

# Xpert<sup>®</sup> *C.difficile/Epi*

**REF** GXCDIFF/EPI-10

**REF** GXCDIFF/EPI-120

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# Xpert<sup>®</sup> *C.difficile*/Epi

For *In Vitro* Diagnostic Use.



## 1 Proprietary Name

Xpert<sup>®</sup> *C.difficile*/Epi

## 2 Common or Usual Name

Xpert *C.difficile*/Epi Assay

## 3 Intended Use

The Cepheid Xpert<sup>®</sup> *C. difficile*/Epi Assay 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 *Clostridium 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 Cepheid GeneXpert Dx System 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 Assay 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 Assay 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.

## 4 Summary and Explanation

*Clostridium difficile* (*C. difficile*) is a Gram-positive, spore-forming anaerobic bacillus that was first linked to disease in 1978.<sup>1</sup> *Clostridium difficile* infection (CDI) ranges from 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+) strains 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 027, PFGE type NAP1 and REA type BI.<sup>8,12</sup> 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.<sup>13,14</sup> 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.<sup>15,16</sup> 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.<sup>17</sup>

## 5 Principle of the Procedure

The GeneXpert Dx System automates and integrates sample purification, 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) assays. (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 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 eliminated. For a full description of the system, see the *GeneXpert Dx System Operator Manual*.

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 Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Cepheid Xpert *C. difficile/Epi* Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection of toxin producing *Clostridium difficile* directly from unformed (liquid or soft) stool specimens of patients suspected of having *Clostridium difficile* infection (CDI). 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 (*tcdCA117*). The combined presence of the genes encoding toxin B and binary toxin and the *tcdCA117* deletion have been associated with a hypervirulent *C. difficile* strain known as 027/NAP1/BI, which has been associated with severe disease outbreaks in healthcare facilities worldwide.<sup>12,13,15</sup> The assay is performed on the Cepheid GeneXpert Dx System.

## 6 Reagents and Instruments

### 6.1 Materials Provided



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

The kits contain the following:

<b>Xpert C. difficile/Epi Assay Cartridges with integrated reaction tubes</b>	<b>10</b>	<b>120</b>
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	• 1 of each per cartridge	• 1 of each per cartridge
• Reagent 1	• 3.0 mL per cartridge	• 3.0 mL per cartridge
• Reagent 2 (Sodium Hydroxide)	• 3.0 mL per cartridge	• 3.0 mL per cartridge
<b>Xpert C. difficile/Epi Assay Reagent pouch</b>	<b>1 per kit</b>	<b>1 per kit</b>
• Sample Reagent (Guanidinium thiocyanate)	• 10 x 2.0 mL per vial	• 120 x 2.0 mL per vial
<b>CD</b>	<b>1 per kit</b>	<b>1 per kit</b>
• Assay Definition File (ADF)		
• Instructions to import ADF into GX software		
• Instructions for Use (Package Insert)		

**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.

## 7 Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer with GeneXpert proprietary software version 4.3 or higher, hand-held barcode scanner, and *GeneXpert Dx System 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® Culture, 139CFA) or Cepheid Single-Use Disposable Swab catalog number SDPS-120 (Copan 138CS01.PH)
- Disposable transfer pipettes

## 8 Materials Available but Not Provided

KWIK-STIKs™ 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.

## 9 Warnings and Precautions

- For *in vitro* diagnostic use.
- For prescription use only.
- Results from Xpert *C. difficile*/Epi Assays 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 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 after removing it from the packaging.
- 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.
- 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.



**10 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 content and/or container in accordance with local, regional, national, and/or international regulations.

**11 Storage and Handling**

- Store the Xpert *C. difficile/Epi* Assay 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.

**12 Specimen Collection and Transport**

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 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.

**13 Procedure****13.1 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 Assay cartridge.
7. Close the cartridge lid.

