GeneXpert
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Xpert® *C. difficile*

REF **GXCDIFFICILE-10**

REF **GXCDIFFICILE-120**

Cepheid® ***In Vitro*** **Diagnostic Medical Device** IVD

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Xpert® *C. difficile*

For *In Vitro* Diagnostic Use Only.

℞ only

1 Proprietary Name

Xpert® *C. difficile*

2 Common or Usual Name

Xpert *C. difficile* Assay

3 Intended Use

The Cepheid Xpert *C. difficile* Assay, performed on the Cepheid GeneXpert® Dx System, is a qualitative *in vitro* diagnostic test for rapid detection of toxin B gene sequences from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI). The test utilizes automated real-time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Xpert *C. difficile* Assay is intended as an aid in the diagnosis of CDI. 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.[1] *Clostridium difficile* infection (CDI) ranges from diarrhea to severe life-threatening pseudomembranous colitis.[2] Mature colonic bacterial flora in a healthy adult is generally resistant to *C. difficile* colonization.[3] However, if the normal colonic flora is altered, resistance to colonization is lost. The most common risk factor is exposure to antibiotics.[4] *C. difficile's* primary virulence factor is cytotoxin B.[5] The genes coding for toxin A (*tcdA*; the enterotoxin) and toxin B (*tcdB*) are parts of the pathogenicity locus (PaLoc).[6,7] 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.[8] 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.[9-11]

In the last several years, there have been outbreaks of CDI caused by "hypervirulent" and fluoroquinolone-resistant strains belonging to PCR ribotype 027, PFGE type NAP1 and REA type BI.[8,12] These strains 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.[13,14]

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.[15,16] 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.[17]

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 RT-PCR assays. 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* Assay includes reagents for the detection of toxigenic *C. difficile*, as well as a Sample Processing Control (SPC). 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* 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 assay is performed on the Cepheid GeneXpert Dx System.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert *C. difficile* kit (GXCDIFFICILE-10) contains sufficient reagents to process 10 specimens or quality control samples. The Xpert *C. difficile* kit (GXCDIFFICILE-120) contains sufficient reagents to process 120 specimens or quality control samples.

The kit contains the following:

Xpert *C. difficile* Assay Cartridges with Integrated Reaction Tubes	**10**	**120**
• **Bead 1, Bead 2 and Bead 3 (freeze-dried)**	**1 of each per cartridge**	**1 of each per cartridge**
• **Reagent 1 (Sodium Hydroxide)**	**3.0 mL per cartridge**	**3.0 mL per cartridge**
• **Reagent 2**	**3.0 mL per cartridge**	**3.0 mL per cartridge**
• **Xpert *C. difficile* Reagent Pouch**	**1**	**1**
• Sample Reagent (guanidinium thiocyanate)	10 x 2.0 mL per vial	120 x 2.0 mL per vial
• **CD**	**1 per kit**	**1 per kit**
• Assay Definition Files (ADF)		
• Instructions to import ADF into GeneXpert software		
• Instructions for Use (Package Insert)		

Note Safety Data Sheets (SDS) for all reagents provided in this assay are available upon request from Cepheid Technical Support, and are available on Cepheid's websites (www.cepheid.com and www.cepheidinternational.com).

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 proprietary software, hand-held barcode scanner, and Operator Manual
- Printer: If a printer is required, contact your Cepheid sales representative to arrange for the purchase of a recommended printer.
- Vortex mixer
- Dry swab for transfer of the specimen, such as the swab found in the Cepheid Sample Collection Device (Cepheid Catalog Number: 900-0370), Cepheid Single-Use Disposable Swab (Cepheid Catalog Number SDPS-120), or the Copan Dual Swab and Transport System (139CFM LQ STUART).
- 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.

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

9 Warnings and Precautions

9.1 General

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[19] and the Clinical and Laboratory Standards Institute.[20]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Performance characteristics were not established for patients less than 2 years of age.
- The Xpert *C. difficile* 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* Assay reagents with other reagents.
- Do not open the Xpert *C. difficile* Assay cartridge lid except when adding sample and reagents or performing a retest.
- Do not use a cartridge that has been dropped or shaken after you have added the sample and reagents.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert *C. difficile* 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.
- Store the Xpert *C. difficile* Assay kit at 2 – 28 °C.

