure

- Non-027/NAP1/BI isolates representing toxinotype XIV will be reported **Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE POSITIVE** using the Xpert *C. difficile*/Epi test.
- 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 test.
- The performance of the Xpert *C. difficile*/Epi test was validated using the procedures provided in this IFU only. Modifications to these procedures may alter the performance of the test.
- Positive results observed with immunocompromised pediatric patients may reflect asymptomatic carriage of *C. difficile*/Epi.
- 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.
- Results from the Xpert *C. difficile*/Epi test 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 Xpert *C. difficile*/Epi test, may result in a false negative result upon retest.





- Inhibition of the Xpert *C. difficile*/Epi test has been observed in the presence of the following substances:  
Zinc oxide paste and Vagisil<sup>®</sup> 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.



## Expected Values

In the Xpert *C. difficile*/Epi test clinical study, a total of 2293 unformed stool specimens were included from seven centers across the United States and Canada. The number and percentage of toxigenic *C. difficile* positive cases by culture, calculated by age and gender, are presented in Table 3 and Table 4, respectively.

**Table 3. Observed Prevalence of Toxigenic *C. difficile* by Age Group<sup>a</sup>**

Age Groups	N	Toxigenic <i>C. difficile</i> Prevalence (includes 027/NAP1/BI)	027/NAP1/BI Prevalence
2-5	16	37.5% (6/16)	12.5% (2/16)
6-21	105	12.4% (13/105)	0.9% (1/105)
22-59	898	16.4% (147/898)	3.3% (30/898)
>60	1274	20.7% (264/1274)	7.2% (92/1274)
<b>Total</b>	<b>2293</b>	<b>18.8% (430/2293)</b>	<b>5.5% (125/2293)</b>

a. Prevalence based on Xpert results.

**Table 4. Observed Prevalence of Toxigenic *C. difficile* by Gender<sup>a</sup>**

Gender	N	Toxigenic <i>C. difficile</i> Prevalence (includes 027/NAP1/BI)	027/NAP1/BI Prevalence
Male	1072	18.2% (195/1072)	5.0% (54/1072)
Female	1221	19.2% (235/1221)	5.8% (71/1221)
<b>Total</b>	<b>2293</b>	<b>18.8% (430/2293)</b>	<b>5.5% (125/2293)</b>

a. Prevalence based on Xpert results.

# ! Specific Performance Characteristics

## Clinical Performance

Performance characteristics of the Xpert *C. difficile*/Epi test were determined in a multi-site prospective investigation study at seven US and Canadian institutions by comparing the Xpert *C. difficile*/Epi test to reference culture followed by cell cytotoxicity testing on the isolates and strain typing on the toxigenic strains by PCR-ribotyping, pulsed-field gel electrophoresis (PFGE) and restriction endonuclease analysis (REA) methods.<sup>22</sup>

Subjects included individuals whose routine care called for *C. difficile* testing. A portion of each leftover unformed stool specimen was obtained for testing by the Xpert *C. difficile*/Epi test. The remaining excess specimen was sent to a central laboratory for reference culture and cytotoxin B testing. Each stool specimen was inoculated onto pre-reduced cycloserine-cefoxitin-fructose agar –direct plate (CCFA-D) and cycloserine-cefoxitin mannitol broth with taurocholate lysozyme cysteine (CCMB-TAL). After 24 hours the CCMB-TAL was subcultured on to a second CCFA-E plate (CCFA- Enriched). This direct-enriched culture method is referred to hereafter as “reference culture”.

If *C. difficile* was isolated from the CCFA-D plate and the isolate was positive by cell cytotoxicity assay, the specimen was classified as “toxigenic *C. difficile* positive” and CCFA-E plate was not further analyzed. If no *C. difficile* was isolated from the CCFA-D plate or if the isolate was negative by cell cytotoxicity assay, the CCFA-E plate was further analyzed.

If CCFA-E was positive for *C. difficile* and the isolate was positive for cell cytotoxicity assay, the specimen was classified as “toxigenic *C. difficile* positive”. The specimen was reported as “negative” if CCFA-E was negative for *C. difficile* or the isolate was tested negative by cell cytotoxicity assay.

Following reference culture testing, the toxigenic *C. difficile* positive isolates were sent to a second set of reference laboratories for strain identification by REA, PFGE, and PCR-ribotyping.

