GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® *C. difficile*

REF **GXCDIFFICILE-CE-10**

Instructions for Use

IVD CE

Cepheid®

In Vitro Diagnostic Medical Device

300-9291, Rev. H
March 2023

Trademark, Patents and Copyright Statements

See Section 27 Revision History for a description of changes.

Xpert® C. *difficile*

For *In Vitro* Diagnostic Use.

1 Proprietary Name

Xpert® C. *difficile*

2 Common or Usual Name

Xpert C. *difficile* Test

3 Intended Use

The Xpert C. *difficile* Test, performed on the Cepheid GeneXpert® Instrument Systems, is a qualitative *in-vitro* diagnostic test for the rapid identification and differentiation of Toxin B, and Binary Toxin from appropriate 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 producing *C. difficile*, which is associated with CDI. The Xpert C. *difficile* Test is intended as an aid in the diagnosis of CDI. Concomitant testing is necessary only if further typing 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 diarrhoea 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 factors are enterotoxin A and cytotoxin B.[5] The genes coding for toxin A (*tcdA*) 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 B1.[8,12] These strains exhibit increased toxin production which is being attributed to deletions in the regulatory gene tcdC.[13,14]

The European Centre for Disease Prevention and Control (ECDC) has identified an urgent need for rapid diagnostic tests with better performance than currently available assays for *C. difficile*.[12] *C. difficile* diagnosis is generally based on the detection of toxin A or B. The labor intensive cell cytotoxicity assay is still considered to be the "gold standard" because of its 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. However, no commercial standardized PCR assays for detection of toxin A and B are currently available.[17]

5 Principle of the Procedure

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction, amplification, and detection of the target sequences in simple or complex samples using real-time PCR and RT-PCR assays. The systems consist of an instrument, computer, and preloaded software for running the tests on collected samples and viewing the

results. The systems require the use of single-use disposable GeneXpert cartridges that hold reagents for PCR and hosts the processes of DNA extraction and PCR. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx System Operator Manual* and/or *GeneXpert Infinity System Operator Manual*.

Xpert C. *difficile* Test includes reagents for the detection of Toxin producing *C. difficile* and Toxin producing *C. difficile*, presumptive 027/NAP1/BI respectively as well as the Sample Processing Control (SPC). The SPC is present to control for an adequate amplification process and to monitor for the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, and all reaction components, including probes and dyes, are present and functional in the cartridge.

The primers and probes in the Xpert C. *difficile* Test detect sequences in the genes for Toxin B (*tcdB*), Binary Toxin (*cdt*), and *tcdC* deletion nt 117 (*tcdC*Δ117).

6 Materials Provided

The Xpert C. *difficile* kit contains sufficient reagents to process 10 specimens or quality control samples.

The kit contains the following:

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

Note Safety Data Sheets (SDS) are available at www.cepheid.com or 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 Storage and Handling

- Store the Xpert C. *difficile* kit at 2 – 28 °C until the expiration date provided on the label.
- Do not use sample reagent or cartridges that have passed the expiration date.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.
- The Sample Reagent is a clear, colorless liquid. Do not use the Sample Reagent if it has become cloudy or discolored.

8 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert software, barcode wand reader, and Operator Manual.
- For GeneXpert Dx System: Software version 4.3 or higher.

Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.

- Vortex mixer
- Disposable, sterile transfer Pipettes
- 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).

9 Warnings and Precautions

- 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 and the Clinical and Laboratory Standards Institute [18,19].
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Performing the Xpert C. *difficile* Test outside the recommended storage temperature ranges and time may produce erroneous or invalid results.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results
- Do not place the sample ID label on the cartridge lid or on the barcode label of the cartridge. Do not use a cartridge with a damaged barcode label.
- The Xpert C. *difficile* Test does not provide susceptibility results. Additional time is required to culture and perform susceptibility testing.
- Do not substitute Xpert C. *difficile* reagents with other reagents.
- Do not open the Xpert C. *difficile* 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* cartridge is used to process one test. Do not reuse processed cartridges.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a 1:10 dilution of household chlorine bleach and then repeat the cleaning of the work area with 70% denatured ethanol. Wipe work surfaces and allow to dry completely before proceeding.
- 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.
- Do not open a cartridge lid until you are ready to perform testing.

