GeneXpert
Powered By CEPHEID INNOVATION

Xpert® C. difficile BT

REF **GXCDIFFBT-CE-10**

Instructions for Use

CE IVD

Cepheid

In Vitro Diagnostic Medical Device

301-6190, Rev. E
2024-07

Trademark, Patents and Copyright Statements

See Section 25 Revision History for a description of changes.

Xpert® C. *difficile* BT

In Vitro Diagnostic Medical Device

1 Proprietary Name

Xpert® C. *difficile* BT

2 Common or Usual Name

Xpert C. *difficile* BT test

3 Intended Use

The Cepheid Xpert C. *difficile* BT test, performed on the Cepheid GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test for rapid detection of *C. difficile tcdB* (toxin B gene), *cdt* (binary toxin gene), and a deletion of a nucleotide at position 117 of the *tcdC* gene from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI). The Xpert C. *difficile* BT test is intended as an aid in the diagnosis of CDI and detection of strains potentially associated with more severe disease. The test utilizes automated real-time polymerase chain reaction (PCR) to detect *tcdB*, *cdt*, and the *tcdC* deletion at base 117 associated with the ribotype 027 strain. Binary toxin is produced by a limited number of *C. difficile* strains, including the 027 strain. Binary toxin together with *tcdB* detection is often an indicator of more severe disease or recurrence of disease. Isolates of *C. difficile* that are negative for *tcdB* but contain binary toxin genes alone may produce symptoms similar to toxigenic *C. difficile* strains, but the clinical significance of such strains is currently uncertain. Concomitant culture is necessary only if further typing or organism recovery is required.

4 Summary and Explanation

C. difficile is a Gram-positive, spore-forming, anaerobic rod that was first linked to disease in 1978.[1]

CDI ranges from mild 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 by other bacterial species, such as *C. difficile*, is lost. The most common risk factor for developing CDI 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 part 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 separate genes (*cdtA* and *cdtB*) and is located outside the PaLoc.[9,10,11]

CDI diagnosis traditionally has been based either on the detection of toxin B directly in stool (the cell culture cytotoxicity neutralization [CCCN] test) or on culture of the organism followed by determination of toxin B production by the isolate (toxigenic culture). Both the CCCN test and toxigenic culture are labor intensive but are still considered to be the "gold standards" because of the specificity of the former and the sensitivity of the latter.[12,13] 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 CCCN test. PCR methods for the detection of genes associated with toxin A and/or toxin B production have been developed and show high sensitivity and specificity as compared to toxigenic culture.[14]

In addition to toxin A and B, recent literature suggests a link between the production of binary toxin and both disease severity and outcome. Bauer et al.[15] showed the presence of binary toxin genes in toxigenic isolates in 23% of the CDI cases in Europe. Binary toxin produced by *cdt* genes is frequently observed in *C.difficile* strains associated with increased severity of CDI. Binary toxin belongs to the family of ADP-ribosylating toxins and consists of *cdtA* genes, the enzymatic

ADP-ribosyltransferase, which modifies actin, and *cdtB*, which binds to host cells and translocates the product of *cdtA* into the cytosol. Multiple clinical studies indicate an association between the presence of binary toxin genes in *C. difficile* and increased 30-day CDI mortality independent of PCR ribotype. There is also literature showing that subjects having severe CDI, fulminant colitis, and/or recurrent CDI are infected more frequently with *C. difficile* ribotypes carrying the genes for binary toxin production (*cdtA/cdtB*) than those without these complications.[16,17]

A subset of binary-producing isolates has mutations in the negative toxin regulator gene (*tcdC*), i.e., a deletion at nucleotide 117 (*tcdC*Δ117) consistent with Ribotype 027 strains. Infection caused by 027/NAP1/BI strains may be associated with a higher rate of mortality and morbidity, including intensive care unit (ICU)-admission and prolonged length of stay. Multivariate analysis demonstrated a significant association between disease severity and the presence of ribotypes carrying the binary toxin gene with or without deletion at nucleotide 117. In the last several years, there have been outbreaks of CDI attributed to a number of emerging "hypervirulent" strains that include fluoroquinoline-resistant strains belonging to PCR ribotype 027, (which are also known as pulsed-field gel electrophoresis group NAP1 and restriction endonuclease assay type BI.)[8,18] Strains of 027 may exhibit increased toxin production, which is attributed to deletions in the regulatory gene *tcdC* and may produce more spores, leading to enhanced persistence in the environment.[19,20] A presumptive positive 027 result may aid in the identification of possible sources of an 027 outbreak.