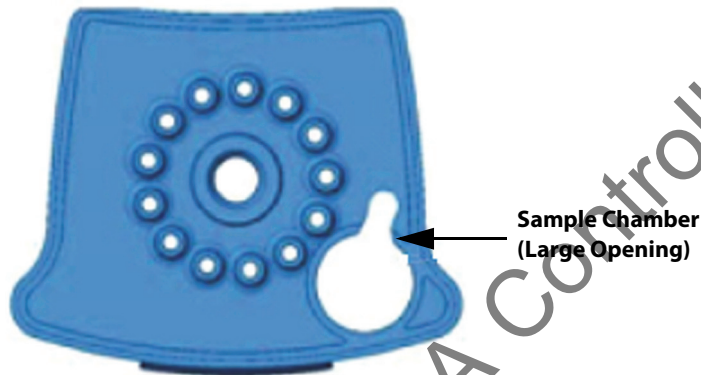


Figure 1. Xpert *C. difficile*/Epi cartridge (top view).

### 13.2 Starting the Test

**Important** Before you start the test, make sure the Xpert *C. difficile*/Epi assay definition is imported into the GeneXpert software.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual*.

**Note** The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Turn on the GeneXpert Dx instrument and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on to the GeneXpert Dx System software using your user name and password.
3. In the GeneXpert Dx System window, click **Create Test**. The **Create Test** window opens.
4. Scan in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the **View Results** window.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the **View Results** window.
6. Scan the barcode on the Xpert *C. difficile*/Epi Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

**Note** If the barcode on the Xpert *C. difficile*/Epi Assay cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test**. In the dialog box that appears, type your password.
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.
11. Dispose of the used cartridges in an appropriate specimen waste container according to your institution's standard practices.

## 14 Viewing and Printing Results

**CONTROL** For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual*.

## 15 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 contains spores of *Bacillus globigii* in the form of a dry spore cake that is included in each cartridge to verify 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 assay. 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 Dx System measures 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.

## 16 Interpretation of Results

The results are interpreted by the GeneXpert Dx System 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 Assay Possible Results**

Results				Interpretations	Example <sup>a</sup>
Toxin B	Binary Toxin	tcdC	SPC		
+	+	+	+/-	Toxigenic <i>C. diff</i> POSITIVE 027-NAP1-BI PRESUMPTIVE POSITIVE	Figure 2
+	+	-	+/-	Toxigenic <i>C. diff</i> POSITIVE 027-NAP1-BI PRESUMPTIVE NEGATIVE	Figure 3
	-	+	+/-		
	-	-	+/-		
-	+	+	+	Toxigenic <i>C. diff</i> NEGATIVE 027-NAP1-BI PRESUMPTIVE NEGATIVE	Figure 4
	+	-	+		
	-	+	+		
	-	-	+		

a. Sample screenshots are provided for positive and negative *C. difficile*/Epi results and an INVALID result. See Figure 2 through Figure 5. ERROR and NO RESULT examples not shown.



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

Result	Interpretation
<b>Toxigenic C.diff POSITIVE; 027 PRESUMPTIVE POSITIVE</b>  <b>(Figure 2)</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) <b>AND</b> both presumptive 027/NAP1/BI targets (Binary Toxin and <i>tcdC</i>Δ117) 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 "Toxigenic <i>C. diff</i> POSITIVE; 027 PRESUMPTIVE POSITIVE" (Figure 2) using the Xpert <i>C. difficile/Epi</i> Assay.</p>
<b>Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE</b>  <b>(Figure 3)</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>tcdC</i>Δ117) 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>  <b>(Figure 4)</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>tcdC</i>Δ117 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>  <b>(Figure 5)</b>	<p>Presence or absence of <i>C. difficile</i> target DNA cannot be determined. Repeat test according to the instructions in the Retest Procedure 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>tcdC</i>Δ117 — NO RESULT</li> </ul> <p>Probe Check — FAIL*; one or more of the probe check results fail.</p> <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>tcdC</i>Δ117 — NO RESULT</li> <li>Probe Check — NA (not applicable)</li> </ul>