9.2 Storage and Handling

- Store the Xpert *C. difficile* Assay cartridges at 2–28 °C, until the expiration date provided on the package label.
- 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 open a cartridge lid until you are ready to perform testing.

9.3 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 stores at 2 – 8 °C. Alternatively, specimens can be kept at room temperature (20 – 30 °C) for up to 24 hours.

10 Chemical Hazards[21,22]

- UN GHS Hazard Pictogram:
- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - Causes skin irritation
  - Causes serious eye irritation
- **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 Procedure

11.1 Preparing the Cartridge

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

To add the sample to the cartridge:

1. Remove the cartridge and 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* Assay cartridge (Figure 1).
7. Close the cartridge lid.

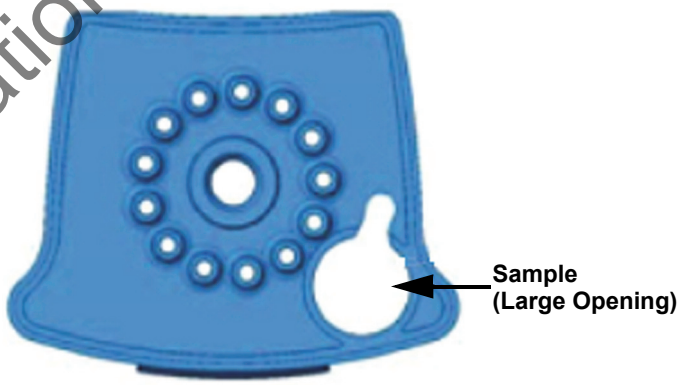

Figure 1. Xpert *C. difficile* Cartridge (Top View)

11.2 Starting the Test

Important **Before starting the test, make sure that the Xpert C. difficile Assay Definition File is imported into the software. This section lists the basic steps of running the test. 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 computer, and then turn on the GeneXpert Dx instrument.
2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.
3. Log on to the GeneXpert Dx System software using your user name and password.
4. In the GeneXpert Dx System window, click **Create Test**. The Scan Cartridge Barcode dialog box appears.
5. Scan the barcode on the Xpert *C. difficile* Assay cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
6. In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the View Results window and all the reports.
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.

12 Viewing and Printing Results

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

13 Quality Control

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** — External controls may be used in accordance with local, state, federal accrediting organizations, as applicable.

14 Interpretation of Results

The results are interpolated 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:

Table 1. Xpert *C. Difficile* Assays and Interpretations

Result	Interpretation
Toxigenic *C. difficile* POSITIVE (Figure 2)	Toxin producing *C. difficile* target DNA sequences are detected. • The toxin producing C. difficile target(s) have Cts within the valid range and endpoint above the minimum setting. • SPC - NA (not applicable), SPC is ignored since *C. difficile* target amplification may compete with this control • Probe Check - PASS; all probe check results pass.
Toxigenic *C. difficile* NEGATIVE (Figure 3)	*C. difficile* target DNA sequences are not detected. • Toxins producing C. difficile targets not detected. • SPC - PASS; SPC has a Ct within the valid range and endpoint above the endpoint minimum setting. • Probe Check - PASS; all probe check results pass.
INVALID (Figure 4)	Presence or absence of *C. difficile* target DNA cannot be determined. Repeat test according to the instructions in the Retest Procedure section below. • SPC - FAIL; SPC target result is negative and the SPC Ct is not within valid range and endpoint below minimum setting. • Probe Check - PASS; all probe check results pass.
ERROR	Presence or absence of *C. difficile* cannot be determined. Repeat test according to instructions in the section below. • Toxin producing *C. difficile* targets - NO RESULT • Probe Check - FAIL*, one or more of the probe check results fail. *If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range.
NO RESULT	Presence or absence of *C. difficile* cannot be determined. Repeat test according to instructions in the section below. • Toxin producing *C. difficile* targets - NO RESULT • Probe Check - NA (not applicable)