Finally, additional studies have reported cases of patients with diarrhea and suspected *C. difficile* infection due to toxinotype XI/PCR ribotype 033, or 033-like strains positive for binary toxin but negative for toxin A and B.[21,22] The clinical significance of such binary toxin positive, toxin B-negative strains is not fully understood.

5 Principle of the Procedure

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid purification and amplification, and detection of the target sequences in simple or complex samples using real-time PCR tests. The systems consist of an instrument, personal computer, and preloaded software for running the tests on clinical specimens and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold reagents for PCR and host the processes of DNA extraction, amplification, and amplicon detection. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the appropriate *GeneXpert Dx System Operator Manual* and/or *GeneXpert Infinity System Operator Manual*.

Xpert C. *difficile* BT test includes reagents for the detection of toxin producing *C. difficile* and a Sample Processing Control (SPC). The SPC indicates adequate processing of the target bacteria and monitors 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 primers and probes in the Xpert C. *difficile* BT test detect sequences in the genes for toxin B (*tcdB*), binary toxin (*cdt*), and the *tcdC*Δ117.

6 Reagents and Instruments

6.1 Materials Provided

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

The kit contains the following:

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

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.

6.2 Storage and Handling

- Store the Xpert C. *difficile* BT kit at 2–28 °C.
- Do not use sample reagent or cartridges that have passed the expiration date.
- Do not open the cartridge lid until you are ready to perform testing.
- Do not use sample reagent that has become cloudy or discolored.
- Do not use a cartridge that has leaked.

6.3 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert Software Version 4.3 or higher, barcode scanner, and operator manual.
- Printer: If a printer is required, contact a Cepheid Sales Representative to arrange for the purchase of a recommended printer.
- Vortex mixer
- Disposable, clean transfer pipettes
- 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 Systems (139C LQ STUART)

7 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.[23,24]

- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Wear clean lab coats and gloves. Change gloves between processing each sample.
- Do not substitute Xpert C. *difficile* BT reagents with other reagents.
- Do not open the Xpert C. *difficile* BT cartridge lid except when adding sample and reagents or to remove sample from the original cartridge to perform a retest in a new cartridge.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.
- Do not place the Sample ID label on the cartridge lid or on the barcode label.
- Each single-use Xpert C. *difficile* BT cartridge is used to process one test. Do not reuse a used cartridge.
- 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.
- In the event of contamination of the work area or equipment with sample or controls, thoroughly clean the contaminated area with a solution of 1:10 dilution of household chlorine bleach and then repeat the cleaning of the work area with 70% ethanol. Wipe work surfaces dry completely before proceeding.

8 Chemical Hazards[25,26]

- 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.

9 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.

10 Procedure

10.1 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.

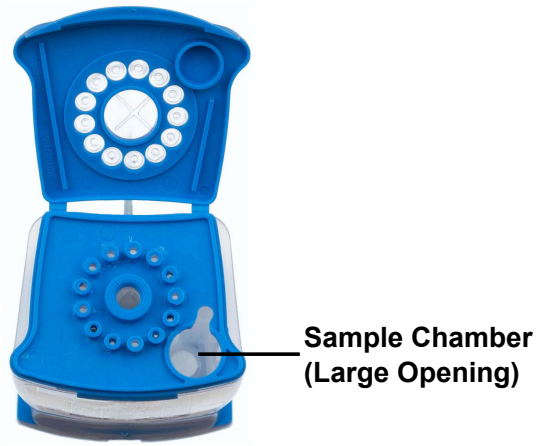

Figure 1. Cartridge (Top View)

10.2 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.

11 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 instrument 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.

12 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 the *Bacillus globigii* in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample. 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 sample-associated inhibition of the real-time PCR Test ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that

the PCR reagents are functional. 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.

13 Interpretation of Results

The results are interpreted by the GeneXpert Instrument Systems from measured fluorescent signals and embedded calculation algorithms and will be shown in the **View Results** window. Possible results are shown in the table below.