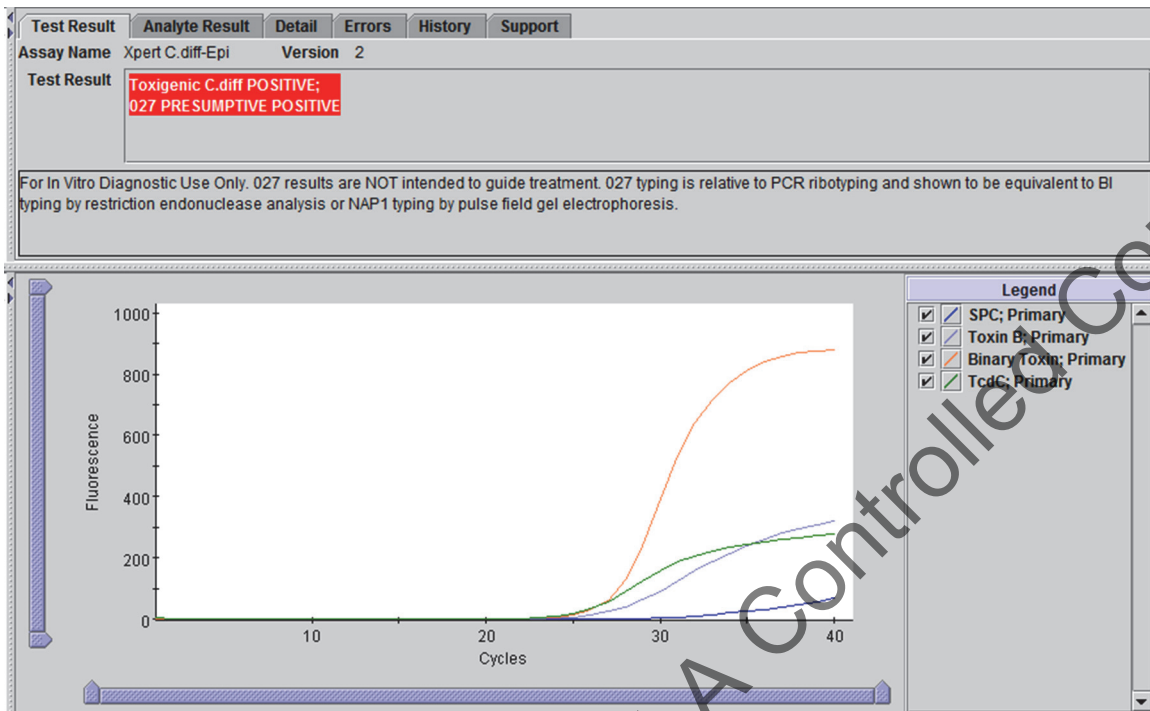


Figure 2. An Example of a Toxigenic C.diff POSITIVE; 027 PRESUMPTIVE POSITIVE Result