10 Chemical Hazards[20,21]

- 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 Specimen Collection and Transport

1. Collect the unformed stool in a clean container. Follow your institution's guidelines for collecting samples for C. *difficile* testing.
2. Label with Patient ID and send to the laboratory for testing.
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.

12 Procedure

12.1 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. Immerse swab in the unformed stool sample briefly. The swab does not need to be completely soaked.
3. Insert the swab into the tube 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 tube, lift the swab a few millimeters from the bottom of the tube and push the stem against the edge of the tube 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, transfer the entire contents of the Sample Reagent to the Sample chamber of the cartridge.
7. Close the cartridge lid.

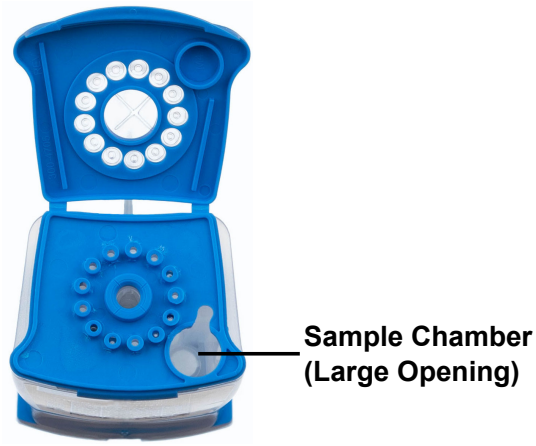

Figure 1. Cartridge (Top View)

12.2 Starting the Test

Important **If you are running a *GeneXpert Dx system*, before you start the test, make sure that the system is running GeneXpert Dx software version 4.7b or higher and that the correct assay definition file is imported into the software.**

Important **If you are running a *GeneXpert Infinity system*, before you start the test, make sure that the system is running Xpertise software version 6.4b or higher and that the correct assay definition file is imported into the software.**

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

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

1. Turn on the GeneXpert instrument:
   - If using the *GeneXpert Dx instrument*, first turn on the GeneXpert Dx instrument, and then turn on the computer. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or
   - If using the *GeneXpert Infinity instrument*, power up the instrument. The Xpertise software will launch automatically. If it does not, double-click the Xpertise software shortcut icon on the Windows® desktop.
2. Log on to the GeneXpert Instrument System software using your username and password.
3. In the **GeneXpert System** window, click **Create Test** (GeneXpert Dx) or **Orders** and **Order Test** (Infinity). The **Create Test** window opens. The **Scan Patient ID barcode** dialog box opens.
4. Scan or type in the Patient ID. 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 and all the reports. The **Scan Sample ID barcode** dialog box opens.
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 and all the reports. The **Scan Cartridge Barcode** dialog box opens.
6. Scan the barcode on the 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 cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen will appear indicating the assay definition file is not loaded on the system. If this screen appears, contact Cepheid Technical Support.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). In the dialog box that appears, type your password, if required.
8. For the *GeneXpert Infinity System*, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed into the waste container.

or

For the *GeneXpert Dx Instrument*:

a) Open the instrument module door with the blinking green light and load the cartridge.
b) Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
c) Wait until the system releases the door lock before opening the module door. Then remove the cartridge.
d) Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

13 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model being used.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the **View Results** window to view and/or generate a PDF report file.