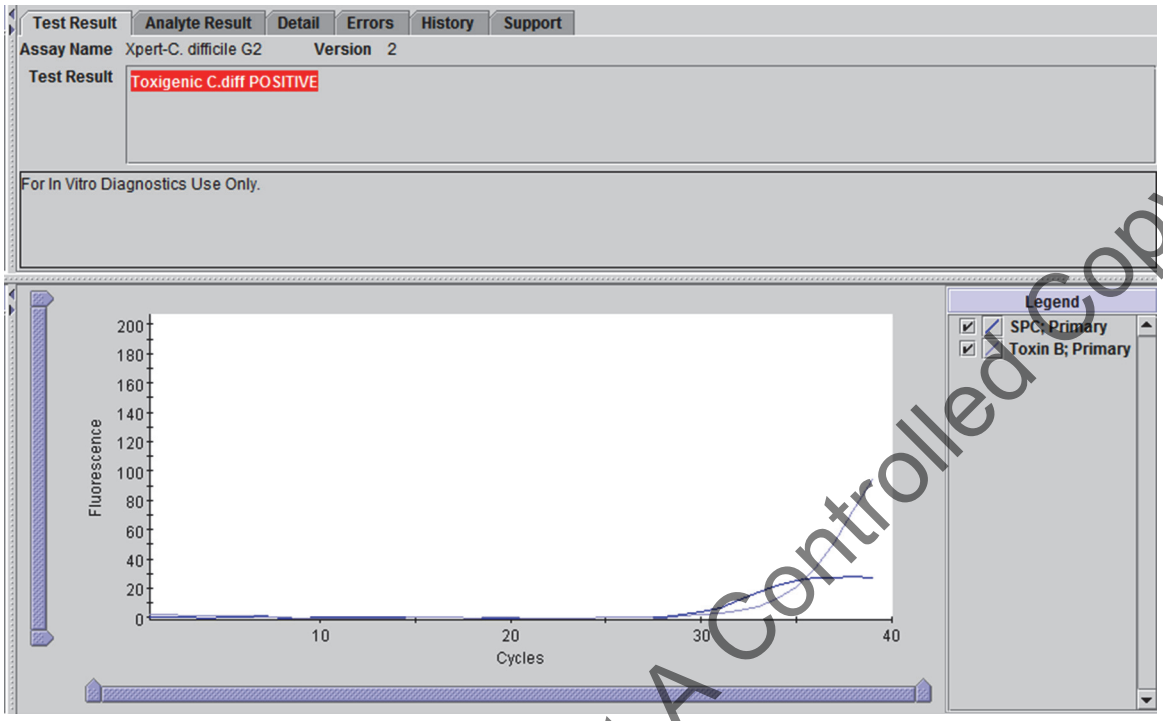

Figure 2. An Example of a Toxigenic *C. difficile* POSITIVE Result

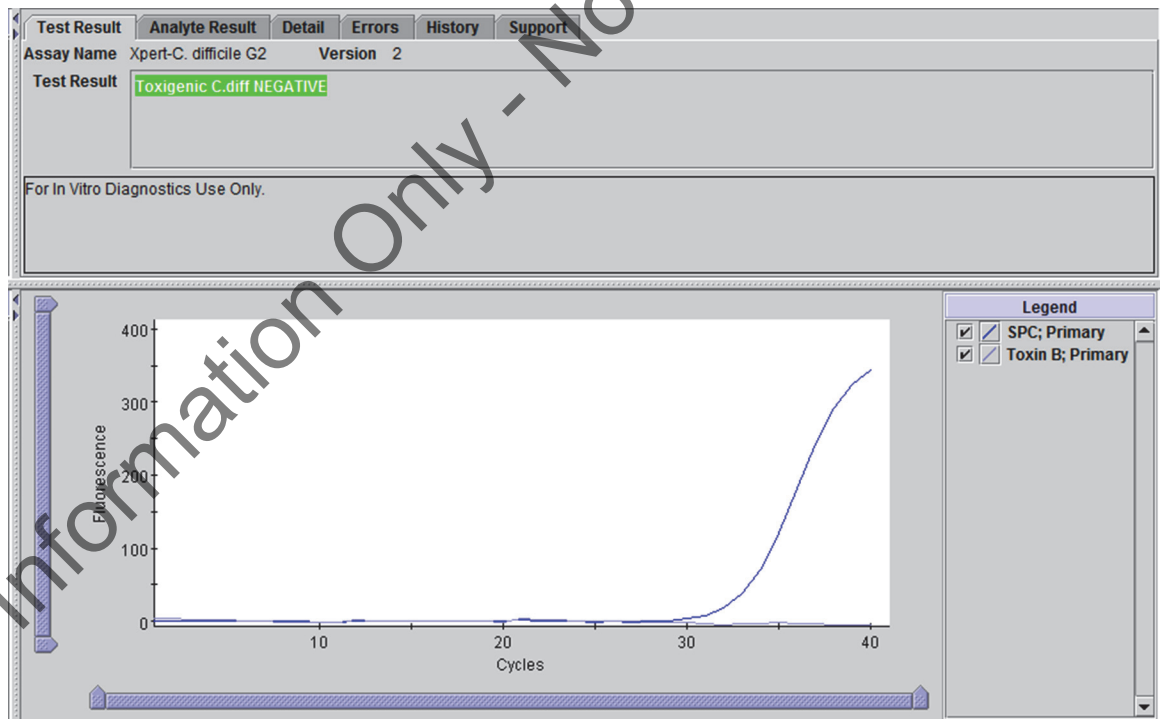

Figure 3. An Example of a