Performance of the Xpert *C. difficile*/Epi test was calculated relative to the results of direct culture with strain typing, for each of the three strain typing methods, and reference culture with strain typing, for each of the three strain typing methods.

A total of 2293 specimens were tested by Xpert *C. difficile*/Epi test, culture, and strain typing.

## Performance vs. Direct Culture

Relative to direct culture with REA strain typing, the Xpert *C. difficile*/Epi test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 98.72% and 90.86%, respectively. The Xpert *C. difficile*/Epi test also



demonstrated a 98.55% positive agreement and 97.65% negative agreement for BI (Table 5).

**Table 5. Xpert C. difficile/Epi test Performance vs. Direct Culture & REA**

Direct Culture & REA					
		Toxin B+ BI +	Toxin B+ BI -	NEG	Total <sup>a</sup>
<b>Xpert C. difficile/Epi test<sup>b</sup></b>	<b>Toxin B+ 027/NAP1/BI+</b>	68	5	47	120
	<b>Toxin B+ 027/NAP1/BI-</b>	1	158	140	299
	<b>NEG</b>	0	3	1860	1863
	<b>Total</b>	69	166	2047	2282
		<b>Toxigenic C. difficile</b>		<b>Toxigenic C. difficile / 027/NAP1/BI</b>	
		Sensitivity:	98.72% (232/235)	Pos Agreement:	98.55% (68/69)
		Specificity:	90.86% (1860/2047)	Neg Agreement:	97.65% (2161/2213)
		Accuracy:	91.67% (2092/2282)	Accuracy:	97.68% (2229/2282)
		PPV <sup>c</sup> :	55.37% (232/419)	PPV:	56.67% (68/120)
		NPV <sup>d</sup> :	99.84% (1860/1863)	NPV:	99.95% (2161/2162)

a. 11 specimens were culture positive but were not strain typed for the following reasons: incomplete restriction endonuclease digestion; or the isolate was not sent. These 11 specimens are not included in the performance characteristics above.

b. Xpert results shown are for first or second attempt. Approximately 3.2% of the specimens were indeterminate on the first attempt.

c. Positive predictive value

d. Negative predictive value

Relative to direct culture with PFGE strain typing, the Xpert C. difficile/Epi test demonstrated a sensitivity and specificity for toxigenic C. difficile of 98.76% and 90.86%, respectively. The Xpert C. difficile/Epi test also demonstrated a 100% positive agreement and 97.61% negative agreement for NAP1 (Table 6).

**Table 6. Xpert C. difficile/Epi test Performance vs. Direct Culture & PFGE**

Direct Culture & PFGE					
		Toxin B+ NAP1 +	Toxin B+ NAP1 -	NEG	Total <sup>a</sup>
<b>Xpert C. difficile/Epi test<sup>b</sup></b>	<b>Toxin B+ 027/NAP1/BI+</b>	71	6	47	124
	<b>Toxin B+ 027/NAP1/BI-</b>	0	161	140	301
	<b>NEG</b>	0	3	1860	1863
	<b>Total</b>	71	169	2047	2288
		<b>Toxigenic C. difficile</b>		<b>Toxigenic C. difficile / 027/NAP1/BI</b>	
		Sensitivity:	98.76% (238/241)	Pos Agreement:	100% (71/71)



Direct Culture & PFGE				
	Toxin B+ NAP1 +	Toxin B+ NAP1 -	NEG	Total <sup>a</sup>
	Specificity:	90.86% (1860/2047)	Neg Agreement:	97.61% (2163/2216)
	Accuracy:	91.70% (2098/2288)	Accuracy:	97.68% (2234/2288)
	PPV <sup>c</sup> :	56.00% (238/425)	PPV:	57.26% (71/124)
	NPV <sup>d</sup> :	99.84% (1860/1863)	NPV:	100% (2164/2164)

- a. 5 specimens were culture positive but were not strain typed for the following reasons: incomplete restriction endonuclease digestion; no growth; or contamination. These 5 specimens are not included in the performance characteristics above.
- b. Xpert results shown are for first or second attempt. Approximately 3.2% of the specimens were indeterminate on the first attempt.
- c. Positive predictive value
- d. Negative predictive value

Relative to direct culture with PCR-ribotyping, the Xpert *C. difficile*/Epi test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 98.78% and 90.86%, respectively. The Xpert *C. difficile*/Epi test also demonstrated a 100% positive agreement and 97.70% negative agreement for 027 (Table 7).