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

Result	Interpretation
Toxigenic C. diff POS, Binary Toxin NEG, 027 NEG See Figure 2.	Toxin-producing *C. difficile* target DNA sequences are detected. ● Toxin-producing *C. difficile* — the toxin-producing *C. difficile* target (toxin B gene) has a Ct within the valid range and an endpoint above the minimum setting. ● Binary toxin gene and the *tcdC* deletion at nt 117 are not detected. ● 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 POS, Binary Toxin POS, 027 NEG See Figure 3.	Toxin-producing *C. difficile* target DNA sequences are detected. ● Toxin-producing *C. difficile* targets (toxin B gene plus binary toxin gene) have Cts within the valid range and endpoints above the minimum setting; the *tcdC* deletion at nt 117 is not detected ● 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 POS, Binary Toxin POS, 027 PRESUMPTIVE POS See Figure 4.	Toxin-producing *C. difficile* and presumptive 027 target DNA sequences are detected. ● All toxin-producing *C. difficile*, presumptive 027 targets (toxin B, binary toxin and *tcdC* deletion at nt 117) 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. diff NEG, Binary Toxin POS, 027 NEG See Figure 5.	*C. difficile* toxin B gene sequences are not detected; however, another DNA target (binary toxin gene) is detected and has a Ct within the valid range and an endpoint above the minimum setting. The clinical significance of binary toxin-positive only isolates has yet to be determined. ● 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 NEG, Binary Toxin NEG, 027 NEG See Figure 6.	*C. difficile* target DNA sequences (Toxin B gene, binary toxin gene) are not detected. ● Toxin-producing *C. difficile* gene sequences (toxin B gene and binary toxin gene) are not detected; other DNA targets for toxigenic *C.difficile* (*tcdC* deletion at nt 117) are 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.

Result	Interpretation
INVALID See Figure 7.	Presence or absence of *C. difficile* target DNA cannot be determined. Repeat test according to the instructions in Section 15. 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 is 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 Section 15. The Probe Check control failed probably because the reaction tube was filled improperly, a probe integrity problem was detected, or the maximum pressure limits were exceeded. • Toxin B — NO RESULT • Binary Toxin — NO RESULT • *tcdC* deletion at nt 117 — NO RESULT • *SPC — NO RESULT • Probe Check — FAIL*; all or one of the probe check results fail. * If the probe check passed, the error is caused by a system component failure.
NO RESULT	Presence or absence of *C. difficile* target DNA cannot be determined. Repeat test according to the instructions in Section 15 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)

Note The screens shown in this section (Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, and Figure 7) are from a GeneXpert Dx System running GeneXpert Dx software.

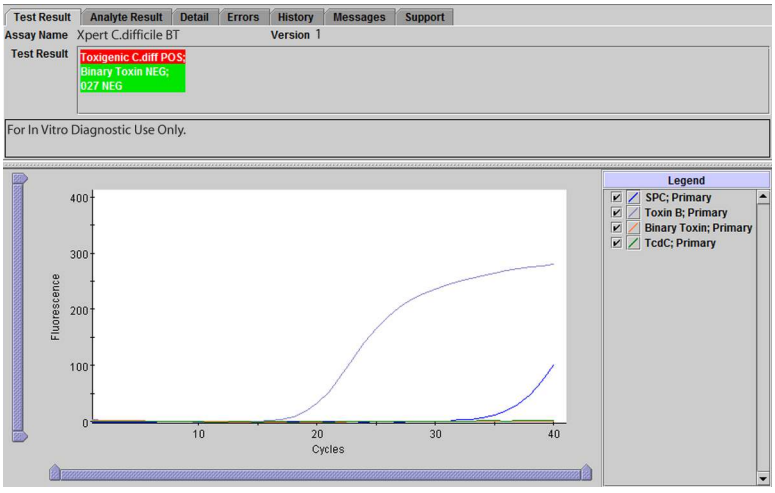

Figure 2. Example of Toxigenic C. diff Positive, Binary Toxin Negative, and 027 Negative Results

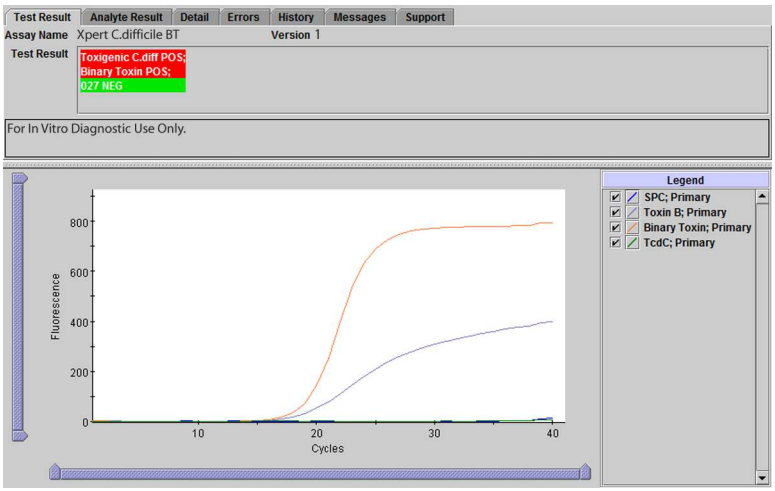

Figure 3. Example of Toxigenic C. diff Positive, Binary Toxin Positive, and 027 Negative Results