14 Quality Control

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

15 Interpretation of Results

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

Table 1. Xpert C. *difficile* Results and Interpretation

Result	Interpretation
Toxigenic C. diff POSITIVE, 027 PRESUMPTIVE NEG See Figure 2	Toxin producing *C. difficile* target DNA sequences are detected ● Toxin producing *C. difficile* — the Toxin producing *C. difficile* target(s) (Toxin B or Toxin B plus either Binary Toxin or *tcdC* deletion nt 117) have a Ct 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. diff POSITIVE, 027 PRESUMPTIVE POS See Figure 3	Toxin producing *C. difficile*, presumptive 027/NAP1/BI target DNA sequences are detected. ● Toxin producing *C. difficile*, presumptive 027/NAP1/BI — all Toxin producing *C. difficile*, presumptive 027/NAP1/BI targets (Toxin B, Binary Toxin and tcdC deletion nt 117) have Ct 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. diff NEGATIVE, 027 PRESUMPTIVE NEG See Figure 4	*C. difficile* target DNA sequences (Toxin B) are not detected. NEGATIVE —Toxin producing *C. difficile* DNA sequences (Toxin B) are not detected, other target DNA for toxigenic *C. diff* (Binary Toxin and *tcdC* deletion nt 117) are not detected. SPC meets acceptance criteria. ● NEGATIVE — *C. difficile* target DNA is 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 See Figure 5	Presence or absence of *C. difficile* target DNA cannot be determined, repeat test according to the instructions in the Retest Procedure section below. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited. ● INVALID — Presence or absence of *C. difficile* target DNA cannot be determined. ● SPC — FAIL; SPC target result is negative and the SPC Ct is not within valid range and endpoint below minimum setting. ● Probe Check — PASS; all probe check results pass.
ERROR	Presence or absence of *C. difficile* target DNA cannot be determined, repeat test according to the instructions in the Retest Procedure section below. The Probe Check control failed probably due to reaction tube was filled improperly, a probe integrity problem was detected or because the maximum pressure limits were exceeded. ● Toxin B (*tcdB*) — NO RESULT ● Binary Toxin (*cdt*) — NO RESULT ● *tcdC*Δ117— NO RESULT ● *SPC — NO RESULT Probe Check — FAIL[a]; all or one of the probe check results fail
NO RESULT	Presence or absence of *C. difficile* target DNA cannot be determined, repeat test according to the instructions in the Retest Procedure section below. Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress). ● Toxin B (*tcdB*) — NO RESULT ● Binary Toxin (*cdt*) — NO RESULT ● *tcdC*Δ117— NO RESULT ● SPC — NO RESULT ● Probe Check — NA (not applicable)

[a] If the probe check passed, the error is caused by a system component failure.