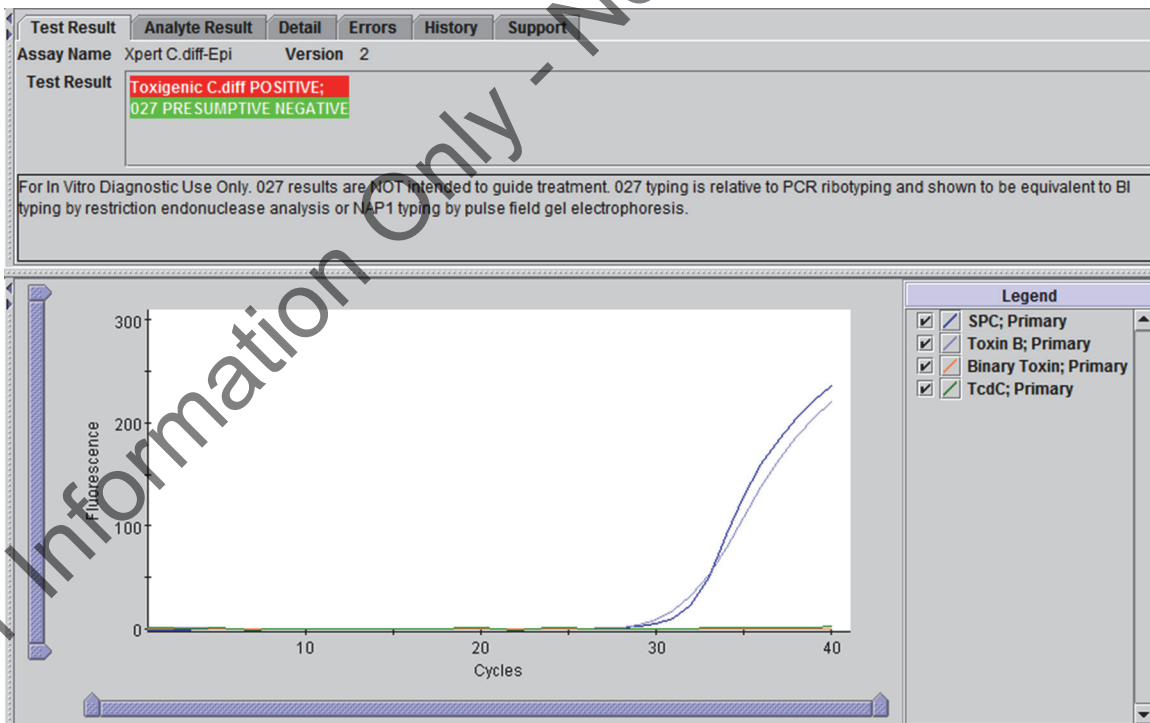


Figure 3. An Example of Toxigenic C.diff POSITIVE; 027 PRESUMPTIVE NEGATIVE Result

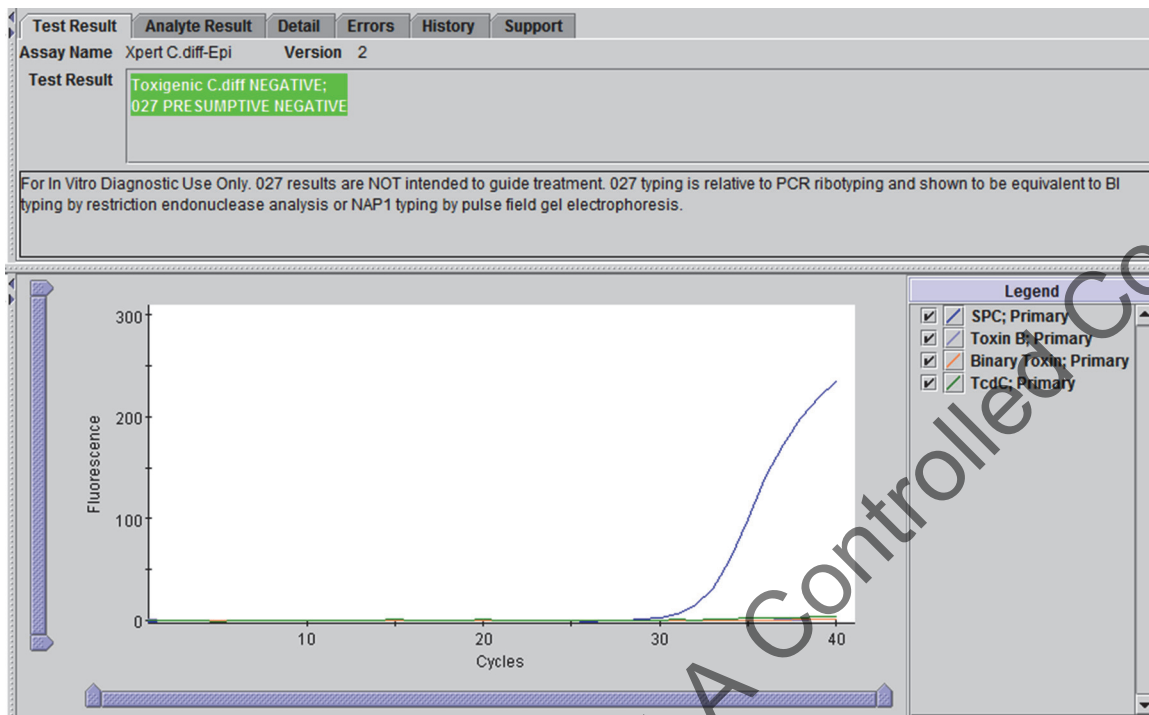


Figure 4. An Example of a Toxigenic C.diff NEGATIVE; 027 PRESUMPTIVE NEGATIVE Result