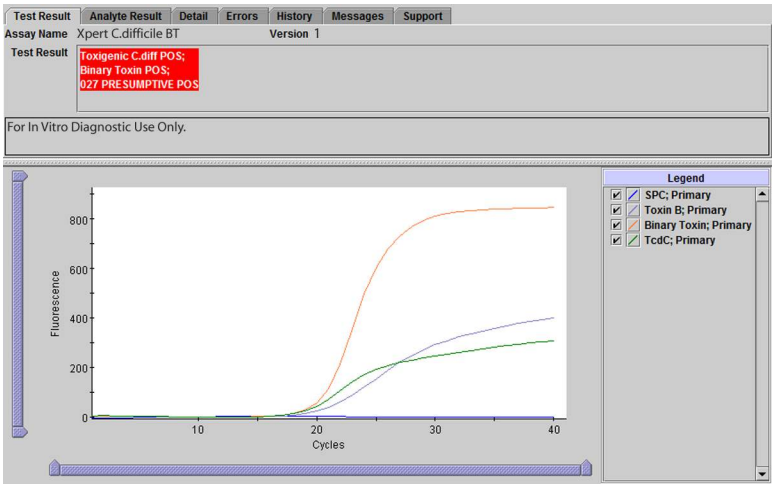

Figure 4. Example of Toxigenic C. diff Positive, Binary Toxin Positive, and 027 Presumptive Positive Results

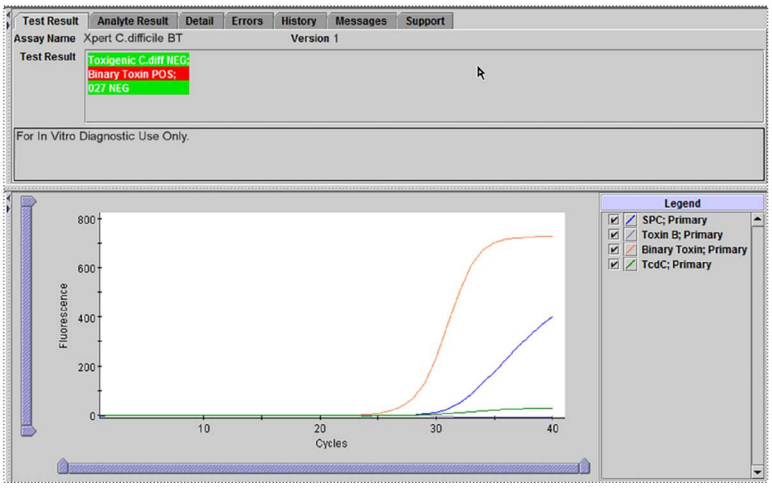

Figure 5. Example of Toxigenic C. diff Negative, Binary Toxin Positive, and 027 Negative Results

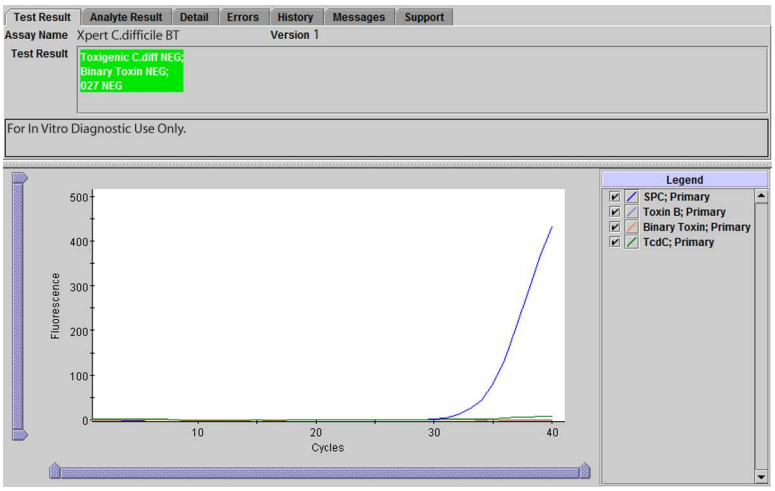

Figure 6. Example of Toxigenic C. diff Negative, Binary Toxin Negative, and 027 Negative Results