Toxigenic *C. difficile* NEGATIVE Result

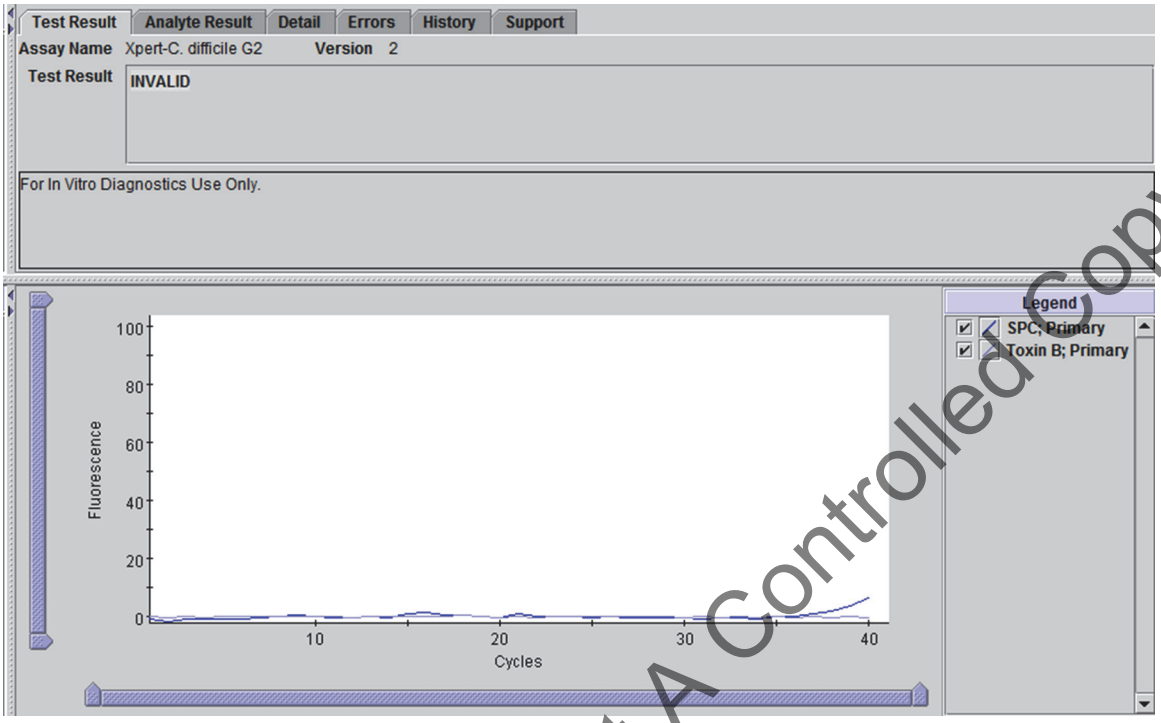

Figure 4. An Example of an INVALID Result

15 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.