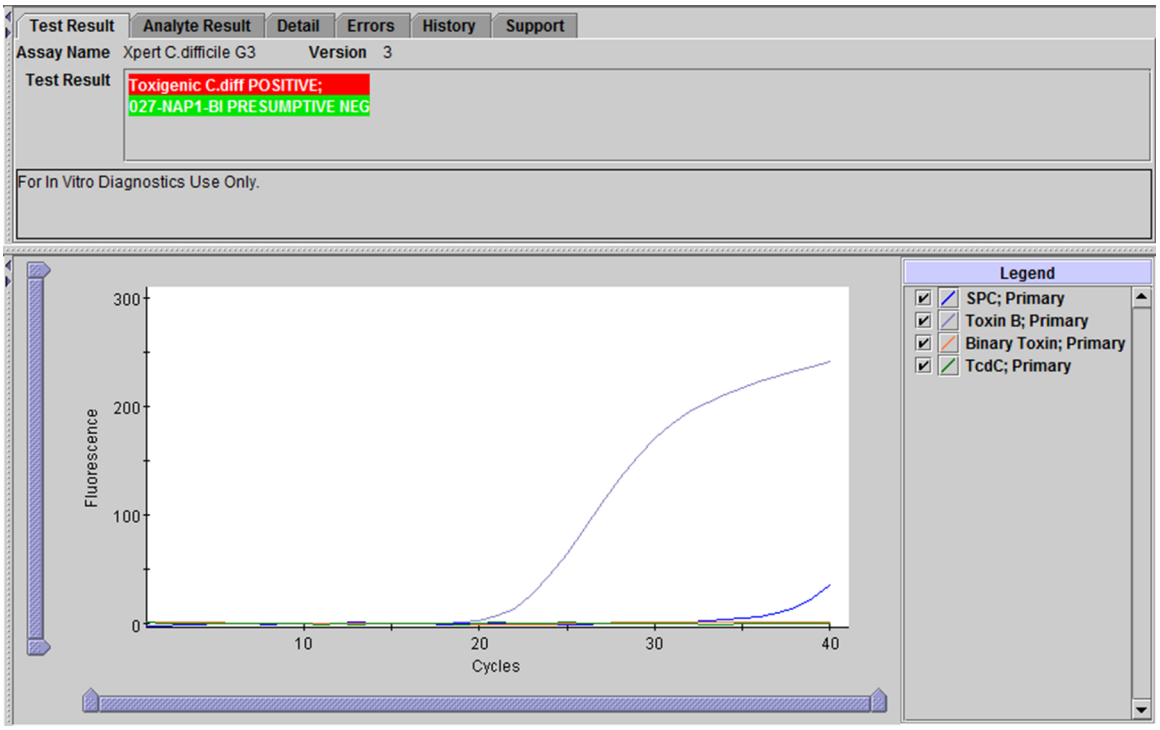

Figure 2. Example of Xpert C. difficile Positive and 027 Presumptive Negative Results

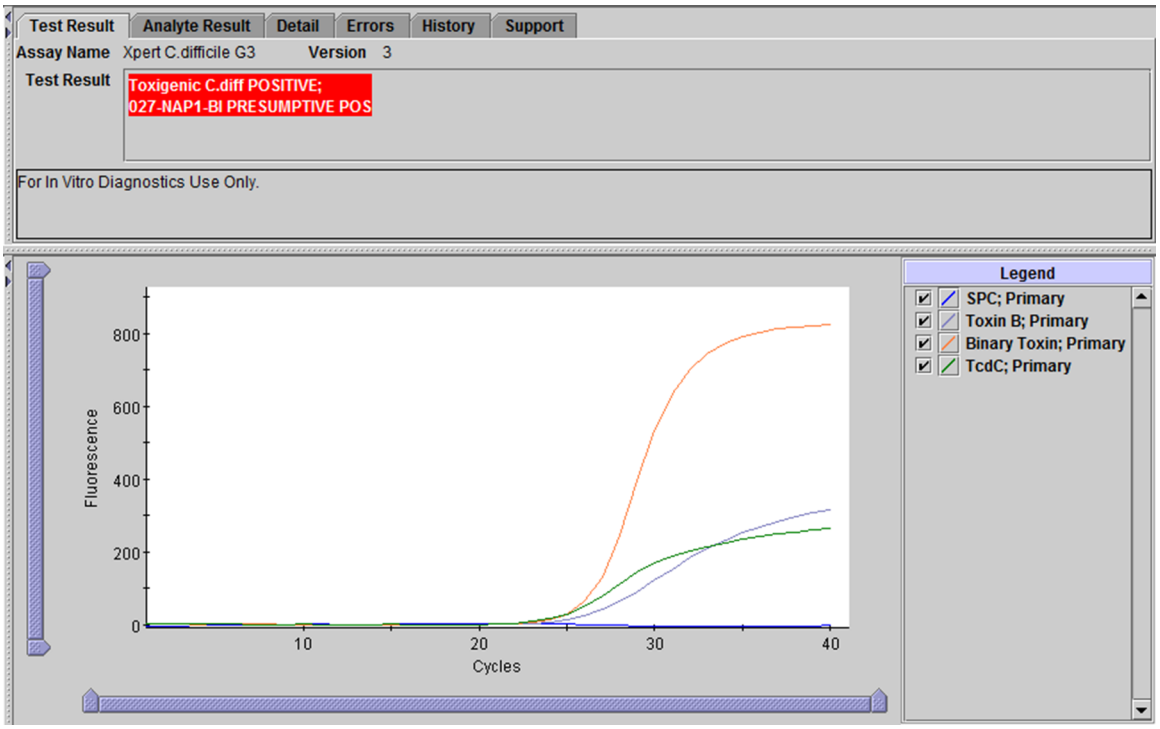

Figure 3. Example of Xpert C. difficile Positive and 027 Presumptive Positive Results