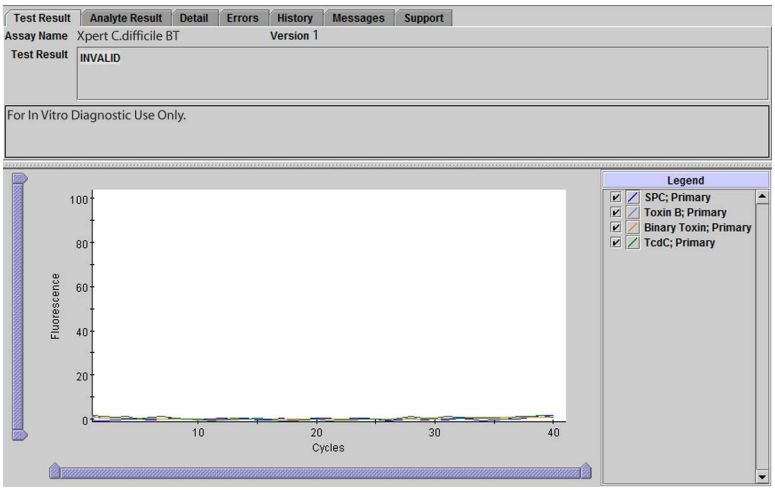

Figure 7. Example of an Invalid Result

14 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to the instructions in Section 15.

- 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.

15 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.

16 Limitations

- Non-027 isolates representing toxinotype XIV will be reported as **Toxigenic C. diff POS; Binary Toxin POS; 027 PRESUMPTIVE POS** using the Xpert C. *difficile* BT test.
- **Toxigenic C. diff NEG; Binary Toxin POS, Presumptive 027 NEG** by Xpert C. *difficile* BT may harbor the Toxin B gene and/or the *tcdC* deletion below the LoD of the test.
- Occasionally, non-027 isolates representing toxinotypes IV, V and X will be reported as **Toxigenic C. diff POS; Binary Toxin POS; 027 PRESUMPTIVE POS** using the Xpert C. *difficile* BT test.
- The performance of the Xpert C. *difficile* BT 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* BT test should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Because of the dilution factor associated with the retest procedure, it is possible that *C. difficile* positive specimens, very near or at the limit of detection (LoD) of the Xpert C. *difficile* BT test, may result in a false negative result upon retest.
- Inhibition of the Xpert C. *difficile* BT test 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.
- 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.
- Positive results obtained with immunocompromised patients may reflect asymptomatic carriage of *C. difficile*.
- Detection of *C. difficile* nucleic acid in stools confirms the presence of the organisms in patients with diarrhea but may not indicate that *C. difficile* is the cause of the diarrhea.
- Performance characteristics were not established for patients <2 years of age.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of targeted C. difficile types resulting in a false negative result.

17 Expected Values

In the Xpert C. *difficile* BT test clinical study, a total of 2293 unformed stool specimens were included from 7 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 shown in the tables below.

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

Age Group	N	Toxigenic *C. difficile* Prevalence (includes 027)	Binary Toxin Prevalence	027 Prevalence
2-5	16	37.5% (6/16)	12.5% (2/16)	12.5% (2/16)
6-21	105	12.4% (13/105)	2.9% (3/105)	0.9% (1/105)
22-59	898	16.4% (147/898)	4.8% (43/898)	3.3% (30/898)
>60	1274	20.7% (264/1274)	9.2% (117/1274)	7.2% (92/1274)
Total	2293	18.8% (430/2293)	7.2% (165/2293)	5.5% (125/2293)

[a] Prevalence based on Xpert results.

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

Gender	N	Toxigenic *C. difficile* Prevalence (includes 027)	Binary Toxin Prevalence	027 Prevalence
Male	1072	18.2% (195/1072)	6.3% (68/1072)	5.0% (54/1072)
Female	1221	19.2% (235/1221)	7.9% (97/1221)	5.8% (71/1221)
Total	2293	18.8% (430/2293)	7.2% (165/2293)	5.5% (125/2293)

[a] Prevalence based on Xpert results.

18 Performance Characteristics

18.1 Clinical Performance

Performance characteristics of the Xpert C. *difficile* BT test were determined in a multi-site prospective investigation study at seven US and Canadian institutions by comparing the Xpert C. *difficile* BT test to reference culture followed by CCCN testing on the isolates and strain typing on the toxigenic strains by PCR-ribotyping.

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* BT test. The remaining excess specimen was sent to a central laboratory for reference culture and cytotoxin B testing. Each stool specimen was inoculated onto pre-reduced cycloserine-cefoxitin-fructose agar –direct plate (CCFA-D) and cycloserine cefoxitin mannitol broth with taurocholate lysozyme cysteine (CCMB-TAL). After 24 hours the CCMB-TAL was subcultured on to a second CCFA-E plate (CCFA- Enriched). This direct-enriched culture method is referred to hereafter as “reference culture”.

If *C. difficile* was isolated from the CCFA-D plate and the isolate was positive by the CCCN test, the specimen was classified as “toxigenic *C. difficile* positive” and the 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 CCCN test, the CCFA-E plate was further analyzed.