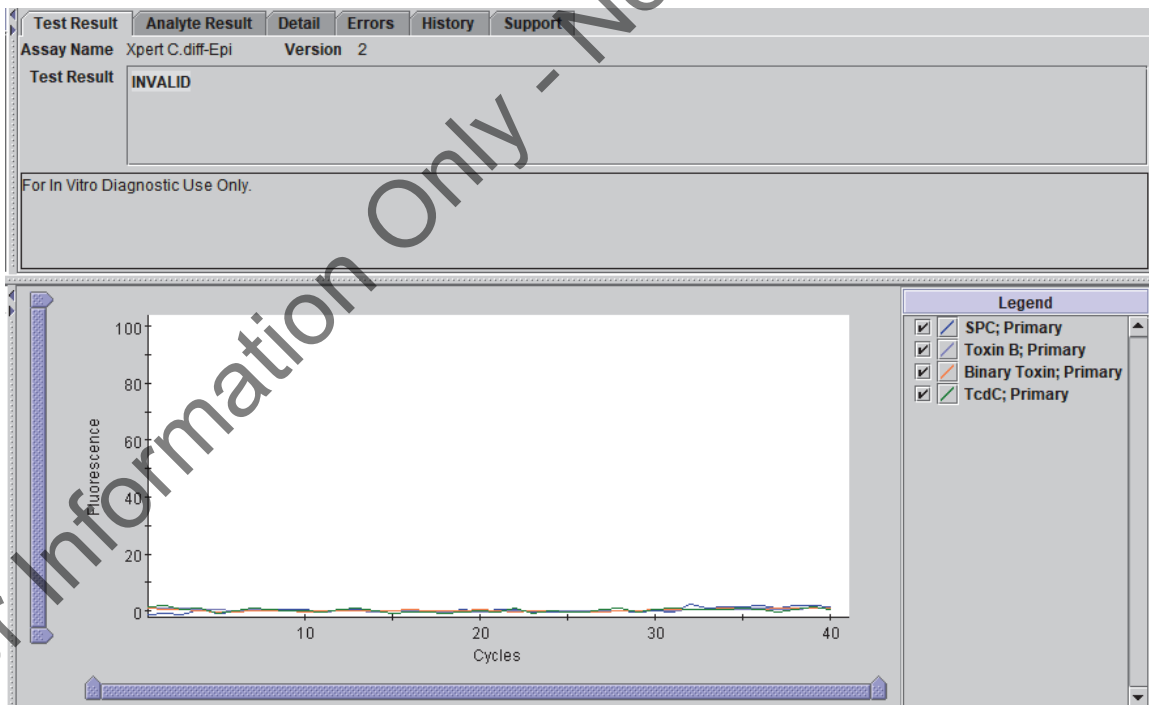


Figure 5. An Example of an INVALID Result

## 17 Reasons to Repeat the Assay

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.

### 17.1 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 Assay 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.

## 18 Limitations

- 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.
- 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 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.

## 19 Expected Values

In the Xpert *C. difficile*/Epi Assay 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.

## 20 Performance Characteristics

### 20.1 Clinical Performance

Performance characteristics of the Xpert *C. difficile*/Epi Assay were determined in a multi-site prospective investigation study at seven US and Canadian institutions by comparing the Xpert *C. difficile*/Epi Assay 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 Assay. 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 Assay 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.

**20.2 Overall Results**

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

**20.3 Performance vs. Direct Culture**

Relative to direct culture with REA strain typing, the Xpert C. difficile/Epi Assay demonstrated a sensitivity and specificity for toxigenic C. difficile of 98.72% and 90.86%, respectively. The Xpert C. difficile/Epi Assay also demonstrated a 98.55% positive agreement and 97.65% negative agreement for BI (Table 5)

**Table 5. Xpert C.difficile/Epi Assay Performance vs. Direct culture & REA**

Direct Culture & REA					
		Toxin B+ BI +	Toxin B+ BI -	NEG	Total <sup>a</sup>
Xpert C.diff/Epi <sup>b</sup>	Toxin B+ 027/NAP1/BI+	68	5	47	120
	Toxin B+ 027/NAP1/BI-	1	158	140	299
	NEG	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 Assay demonstrated a sensitivity and specificity for toxigenic C. difficile of 98.76% and 90.86%, respectively. The Xpert C. difficile/Epi Assay also demonstrated a 100% positive agreement and 97.61% negative agreement for NAP1 (Table 6).