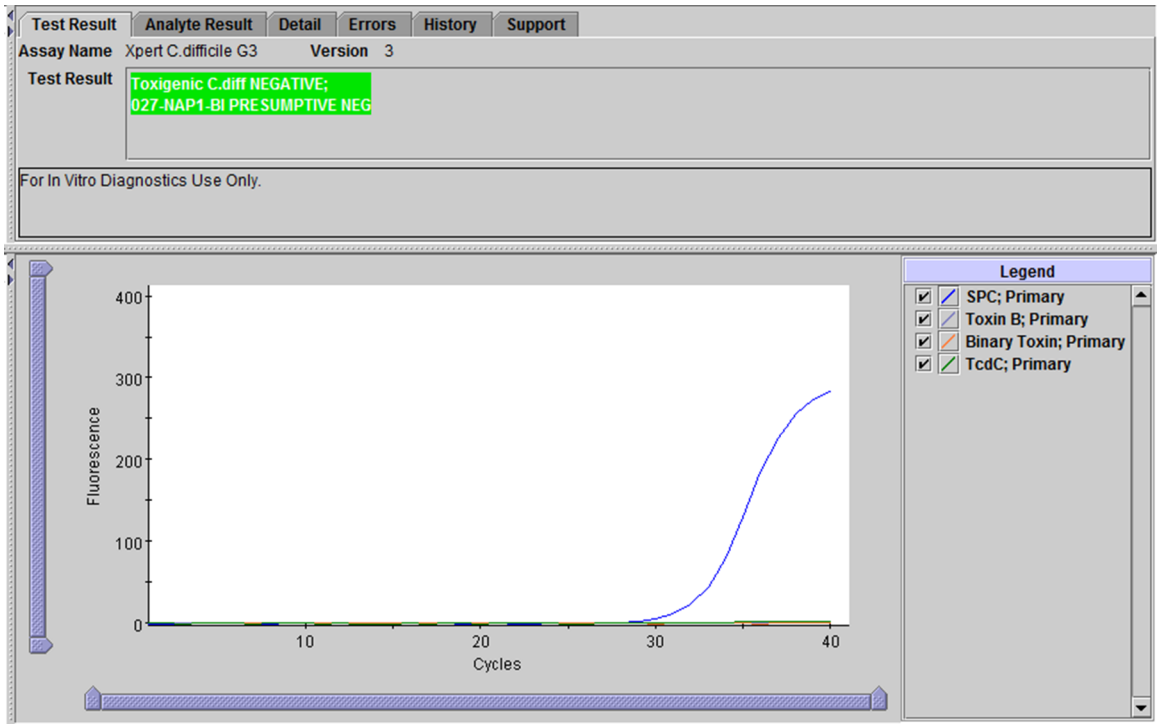

Figure 4. Example of C. difficile Negative and 027 Presumptive Negative Results

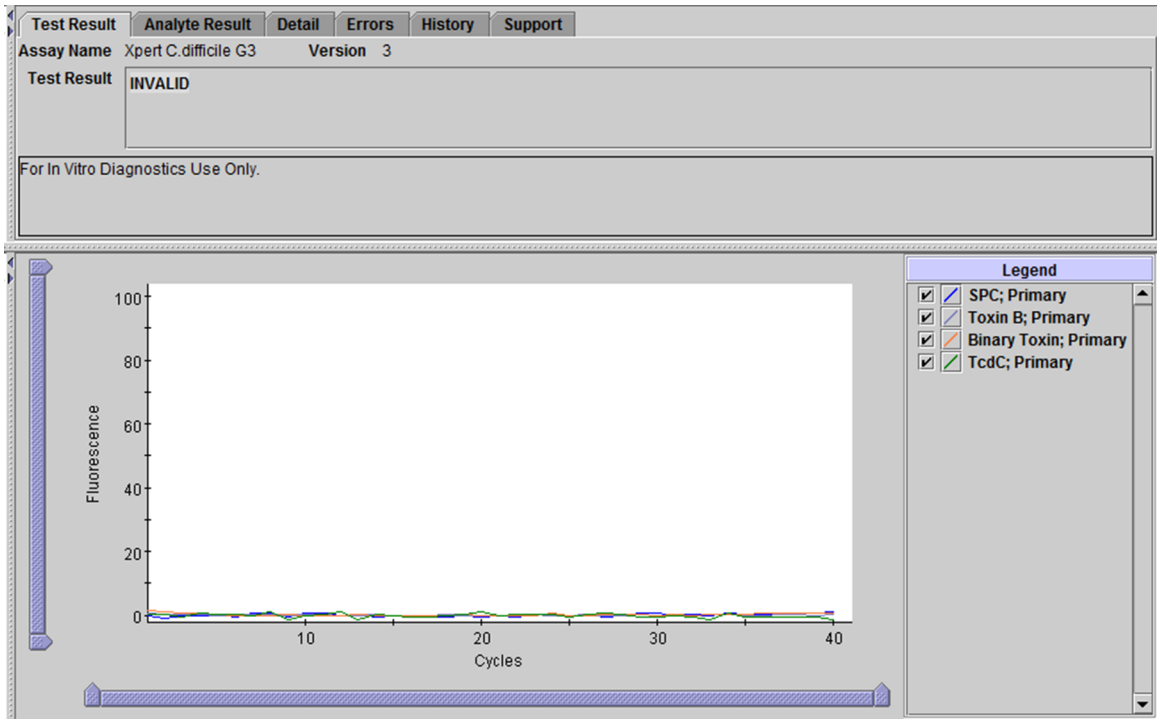

Figure 5. Example of an Invalid Result

16 Reasons to Repeat the Test

16.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to the instructions in Section 16.2.

- 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 may have failed and the test was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded, or a valve positioning error was detected.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

16.2 Retest Procedure

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

1. Remove a new cartridge from the kit.
2. Transfer all remaining contents from the Sample Chamber to a new Sample Reagent vial using a disposable transfer pipette.
3. Vortex and add the entire contents of the Sample Reagent to the Sample Chamber of the new Xpert C. *difficile* cartridge.
4. Close the lid and start the new test.

For retest after 3 hours of an indeterminate result, repeat the test with a new swab sample from the original patient specimen.

17 Limitations

Non-027/NAP1/BI isolates representing toxinotype XIV will be reported **Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE POSITIVE** using the Xpert C. *difficile* Test.

Occasionally, non-027/NAP1/BI isolates representing toxinotype IV, V and X will be reported **Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE POSITIVE** using the Xpert C. *difficile* Test.

The performance of the Xpert C. *difficile* Test 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* 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 the detection of *C. difficile* is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.

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* Test, may result in a false negative result upon retest.

Rerunning the Xpert C. *difficile* Test when results are **INVALID**, **ERROR**, or **NO RESULT** should depend on practices and policies within each facility. Alternate procedures should be available. For culturing, remaining swab specimens should be placed in appropriate transport systems and cultured within 4 days.

A positive test result does not necessarily indicate the presence of viable organism. It is however, presumptive for the presence of *C. difficile*.

Outbreaks of CDI may be caused by strains other than 027/NAP1/BI.

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.