15.1 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* 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.

16 Limitations

- This test detects but does not differentiate the NAP1 (Ribotype 027) strain from other toxigenic strains of *C. difficile.*
- This test targets the *tcdB* gene for Toxin B production. This test will not detect strains of *C. difficile* that do not contain the *tcdB* gene.
- Positive results observed with immunocompromised pediatric patients may reflect asymptomatic carriage of *C. difficile.*
- 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.
- The performance of the Xpert *C. difficile* Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Results from the Xpert *C. difficile* 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 Xpert *C. difficile* Assay, may result in a false negative result upon retest.
- Inhibition of the Xpert *C. difficile* Assay has been observed in the presence of the following substances: Zinc oxide paste and Vagisil® cream.
- 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.

17 Expected Values

In the Xpert *C. difficile* Assay clinical study, a total of 2296 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 2 and Table 3, respectively.

Table 2. Observed Prevalence of Toxigenic *C. difficile* by Age Group[a]

Age Group	N	Toxigenic *C. difficile* Prevalence
2-5	16	25.0% (416)
6-21	105	10.5% (11/105)
22-59	898	12.9% (116/898)
>60	1277	16.2% (207/1277)

a Prevalence based on reference culture.

Table 3. Observed Prevalence of Toxigenic *C. difficile* by Gender[a]

Gender	N	Toxigenic *C. difficile* Prevalence
Male	1074	13.8% (148/1074)
Female	1222	15.5% (190/1222)

a Prevalence based on reference culture

18 Performance Characteristics

18.1 Clinical Performance

Performance characteristics of the Xpert *C. difficile* Assay were determined in a multi-site prospective investigation study at seven US and Canadian institutions by comparing the Xpert *C. difficile* Assay to reference culture followed by cell cytotoxicity testing on the isolates.

Subjects included individuals whose routine care called for *C. difficile* testing. A portion of the leftover unformed stool specimens were obtained for testing by the Xpert *C. difficile* Assay. The remaining excess specimen was sent to a central laboratory for reference culture and cytotoxin B isolate 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 is negative for *C. difficile* or the isolate was tested negative by cell cytotoxicity assay.

Performance of the Xpert *C. difficile* Assay was calculated relative to the results of direct culture and reference culture.

19 Overall Results

A total of 2296 specimens were tested by Xpert *C. difficile* Assay and culture.

19.1 Performance Versus Direct Culture

Relative to direct culture with, the Xpert *C. difficile* Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 98.79% and 90.82%, respectively (Table 4).

Table 4. Xpert *C. difficile* Assay Performance vs. Direct Culture

		Direct Culture		
		C. diff	**NEG**	**Total**
Xpert *C. difficile*	**Toxin B+**	245 (240)	188 (183)	433 (423)
	NEG	3 (3)	1860 (1795)	1863 (1798)
	Total	248 (243)	2048 (1978)	2296 (2221)
		Sensitivity:	98.79%	
		Specificity:	90.82%	
		Accuracy:	91.68%	
		PPV[a]	56.58%	
		NPV[b]	99.83%	
		Prevalence:	10.80%	

a Positive predictive value
b Negative predictive value
( ) Xpert C. difficile results on first attempt

19.2 Performance Versus 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, the Xpert *C. difficile* Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.49% and 94.02%, respectively (Table 5).

Table 5. Xpert *C. difficile* Assay Performance vs. Reference Culture

		Reference Culture		
		C. diff	**NEG**	**Total**
Xpert *C. difficile*	**Toxin B+**	316 (310)	117 (113)	433 (423)
	NEG	22 (22)	1841 (1776)	1863 (1798)
	Total	338 (332)	1958 (1889)	2296 (2221)
		Sensitivity:	93.49%	
		Specificity:	94.02%	
		Accuracy:	93.95%	
		PPV[a]	72.98%	
		NPV[b]	98.82%	
		Prevalence:	14.72%	

a Positive predictive value
b Negative predictive value
( ) Xpert C. difficile results on first attempt

20 Antibiotic Usage

Among the 2296 cases included in the main dataset, antibiotic use within the 2 months prior to sample collection was reported for 1633 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.