If CCFA-E was positive for *C. difficile* and the isolate was positive for the CCCN test, 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 found to be negative by the CCCN test.

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

Performance of the Xpert C. *difficile* BT test was calculated relative to the results of direct culture with strain typing and reference culture with strain typing.

18.2 Overall Results

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

18.2.1 Performance Results vs. Direct Culture

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

Table 4. Xpert C. *difficile* BT Test Performance vs. Direct Culture and PCR-Ribotyping

Direct Culture and PCR-Ribotyping					
		Toxin B+ 027+	**Toxin B+ 027-**	**NEG**	**Total**
Xpert C. *difficile* BT[b]	**Toxin B+ 027+**	74	4	47	125
	Toxin B+ 027-	0	164	140	304[a]
	NEG	0	3	1860	1863
	Total	74	171	2047	2292[a]
		Toxigenic *C. difficile*		**Toxigenic *C. difficile* / 027**	
		Sensitivity: 98.78% (242/245) Specificity: 90.86% (1860/2047) Accuracy: 91.71% (2102/2292) PPV[c]: 56.41% (242/429) NPV[d]: 99.84% (1860/1863)		Pos Agreement: 100% (74/74) Neg Agreement: 97.70% (2167/2218) Accuracy: 97.77% (2241/2292) PPV: 59.20% (74/125) 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 first attempt.

c. Positive predictive value

d. Negative predictive value

18.2.2 Performance vs. Reference Culture

Relative to reference culture with PCR-ribotyping, the Xpert *C. difficile* BT test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.39% and 94.02%, respectively. The Xpert *C. difficile* BT test also demonstrated a 98.89% positive agreement and 98.36% negative agreement for 027 (see Table 5).

Table 5. Xpert *C. difficile* BT Test Performance vs. Reference Culture and PCR-Ribotyping

		Reference Culture and PCR-Ribotyping			
		Toxin B+ 027+	**Toxin B+ 027-**	**NEG**	**Total**
Xpert *C. difficile* BT[b]	**Toxin B+ 027+**	89	5	31	125
	Toxin B+ 027-	0	217	86	303[a]
	NEG	1	21	1841	1863
	Total	90	243	1958	2291[a]
		Toxigenic *C. difficile*		**Toxigenic *C. difficile* / 027**	
		Sensitivity: 93.39% (311/333) Specificity: 94.02% (1841/1958) Accuracy: 93.93% (2152/2291) PPV[c]: 72.66% (311/428) NPV[d]: 98.82% (1841/1863)		Pos Agreement: 98.89% (89/90) Neg Agreement: 98.36% (2165/2201) Accuracy: 98.38% (2254/2291) PPV: 71.20% (89/125) NPV: 99.95% (2165/2166)	

a. Two isolates were not typeable due to contamination: the specimens are 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 first attempt.

c. Positive predictive value.

d. Negative predictive value.

18.2.3 Summary

The table below lists the total number of specimens for each different Test Result out of the 2293 specimens included in the clinical Performance data analysis.

Table 6. Xpert C. *difficile* BT Test Overall Performance

Test Result	N
Toxigenic C. diff POS; Binary Toxin NEG; O27 NEG	272
Toxigenic C. diff POS; Binary Toxin POS; O27 NEG	36
Toxigenic C. diff POS; Binary Toxin POS; O27 PRESUMPTIVE POS	122
Toxigenic C. diff NEG; Binary Toxin POS; O27 NEG	7[a]
Toxigenic C. diff NEG; Binary Toxin NEG; O27 NEG	1856
Total	**2293**

[a] In additional testing, 4 of 7 strains were shown to harbor the toxin B gene.

18.2.4 Antibiotic Usage

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

19 Analytical Performance

19.1 Analytical Specificity

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

Of the bacterial species that were tested, 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 $1.1x10^8$ to $2.2x10^{10}$ CFU/swab. Positive and negative controls were included in the study.

Under the conditions of the study, all isolates were reported **Toxigenic C. diff NEG; Binary Toxin NEG; 027 NEG** (see Table 7). The analytical specificity was 100%.

An additional series of non-*difficile Clostridium* species were tested to demonstrate the specificity of the binary toxin assay.