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

Direct Culture & PFGE					
		Toxin B+ NAP1 +	Toxin B+ NAP1 -	NEG	Total <sup>a</sup>
Xpert C.diff/ Epi <sup>b</sup>	Toxin B+ 027/NAP1/BI+	71	6	47	124
	Toxin B+ 027/NAP1/BI-	0	161	140	301
	NEG	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)
		Specificity:	90.86% (1860/2047)	Neg Agreement:	97.61% (2163/2216)

Table 6. Xpert C.difficile/Epi Assay Performance vs. Direct Culture &amp; PFGE

Direct Culture & PFGE				
	Toxin B+ NAP1 +	Toxin B+ NAP1 -	NEG	Total <sup>a</sup>
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 Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 98.78% and 90.86%, respectively. The Xpert *C. difficile*/Epi Assay also demonstrated a 100% positive agreement and 97.70% negative agreement for 027 (Table 7).

Table 7. Xpert C.difficile/Epi Assay Performance vs. Direct Culture &amp; PCR-Ribotyping

Direct Culture & PCR-Ribotyping					
		Toxin B+ 027 +	Toxin B+ 027 -	NEG	Total <sup>a</sup>
Xpert C.diff/ Epi <sup>b</sup>	Toxin B+ 027/NAP1/BI+	74	4	47	125
	Toxin B+ 027/NAP1/BI-	0	164	140	304
	NEG	0	3	1860	1863
	Total	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

**20.4 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 Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.35% and 94.02%, respectively. The Xpert *C. difficile*/Epi Assay also demonstrated a 96.51% positive agreement and 98.31% negative agreement for BI (Table 8).

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

Reference Culture & REA					
		Toxin B+ BI +	Toxin B+ BI -	NEG	Total <sup>a</sup>
Xpert <i>C. diff</i> /Epi <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 Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.60% and 94.02%, respectively. The Xpert *C. difficile*/Epi Assay also demonstrated a 97.73% positive agreement and 98.27% negative agreement for NAP1 (Table 9).

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

		Reference Culture & PFGE			
		Toxin B+ NAP1 +	Toxin B+ NAP1 -	NEG	Total <sup>a</sup>
Xpert <i>C. diff</i> /Epi <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>	<b>88</b>	<b>240</b>	<b>1958</b>	<b>2286</b>
		<b>Toxigenic <i>C. difficile</i></b>		<b>Toxigenic <i>C. difficile</i> / 027/NAP1/BI</b>	
		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 Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.39% and 94.02%, respectively. The Xpert *C. difficile*/Epi Assay also demonstrated a 98.89% positive agreement and 98.36% negative agreement for 027 (Table 10).

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

Reference Culture & PCR-Ribotyping					
		Toxin B+ 027 +	Toxin B+ 027 -	NEG	Total <sup>a</sup>
Xpert <i>C. diff</i> /Epi <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>	<b>90</b>	<b>243</b>	<b>1958</b>	<b>2291</b>
<b>Toxigenic <i>C. difficile</i></b>			<b>Toxigenic <i>C. difficile</i> / 027/NAP1/BI</b>		
Sensitivity: 93.39% (311/333)			Pos Agreement: 98.89% (89/90)		
Specificity: 94.02% (1841/1958)			Neg Agreement: 98.36% (2165/2201)		
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)		

- a. 2 specimens were culture positive but were not strain typeable due to contamination and 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

## 21 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 assay performance.

## 22 Analytical Specificity

Fifty-five (55) strains were collected, quantitated and tested using the Xpert *C. difficile*/Epi Assay. 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* Clostridium 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%.