**Table 7. Xpert *C. difficile*/Epi test Performance vs. Direct Culture & PCR-Ribotyping**

Direct Culture & PCR-Ribotyping					
		Toxin B+ 027 +	Toxin B+ 027 -	NEG	Total <sup>a</sup>
<b>Xpert <i>C. difficile</i>/Epi test<sup>b</sup></b>	<b>Toxin B+ 027/NAP1/BI+</b>	74	4	47	125
	<b>Toxin B+ 027/NAP1/BI-</b>	0	164	140	304
	<b>NEG</b>	0	3	1860	1863
	<b>Total</b>	74	171	2047	2292
		<b>Toxigenic <i>C. difficile</i></b>		<b>Toxigenic <i>C. difficile</i> / 027/NAP1/BI</b>	
		Sensitivity:	98.78% (242/245)	Pos Agreement:	100% (74/74)
		Specificity:	90.86% (1860/2047)	Neg Agreement:	97.70% (2167/2218)
		Accuracy:	91.71% (2102/2292)	Accuracy:	97.77% (2241/2292)
		PPV <sup>c</sup> :	56.41% (242/429)	PPV:	59.20% (74/125)
		NPV <sup>d</sup> :	99.84% (1860/1863)	NPV:	100% (2218/2218)

- a. One isolate was not typeable due to contamination; this specimen is not included in the performance statistics.
- b. Xpert results shown are for first or second attempt. Approximately 3.2% of the specimens were indeterminate on the first attempt.
- c. Positive predictive value
- d. Negative predictive value



## Performance vs. Reference Culture

Reference (enriched) culture is a more sensitive method for detection of *C. difficile* in symptomatic patients, for example, it enhances detection of low number of organisms in samples due to prior antibiotic treatment and potential loss of viability due to specimen transport.

Relative to reference culture with REA strain typing, the Xpert *C. difficile*/Epi test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.35% and 94.02%, respectively. The Xpert *C. difficile*/Epi test also demonstrated a 96.51% positive agreement and 98.31% negative agreement for BI (Table 8).

**Table 8. Xpert *C. difficile*/Epi test Performance vs. Reference Culture & REA**

Reference Culture & REA					
		Toxin B+ BI +	Toxin B+ BI -	NEG	Total <sup>a</sup>
Xpert <i>C. difficile</i> /Epi test <sup>b</sup>	Toxin B+ 027/NAP1/BI+	83	6	31	120
	Toxin B+ 027/NAP1/BI-	2	204	86	292
	NEG	1	20	1841	1862
	<b>Total</b>	86	230	1958	2274
		Toxigenic <i>C. difficile</i>		Toxigenic <i>C. difficile</i> / 027/NAP1/BI	
		Sensitivity:	93.35% (295/316)	Pos Agreement:	96.51% (83/86)
		Specificity:	94.02% (1841/1958)	Neg Agreement:	98.31% (2151/2188)
		Accuracy:	93.93% (2136/2274)	Accuracy:	98.24% (2234/2274)
		PPV <sup>c</sup> :	71.60% (295/412)	PPV:	69.17% (83/120)
		NPV <sup>d</sup> :	98.87% (1841/1862)	NPV:	99.86% (2151/2154)

a. 19 specimens were culture positive but were not strain typed for the following reasons: incomplete restriction endonuclease digestion; or the isolate was not sent. These 19 specimens are not included in the performance characteristics above.

b. Xpert results shown are for first or second attempt. Approximately 3.2% of the specimens were indeterminate on the first attempt.

c. Positive predictive value

d. Negative predictive value

Relative to reference culture with PFGE strain typing, the Xpert *C. difficile*/Epi test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.60% and 94.02%, respectively. The Xpert *C. difficile*/Epi test also demonstrated a 97.73% positive agreement and 98.27% negative agreement for NAP1 (Table 9).