Table 7. Binary Toxin Gene Specificity Study Results

Genus	Species	Number Tested	Toxin A/B	Binary Toxin
Clostridium	*aldenense*	2	neg	neg
Clostridium	*aminovalericum-like*	2	neg	neg
Clostridium	*baratii*	2	neg	neg
Clostridium	*bartletti*	1	neg	neg
Clostridium	*bifermentans*	2	neg	neg
Clostridium	*bolteae*	2	neg	neg
Clostridium	*butyricum*	2	neg	neg
Clostridium	*cadaveris*	2	neg	neg
Clostridium	*celerecrescens*	2	neg	neg
Clostridium	*citroniae*	2	neg	neg
Clostridium	clostridioforme	2	neg	neg
Clostridium	*cochlearium*	1	neg	neg
Clostridium	*colicanis*	2	neg	neg
Clostridium	*disporicum*	1	neg	neg
Clostridium	*fallax*	2	neg	neg
Clostridium	*glycolicum*	2	neg	neg
Clostridium	*hastiforme*	1	neg	neg
Clostridium	*hathewayi*	2	neg	neg
Clostridium	*hylemonae*	2	neg	neg

Genus	Species	Number Tested	Toxin A/B	Binary Toxin
Clostridium	*innocuum*	2	neg	neg
Clostridium	*lactatifermentans*	2	neg	neg
Clostridium	*lavalense*	1	neg	neg
Clostridium	*limosum*	2	neg	neg
Clostridium	*mangenotii*	1	neg	neg
Clostridium	*mayombei-like*	1	neg	neg
Clostridium	*novyi*	2	neg	neg
Clostridium	*paraputrificum*	2	neg	neg
Clostridium	*perfringens*	2	neg	neg
Clostridium	*perfringens Type E*	3	neg	neg
Clostridium	*ramosum*	2	neg	neg
Clostridium	*sardiniense*	1	neg	neg
Clostridium	*scindens*	2	neg	neg
Clostridium	*septicum*	2	neg	neg
Clostridium	*sordellii*	2	neg	neg
Clostridium	*species*	19	neg	neg
Clostridium	*spiroforme*	1	neg	neg
Clostridium	*sporogenes*	2	neg	neg
Clostridium	*subterminale group*	3	neg	neg
Clostridium	*symbiosum*	2	neg	neg
Clostridium	*tertium*	2	neg	neg
Clostridium	*tetani*	1	neg	neg
Clostridium	*xylano/aerotolerans*	1	neg	neg
Clostridium	*difficile* RT 027	5	+	+
Clostridium	*difficile* RT 078	2	+	+

All the non-binary toxin containing isolates were negative with the Xpert C. *difficile* BT test.

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

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

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

Table 8. 95% Confidence Intervals for Analytical LoD–*C. difficile*

Strain ID	Toxinotype	$LoD_{95\%}$ (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)[a]	III	23	19	31
LUMC-5 (027)[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

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

In addition to the LoD determination, 18 *C. difficile* strains representing toxinotypes 0 plus 12 variant toxinotypes, including four 027 toxinotype III isolates, were tested using the Xpert *C. difficile* BT 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* BT Assay correctly identified all 18 strains tested as Toxigenic C. diff POS. 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* BT Assay. All four 027 isolates representing toxinotype III were correctly identified as Toxigenic C. diff POS; Binary Toxin POS; 027 PRESUMPTIVE POS.

Seven *C. difficile* isolates of PCR ribotype 033 and three additional *C. difficile* isolates of related PCR ribotype that were negative for tcdA and tcdB but produced binary toxin (CDT)[22] were tested with the Xpert *C. difficile* BT Assay. All 10 isolates yielded positive results for binary toxin only (see Table 9), confirming the ability of the assay to detect isolates that are Toxin A-, toxin B-, binary toxin +).

Table 9. Testing Organisms that Produce Binary Toxin Only (Toxin A-, Toxin B) with Xpert *C. difficile* BT Test

Organism	Strain ID	PCR Ribotype	Test Result
C. difficile	CD12-066	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	CD12-203	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	CD13-022	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	06-08-02	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	06-20-01	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	NT077	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	AI-0016	238	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	WA-0012	239	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	ES-0145	288	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	R-0010	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG

19.3 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* BT test. Potentially interfering substances include, but are not limited to, Vagisil cream and zinc oxide paste (see Section 16, Limitations). The 19 substances listed in the table below showed no detectable interference with the Xpert C. *difficile* BT test.