## 23 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* Assay. The fecal matrix consisted of human liquid feces (*C. difficile* negative by Xpert *C. difficile/Epi* Assay) 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*s

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
LUMC-6	VIII	60	50	74
9101	XII	41	34	49

<sup>a</sup>By PCR-ribotyping/pulsed-field gel electrophoresis/restriction endonuclease analysis

The results of this study indicate that the Xpert *C. difficile/Epi* Assay 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* Assay. *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* Assay 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* Assay. All four 027/NAP1/BI isolates representing toxinotype III were correctly identified as **Toxigenic C. diff POSITIVE; 027-NAP1-BI PRESUMPTIVE POSITIVE**.

## 24 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 Assay. Potentially interfering substances include, but are not limited to, Vagisil cream and zinc oxide paste (see Section 18, Limitations). The 19 substances listed in Table 12 showed no detectable interference with the Xpert *C. difficile*/Epi Assay.

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

Substance	Substance
Whole Blood Karolinska University Hospital	K-Y Jelly/Gelée® McNeil-PPC
Mucin (porcine) Sigma	Vaseline Unilever
Kaopectate® Chattem	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-HDTM High Density Barium Sulfate for suspension E-Z-EM Canada
Hydrocortisone Cream Longs Drugs	

## 25 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 Assay was used at each of the 3 testing sites. Xpert *C. difficile*/Epi Assays were performed according to the Xpert *C. difficile*/Epi Assay 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)	90.0% (54/60)
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)	100% (20/20)	100% (20/20)	100% (60/60)
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 Assay was used at each of the 3 testing sites. Xpert *C. difficile*/Epi Assays were performed according to the Xpert *C. difficile*/Epi Assay 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

## 26 References

1. Larson HE, Price AB, Honour P, Borriello SP. *Clostridium difficile* and the aetiology of pseudomembranous colitis. *Lancet* 1978;1:1063-1066.
2. Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. *N Engl J Med* 2002; 31:334-339
3. Borriello SP. The influence of the normal flora on *Clostridium difficile* colonization of the gut. *Ann Med* 1990;22:61-67.
4. Bignardi GE. Risk factors for *Clostridium difficile* infection. *J Hosp Infect.* 1998; 40:1-15.
5. Kelly CP, Pothoulakis C, Lamont JT. *Clostridium difficile* colitis. *N Engl J Med* 1994; 330:257-262.
6. 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.
7. Hammond GA, Johnson JL. The toxigenic element of *Clostridium difficile* strain VPI 10463. *Microb Pathog.* 1995;19:203-213.
8. 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.
9. Gonçalves C, Decré D, Barbut F, et al. Prevalence and characterization of a binary toxin (actin-specific ADP-ribosyltransferase) from *Clostridium difficile*. *J Clin Microbiol.* 2004;42:1933-1939
10. 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.
11. Popoff MR, Rubin EJ, Gill DM, Boquet P. Action-specific ADP-ribotransferase produced by a *Clostridium difficile* strain. *Infect Immun.* 1988;56:2299-2306.
12. 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.
13. 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.
14. 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.
15. 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.
16. Wilkins TD, Lyerly DM. *Clostridium difficile* testing: after 20 years, still challenging. *Clin Microbiol.* 2003 Feb;41:531-534.
17. Delmee M. Laboratory diagnosis of *Clostridium difficile* disease. *Clin Microbiol Infect.* 2001;7:411-416.
18. Poutanen SM, Simor AE. *Clostridium difficile*-associated diarrhea in adults. *CMAJ.* 2004;171:51-58.
19. 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.
20. 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).
21. 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.
22. 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.
23. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
24. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

## 27 Cepheid Headquarters Locations

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## 28 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

### Contact Information

#### United States

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Email: techsupport@cepheid.com

#### France
















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Contact information for all Cepheid Technical Support offices is available on our website:

[www.cepheid.com/en/CustomerSupport](http://www.cepheid.com/en/CustomerSupport)



## 29 Table of Symbols

Symbol	Meaning
	Catalog number
	<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
	For prescription use only
	Temperature limitation
	Biological risks
	Warning



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