Table 9. Xpert *C. difficile*/Epi test Performance vs. Reference Culture & PFGE

Direct Culture & PFGE					
		Toxin B+ NAP1 +	Toxin B+ NAP1 -	NEG	Total <sup>a</sup>
Xpert <i>C. difficile</i> /Epi test <sup>b</sup>	Toxin B+ 027/NAP1/BI+	86	7	31	124
	Toxin B+ 027/NAP1/BI-	1	213	86	300
	NEG	1	20	1841	1862
	<b>Total</b>	88	240	1958	2286
		Toxigenic <i>C. difficile</i>		Toxigenic <i>C. difficile</i> / 027/NAP1/BI	
		Sensitivity:	93.60% (307/328)	Pos Agreement:	97.73% (86/88)
		Specificity:	94.02% (1841/1958)	Neg Agreement:	98.27% (2160/2198)
		Accuracy:	93.96% (2148/2286)	Accuracy:	98.25% (2246/2286)
		PPV <sup>c</sup> :	72.41% (307/424)	PPV:	69.35% (86/124)
		NPV <sup>d</sup> :	98.87% (1841/1862)	NPV:	99.91% (2160/2162)

- a. 7 specimens were culture positive but were not strain typed for the following reasons: incomplete restriction endonuclease digestion; no growth; or contamination. These 7 specimens are not included in the performance characteristics above.
- b. Xpert results shown are for first or second attempt. Approximately 3.2% of the specimens were indeterminate on the first attempt.
- c. Positive predictive value
- d. Negative predictive value

Relative to reference culture with PCR-ribotyping, the Xpert *C. difficile*/Epi test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.39% and 94.02%, respectively. The Xpert *C. difficile*/Epi test also demonstrated a 98.89% positive agreement and 98.36% negative agreement for 027 (Table 10).

Table 10. Xpert *C. difficile*/Epi test Performance vs. Reference Culture & PCR-Ribotyping

Direct Culture & PCR-Ribotyping					
		Toxin B+ 027 +	Toxin B+ 027 -	NEG	Total <sup>a</sup>
Xpert <i>C. difficile</i> /Epi test <sup>b</sup>	Toxin B+ 027/NAP1/BI+	89	5	31	125
	Toxin B+ 027/NAP1/BI-	0	217	86	303
	NEG	1	21	1841	1863
	<b>Total</b>	90	243	1958	2291
		Toxigenic <i>C. difficile</i>		Toxigenic <i>C. difficile</i> / 027/NAP1/BI	
		Sensitivity:	93.39% (311/333)	Pos Agreement:	98.89% (89/90)
		Specificity:	94.02% (1841/1958)	Neg Agreement:	98.36% (2165/2201)



Direct Culture & PCR-Ribotyping				
	Toxin B+ 027 +	Toxin B+ 027 -	NEG	Total <sup>a</sup>
	Accuracy:	93.93% (2152/2291)	Accuracy:	98.38% (2254/2291)
	PPV <sup>c</sup> :	72.66% (311/428)	PPV:	71.20% (89/125)
	NPV <sup>d</sup> :	98.82% (1841/1863)	NPV:	99.95% (2165/2166)

- 2 specimens were culture positive but were not strain typeable due to contamination and are not included in the performance characteristics above.
- Xpert results shown are for first or second attempt. Approximately 3.2% of the specimens were indeterminate on the first attempt.
- Positive predictive value
- Negative predictive value

## Antibiotic Usage

Among the 2293 cases included in the main dataset, antibiotic use within the 2 months prior to sample collection was reported for 1630 and no antibiotic use was confirmed for 570; for 93 cases, antibiotic status was unknown. Antibiotic use did not cause a statistically significant difference in test performance.

## Analytical Performance

### Analytical Sensitivity

Studies were performed to determine the 95% confidence intervals for the analytical limit of detection (LoD) of *C. difficile* diluted into a fecal matrix of human origin that can be detected by the Xpert *C. difficile*/Epi test. The fecal matrix consisted of human liquid feces (*C. difficile* negative by Xpert *C. difficile*/Epi test) diluted in PBS with 15% glycerol. The LoD is defined as the lowest number of colony forming units (CFU) per swab that can be reproducibly distinguished from negative samples with 95% confidence.

Replicates of 20 were evaluated at each *C. difficile* concentration tested (CFU/swab) for 7 different *C. difficile* strains representing toxinotypes 0 (two strains), III (two strains), IV, V and VIII (one of each strain).

The estimate and confidence intervals were determined using logistic regression with data (number of positive results per number of replicates at each level) over the range of CFUs tested. The confidence intervals were determined using maximum likelihood estimates on the logistic model parameters using the large sample variance-covariance matrix. The LoD point estimates and 95% upper and lower confidence intervals for each *C. difficile* toxinotype tested are summarized in [Table 11](#).