Table 10. Substances Tested and Showing No Test Interference

Substance	Substance
Whole Blood Karolinska University Hospital	K-Y Jelly/Gelée® McNeil-PPC
Mucin (porcine) Sigma	Vaseline Unilever
Kaopectate® Chattem	Dulcolax® Boehringer Ingelheim Pharmaceuticals
Immodium® McNeil-PPC	Preparation H Portable Wipes Wyeth Consumer Healthcare
Pepto-Bismol® Proctor & Gamble	Vaginal Contraceptive Film (VCF) Apothecus Pharmaceutical
Preparation H® Wyeth Consumer Healthcare	Vancomicin 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	

20 Reproducibility

A panel of 7 specimens with varying concentrations of toxigenic *C. difficile* and *C. difficile* Ribotype 027 were tested on 10 different days by 2 different operators at each of the 3 sites (7 specimens x 2 operators/ day x 10 days x 3 sites). One lot of Xpert C. *difficile* BT test was used at each of the 3 testing sites. Xpert C. *difficile* BT tests were performed according to the Xpert C. *difficile* BT test procedure. Results are summarized in the next two tables below.

Table 11. Summary of Reproducibility Results (All)

Specimen ID	% Agreement [a]			% Total Agreement by Sample
	Site 1	Site 2	Site 3	
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% (54/60)
Toxigenic *C. difficile* Moderate Positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* Ribotype 027 High Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* Ribotype 027 Low Positive	100% (20/20)	95% (19/20)	95% (19/20)	96.7% (58/60)
Toxigenic *C. difficile* Ribotype 027 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 12. 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 high neg	32.11	0.65	2.03%
027 low pos	31.93	0.72	2.26%
027 mod pos	31.96	0.61	1.90%
Neg	32.26	0.72	2.22%
***tcdB* (Toxin B)**			
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 high neg	39.11	0.98	2.50%
027 low pos	35.49	0.58	1.65%
027 mod pos	32.10	0.63	1.97%

An additional panel of 6 specimens, 3 negative and 3 toxigenic *C. difficile* high-negative, were tested on 5 different days by 2 different operators at each of the 3 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* BT test was used at each of the 3 testing sites. Xpert C. *difficile* BT tests were performed according to the Xpert C. *difficile* BTtest procedure. Results are summarized in the table below.

Table 13. Summary of Additional Reproducibility Specimen Results

Specimen ID	% Agreement [a]			% Total Agreement by Sample
	Site 1	Site 2	Site 3	
Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Toxigenic *C. difficile* High Negative[b]	60% (18/30)	60% (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

21 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. Goncalves C, Decre D, Barbut F, et al. Prevalence and characterization of a binary toxin (actin-specific ADP- ribosyl-transferase) from *Clostridium difficile*. J Clin Microbiol 2004;42:1933-1939
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;186:307-12.
11. Popoff MR, Rubin EJ, Gill DM, Boquet P. Action-specific ADP-ribotransferase produced by a *Clostridium difficile* strain. Infect Immun 1998;56:2299-2306.
12. 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 Jun;44(6):2147-2152.
13. Wilkins TD, Lyerly DM. *Clostridium difficile* testing: after 20 years, still challenging. Clin Microbiol. 2003 Feb;41:531-534.
14. Delmee M. Laboratory diagnosis of *Clostridium difficile* disease. Clin Microbiol Infect. 2001;7:411-416.
15. Bauer MP, Notermans DW, van Benthem BH, Brazier JS, et al. *Clostridium difficile* infection in Europe: a hospital-based survey. Lancet. 2011;377:63-73.
16. Walker AS, Eyre DW, Wyllie DH, et al. Relationship between bacterial strain type, host biomarkers, and mortality in *Clostridium difficile* infection. Clin Infect Dis. 2013; 56:1589-1600.
17. See I, Mu Y, Cohen J, Beldavs ZG, et al. NAP1 strain type predicts outcomes from *Clostridium difficile* infection. *Clin Infect Dis* 2014;58:1394-1400.
18. 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.
19. 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.
20. 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.
21. Eckert C, Emirian A, Le Monnier A, et al. Prevalence and pathogenicity of binary toxin-positive *Clostridium difficile* strains that do not produce toxins A and B. *New Microbes New Infect*. 2014:8;3:12-7.
22. Androga GO, McGovern AM, Elliott B, et al. Evaluation of the Cepheid Xpert *C. difficile/Epi* and meridian bioscience illumigene *C. difficile* assays for detecting *Clostridium difficile* ribotype 033 strains. *J Clin Microbiol.* 2015;53:973-5
23. 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.
24. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to latest edition).
25. 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).
26. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).
27. 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.

22 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

23 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.

24 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	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
CE	CE marking – European Conformity
	Temperature limitation
	Biological risks
	Warning
CH REP	Authorized Representative in Switzerland
	Importer

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Sweden

CH REP

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

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

IVD CE

25 Revision History

Description of Changes: 301-6190, Rev. D to Rev. E

Section	Description of Change
Analytical Sensitivity	Corrected error in "Analytical Sensitivity" section.
Table of Symbols	Corrected error in "Table of Symbols" section.