21 Analytical Specificity

Fifty-five (55) strains were collected, quantitative and tested using the Xpert *C. difficile* 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 microaerophillic (2).

Each strain was tested in triplicate at concentrations ranging from $1.1x10^8$ to $2.2x10^{10}$ CF U/swab. Positive and negative controls were included in the study. Under the conditions of the study, all isolates were reported **Toxigenic C. diff NEGATIVE**. The analytical specificity was 100%.

22 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* Assay. The fecal matrix consisted of human liquid feces (*C. difficile* negative by Xpert C. difficile Assay) diluted in PBS with 15% glycerol. The LoD is defined as the lowest number of colony forming units (CF U) per swab that can be reproducibly distinguished from negative samples with 95% confidence.

Replicates of 20 were evaluated at each *C. difficile* concentration tested (CF U/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 CF Us 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 6.

Table 6. 95% Confidence Intervals for Analytical LoD - *C. difficile*

Strain ID	Toxinotype	LoD95% (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)[a]	III	23	19	31
LUMC-5 (027/NAP1/BI)[a]	III	75	45	176
LUMC-7	V	45	34	104
LUMC-6	VIII	60	50	74
9101	XII	41	34	49

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

The results of this study indicate that the Xpert *C. difficile* Assay will produce a positive *C. difficile* result 95% of the time with 95% confidence for a fecal sample containing 460 CFU.

In addition to the LoD determination, eighteen *C. difficile* strains representing 12 variant toxinotypes, including four 027/NAP1/ BI toxinotype III isolates, were tested using the Xpert *C. difficile* 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 CF U/swab and tested in triplicate.

Under the conditions of this study, the Xpert *C. difficile* Assay correctly identified all 18 toxinotypes 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**.

23 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* Assay. Potentially interfering substances include, but are not limited to, Vagisil cream and zinc oxide paste. The 19 substances listed in Table 7 showed no detectable interference with the Xpert *C. difficile* Assay.

Table 7. 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-HD™ High Density Barium Sulfate for suspension E-Z-EM Canada
Hydrocortisone Cream Longs Drugs	

24 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* Assay was used at each of the 3 testing sites. Xpert C. difficile Assays were performed according to the Xpert *C. difficile* Assay procedure. Results are summarized in Table 8 and Table 9.

Table 8. Summary of Reproducibility Results (All)

	% Agreement[a]			
Specimen ID	**Site 1**	**Site 2**	**Site 3**	**% Total Agreement by Sample**
Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* High Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* Low Positive	100% (20/20)	85% (17/20)	85% (17/20)	90.0% (54/60)
Toxigenic *C. difficile* 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 9. Summary of Ct Value Results by Sample Level and Probe

SPC			
Level	**Ave**	**StdDev**	**CV**
Toxigenic C. diff high neg	32.17	0.59	1.83%
Toxigenic C. diff low pos	32.14	0.53	1.66%
Toxigenic C. diff 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			
Level	**Ave**	**StdDev**	**CV**
Toxigenic C. diff high neg	39.59	0.70	1.77%
Toxigenic C. diff low pos	35.88	0.81	2.24%
Toxigenic C. diff 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* Assay was used at each of the 3 testing sites. Xpert *C. difficile* Assays were performed according to the Xpert *C. difficile* Assay procedure. Results are summarized in Table 10.

Table 10. Summary of Additional Reproducibility Specimen Results

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

[a] (# negative results / total high negative samples run)
[b] 20-80% agreement expected for high negative sample

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26 Assistance

For assistance, contact Cepheid using one of the following contact details. Make sure you provide the instrument serial number and reagent lot ID when you call or email.

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
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www.cepheid.com	www.cepheidinternational.com/

27 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

Region	Telephone	Email
US	+ 1 888 838 3222	techsupport@cepheid.com
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Contact information for other Cepheid offices is available on our website at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab. Select the **Contact Us** option.

28 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
2	Do not reuse
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
°C	Temperature limitation
	Biological risks
Rx only	For prescription use only

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IVD