**Table 11. 95% Confidence Intervals for Analytical LoD-C.difficile**

Strain ID	Toxinotype	LoD <sub>95%</sub> (CFU/swab)	Lower 95% CI	Upper 95% CI
VPI 10463 (CCUG19126)	0	255	190	632
90556-M6S (ATCC9689)	0	460	419	587
LUMC-1 (027/NAP1/BI) <sup>a</sup>	III	23	19	31
LUMC-5 (027/NAP1/BI) <sup>a</sup>	III	75	45	176
LUMC-7	V	45	34	104





Strain ID	Toxinotype	LoD <sub>95%</sub> (CFU/swab)	Lower 95% CI	Upper 95% CI
LUMC-6	VIII	60	50	74
9101	XII	41	34	49

a. By PCR-ribotyping/pulsed-field gel electrophoresis/restriction endonuclease analysis

The results of this study indicate that the Xpert *C. difficile*/Epi test will produce a positive *C. difficile* result 95% of the time for a fecal sample containing 460 CFU/swab and a presumptive positive 027/NAP1/BI result 95% of the time for a swab containing 75 CFU.

In addition to the LoD determination, eighteen *C. difficile* strains representing toxinotypes 0 plus 12 variant toxinotypes, including four 027/NAP1/BI toxinotype III isolates, were tested using the Xpert *C. difficile*/Epi test. *C. difficile* strains were selected to broadly represent the majority of *C. difficile* toxinotypes encountered in practice. Stock cultures were prepared by suspending the bacterial growth from agar plates in PBS buffer containing 15% glycerol. The concentration of each stock was adjusted to 1.4-5.9 McFarland units. All strains were serially diluted to approximately 900 CFU/swab and tested in triplicate.

Under the conditions of this study, the Xpert *C. difficile*/Epi test correctly identified all 18 strains tested as **Toxigenic C. diff POSITIVE**. Included in the panel were 8 toxinotypes reported to be positive for binary toxin (CDT) production as well. All were CDT positive using the Xpert *C. difficile*/Epi test. All four 027/NAP1/BI isolates representing toxinotype III were correctly identified as **Toxigenic C. diff POSITIVE; 027-NAP1-BI PRESUMPTIVE POSITIVE**.

## Analytical Specificity

Fifty-five (55) strains were collected, quantitated and tested using the Xpert *C. difficile*/Epi test. The strains originated from the American Type Culture Collection (ATCC), Culture Collection University of Göteborg (CCUG), German Collection of Microorganisms and Cell Cultures (DSMZ), the Centers for Disease Control and Prevention (CDC), the Institute of Public Health, Maribor, Slovenia and Swedish Institute for Infectious Disease Control (SMI).

Of the tested species, ten (10) non-toxigenic *C. difficile* strains and eleven (11) non-*C. difficile* Clostridiodes species were included. The organisms tested were identified as either Gram-positive (37) or Gram-negative (18). The organisms were further classified as aerobic (24), anaerobic (29) or microaerobic (2).

Each strain was tested in triplicate at concentrations ranging from  $1.1 \times 10^8$  to  $2.2 \times 10^{10}$  CFU/swab. Positive and negative controls were included in the study. Under the conditions of the study, all isolates were reported **Toxigenic C. diff NEGATIVE; 027-NAP1-BI PRESUMPTIVE NEG**. The analytical specificity was 100%.

## Interfering Substances

Twenty-one (21) biological and chemical substances occasionally used or found in stool specimens were tested for interference with the Xpert *C. difficile*/Epi test. Potentially interfering substances include, but are not limited to, Vagisil cream and zinc oxide paste. The 19 substances listed in [Table 12](#) showed no detectable interference with the Xpert *C. difficile*/Epi test.

**Table 12. Substances Tested and Showing No Test Interference**

Substance	Substance
Whole Blood Karolinska University Hospital	K-Y Jelly/Gelée® McNeil-PPC
Mucin (porcine) Sigma	Vaseline Unilever
Kaopectate® Chatterm	Dulcolax® Boehringer Ingelheim Pharmaceuticals
Imodium® McNeil-PPC	Preparation H Portable Wipes Wyeth Consumer Healthcare
Pepto-Bismol® Procter & Gamble	Vaginal Contraceptive Film (VCF) Apothecus Pharmaceutical
Preparation H® Wyeth Consumer Healthcare	Vancomycin Fluka
Fleet® CB Fleet Company	Metronidazole Actavis
Fecal fats Karolinska University Hospital	Anusol® Plus TM Warner-Lambert Company
Monistat® McNeil-PPC	E-Z-HD™ High Density Barium Sulfate for suspension E-Z-EM Canada
Hydrocortisone Cream Longs Drugs	