Performance characteristics of this test have been established with the specimen type listed in the Intended Use Section only. The performance of this test with other specimen types or samples has not been evaluated.

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.

18 Interfering Substances

Potential inhibitory substances tested include blood, excess feces and mucus. Substances were tested in replicates of three with *C. difficile* bacteria strain 027/NAP1/BI spiked near the analytical Limit of Detection (~3x LoD) and higher (~50x LoD). Excess feces material was evaluated with real clinical samples in a multi-site investigation study. Inhibitory effect is occasionally seen in the presence of excess feces on the swab. No significant inhibitory effects were observed in the presence of blood or mucus.

19 Performance Characteristics

Performance characteristics of the Xpert C. *difficile* Test were determined in a prospective investigation study at two sites in Europe by comparing the Xpert C. *difficile* Test on the GeneXpert System (Xpert C. *difficile* Test) with toxinogenic culture followed by PCR ribotyping of culture positive samples. To be enrolled in the study, specimens had to be from individuals for whom cultures were indicated and/or ordered, according to institutional practices.

19.1 Overall Results

A total of 285 specimens were tested for C. difficile by the Xpert C. *difficile* Test and compared to the direct culture method (see table below).

Table 2. Performance Characteristics of the Xpert C. *difficile* Test as Compared to Direct Culture

		Toxinogenic Culture				
		C. difficile	027/NAP1/ BI pos	Negative		
Xpert C. *difficile*	Toxin B+	34	0	16	Sensitivity	100%
	027/NAP1/BI	0	0	1	Specificity	93%
	Negative	0	0	234		

20 Performance Characteristics of the 027/NAP1/BI

To determine the performance characteristics of the 027/NAP1/BI strain, clinical samples were evaluated in-house by Xpert C. *difficile*, cultured and PCR-ribotyped. The data of the study is provided in the table below. Negative in this case means toxinogenic *C. difficile* strains that are not 027/NAP1/BI.

Table 3. Performance Characteristics of the Xpert C. *difficile* Test as Compared to PCR Ribotyping

		Toxinogenic Culture and PCR-Ribotypings			
		027/NAP1/ BI Pos	027/NAP1/ BI Neg		
Xpert C. *difficile*	027/NAP1/ BI Pos	14	1	Sensitivity	100%
	027/NAP1/ BI Neg	0	10	Specificity	91%

21 Analytical Specificity

Cultures from American Type Culture Collection (ATCC) and Culture Collection, University of Göteborg (CCUG) representing organisms closely related to *C. difficile* as well as normal and pathogenic rectal flora were tested in a cross-reactivity study. Two strains of non-toxin producing *C. difficile* were tested using the Xpert C. *difficile* Test. The organisms tested were represented by 24 aerobic, 14 anaerobic and two microaerophilic species. Three replicates of each isolate were

tested at a concentration of at least 10^9 CFU per reaction. Under the conditions of the study, all isolates were reported toxinogenic *C. difficile* negative; none of the isolates were detected by the Xpert C. *difficile* Test. Positive and Negative controls were included in the study. The Analytical specificity was 100%.

22 Analytical Sensitivity

Additional studies were performed to determine the 95% confidence interval for the analytical limit of detection (LoD) of this assay. The limit of detection is defined as the lowest number of colony forming units (CFU) per sample that can be reproducibly distinguished from negative samples with 95% confidence. Replicates of 20 were evaluated at six concentrations (100, 300, 600, 1200, 2400 and 4800 CFU/sample).

Under the conditions of the study and using a maximum valid Ct setting of 37, for *tcdB* and *cdt* and 40 for *tcdC* results indicate that the LoD point estimate for toxigenic *C. difficile* is 1657 CFU/swab with a 95% confidence interval ranging from 1157 CFU/swab to 3561 CFU/swab and for toxigenic *C. difficile* strain 027/NAP1/BI 2058 CFU/sample with a 95% confidence interval ranging from 1581 CFU/swab to 3441 CFU/swab.