## Reproducibility

A panel of 7 specimens with varying concentrations of toxigenic *C. difficile* and *C. difficile*, 027/NAP1/BI were tested on 10 different days by two different operators at each of the three sites (7 specimens x 2 operators/ day x 10 days x 3 sites). One lot of Xpert *C. difficile*/Epi test was used at each of the 3 testing sites. Xpert *C. difficile*/Epi tests were performed according to the Xpert *C. difficile*/Epi test procedure. Results are summarized in [Table 13](#) and [Table 14](#).

**Table 13. Summary of Reproducibility Results (all)**

Specimen ID	% Agreement <sup>a</sup>			% Total Agreement by Sample
	Site 1	Site 2	Site 3	
Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic <i>C. difficile</i> High Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic <i>C. difficile</i> Low Positive	100% (20/20)	85% (17/20)	85% (17/20)	90% (54/60)



Specimen ID	% Agreement <sup>a</sup>			% Total Agreement by Sample
	Site 1	Site 2	Site 3	
Toxigenic <i>C. difficile</i> Moderate Positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
027/NAP1/BI High Negative	100% (20/20)	95% (19/20)	95% (19/20)	96.7% (58/60)
027/NAP1/BI Low Positive	100% (20/20)	95% (19/20)	95% (19/20)	96.7% (58/60)
027/NAP1/BI Moderate Positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
% Total Agreement by Site	100% (140/140)	97.1% (136/140)	97.1% (136/140)	98.1% (412/420)

a. For negative and high negative samples, %Agreement = (# negative results/total samples run); for low and moderate positive samples, %Agreement = (# positive results/total samples run).

**Table 14. Summary of Ct Value Results by Sample Level and Probes**

SPC			
Level	Ave	StdDev	CV
Toxigenic <i>C. diff</i> high neg	32.17	0.59	1.83%
Toxigenic <i>C. diff</i> low pos	32.14	0.53	1.66%
Toxigenic <i>C. diff</i> mod pos	31.98	0.47	1.47%
027/NAP1/BI high neg	32.11	0.65	2.03%
027/NAP1/BI low pos	31.93	0.72	2.26%
027/NAP1/BI mod pos	31.96	0.61	1.90%
Neg	32.26	0.72	2.22%
tcdB (Toxin B)			
Level	Ave	StdDev	CV
Toxigenic <i>C. diff</i> high neg	39.59	0.70	1.77%
Toxigenic <i>C. diff</i> low pos	35.88	0.81	2.24%
Toxigenic <i>C. diff</i> mod pos	32.17	0.45	1.39%
027/NAP1/BI high neg	39.11	0.98	2.50%
027/NAP1/BI low pos	35.49	0.58	1.65%
027/NAP1/BI mod pos	32.10	0.63	1.97%

An additional panel of 6 specimens, three negative and three toxigenic *C. difficile* high negative, were tested on 5 different days by two different operators at each of the three sites (6 specimens x 2 operators/ day x 5 days x 3 sites). The high negative specimens were prepared at a concentration below LoD such that they were expected to give a negative result 20 to 80% of the time. One lot of Xpert *C. difficile*/Epi test was used at each of the 3 testing sites. Xpert *C. difficile*/Epi tests were performed according to the Xpert *C. difficile*/Epi test procedure. Results are summarized in [Table 15](#).

**Table 15. Summary of Additional Reproducibility Specimen Results**

Specimen ID	% Agreement <sup>a</sup>			% Total Agreement by Sample
	Site 1	Site 2	Site 3	
Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Toxigenic <i>C. difficile</i> High Negative <sup>b</sup>	60.0% (18/30) <sup>b</sup>	60.0% (18/30) <sup>b</sup>	53.3% (16/30) <sup>b</sup>	57.8% (52/90) <sup>b</sup>

a. (# negative results / total high negative samples run)

b. 20-80% agreement expected for high negative sample

# ? Appendix

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## Table of Symbols

Symbol	Meaning
	Catalog number
	For prescription use only
	<i>In vitro</i> diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for $n$ tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Warning



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## Revision History

**Description of Changes:** 303-0936, Rev. A to B

**Purpose:** Add retest procedure

Section	Description of Change
Results	Add retest procedure back into the test IFU. This was deleted by mistake in last revision.