23 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;22-61-7
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 VT, Hundsberger P, Leukel M, Sauerborn and C. von Eichel-Striber. 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 SPMM, Merrigan D, Lyerly DN Gerding, Johnson S. Toxin gene analysis of a variant strain of *Clostridium difficile* that causes human clinical disease. Infect. Immun 2000;68:5480-5487.
9. Goncalves C, Decre D, Barbut F, Burghoffer B, Petit JC. Prevalence and characterization of a binary toxin (actin-specific ADP-ribosyl-transferase) from *Clostridium difficile*. J Clin Microbiol 2004;42:1933-9
10. Stubbs S, Rupnik M, Gibert M, Brazier J, Duerden B, Popoff M. Production of actin-specific ADP-ribosyltransferase (binary toxin) by strains of *Clostridium difficile*. FEMS Microbiol Lett 2000;186:307-12.
11. Popoff MR, Rubin EJ, Gill DM, Boquet P. Action-specific ADP-ribotransferase produced by a *Clostridium difficile* strain. Infect Immun 1998;56:2299-306.
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; Oct;12 Suppl 6:2-18. Review.
13. Curry SR, Marsh JW, Muto CA, O'Leary MM, Pasculle AW, Harrison LH. *tcdC* genotypes associated with severe *TcdC* truncation in an epidemic clone and other strains of *Clostridium difficile*, J Clin Microbiol. 2007 Jan;45(1):215-21. Epub 2006 Oct 11. Erratum in: J Clin Microbiol. 2007 Jun;45(6):2103.
14. MacCannell DR, Louie TJ, Gregson DB, Laverdiere M, Labbe AC, Laing F, Henwick S. Molecular analysis of *Clostridium difficile* PCR ribotype 027 isolates from Eastern and Western Canada, J Clin Microbiol. 2006 Jun;44(6):2147-52.
15. Wilkins TD, Lyerly DM. *Clostridium difficile* testing: after 20 years, still challenging. Clin Microbiol. 2003 Feb;41(2):531-4. Review.
16. Delmee M. Laboratory diagnosis of *Clostridium difficile* disease. Clin Microbiol Infect. 2001 Aug;7(8):411-6. Review.
17. Poutanen SM, Simor AE. *Clostridium difficile*-associated diarrhea in adults. CMAJ. 2004 Jul 6;171(1):51-8. Review.
18. 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.
19. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to latest edition).
20. 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).
21. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

24 Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

European Headquarters

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300
Fax: + 33 563 825 301
www.cepheidinternational.com

25 Technical Assistance

Before Contacting Us

Collect the following information before contacting Cepheid Technical Support:

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

United States Technical Support

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France Technical Support

Telephone: + 33 563 825 319
Email: support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

26 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* 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
	CE marking – European Conformity
	Temperature limitation
	Biological risks
	Authorized Representative in Switzerland
	Importer

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna,
Sweden

Tel: +46 8 6843 7000

Fax: +46 8 6843 7010

Cepheid Switzerland GmbH
Zürcherstrasse 66
Postfach 124, Thalwil
CH-8800
Switzerland

Cepheid Switzerland GmbH
Zürcherstrasse 66
Postfach 124, Thalwil
CH-8800
Switzerland

27 Revision History

Description of Changes: 300-9291, Rev. G to Rev. H

Purpose: To correct graphics and align with the requirements of Regulation (EU) 2017/746

Section	Description of Change
Trademark, Patents and Copyright Statements	Updated legal information to current Cepheid legal standards.
12	Updated wording of Procedure sections for consistency between Cepheid Instructions for Use.
15	Ordered figures to match result order in Table 1.
16.2	Updated wording of Retest Procedure section for consistency between Cepheid Instructions for Use.
26	Added CH REP and Importer symbols and definitions to Table of Symbols. Added CH REP and Importer information with Switzerland address.
27	Updated revision history table.
Throughout	Instances of "assay" used as a brand name changed to "test".