Assay Training: Xpert® *C.difficile* BT

Technical Training for CE-IVD product only

Cepheid®
A better way.

301-6996, Rev. A July 2016

Training Agenda

- **Xpert *C.difficile* BT Training**
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Precautions
  - Preparing cartridge
- Quality Control
- Results analysis
- Discussion and Q&A

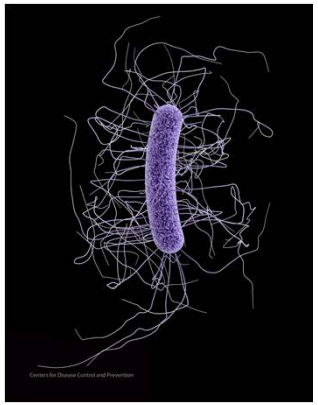
Xpert *C.difficile* BT Training Objectives

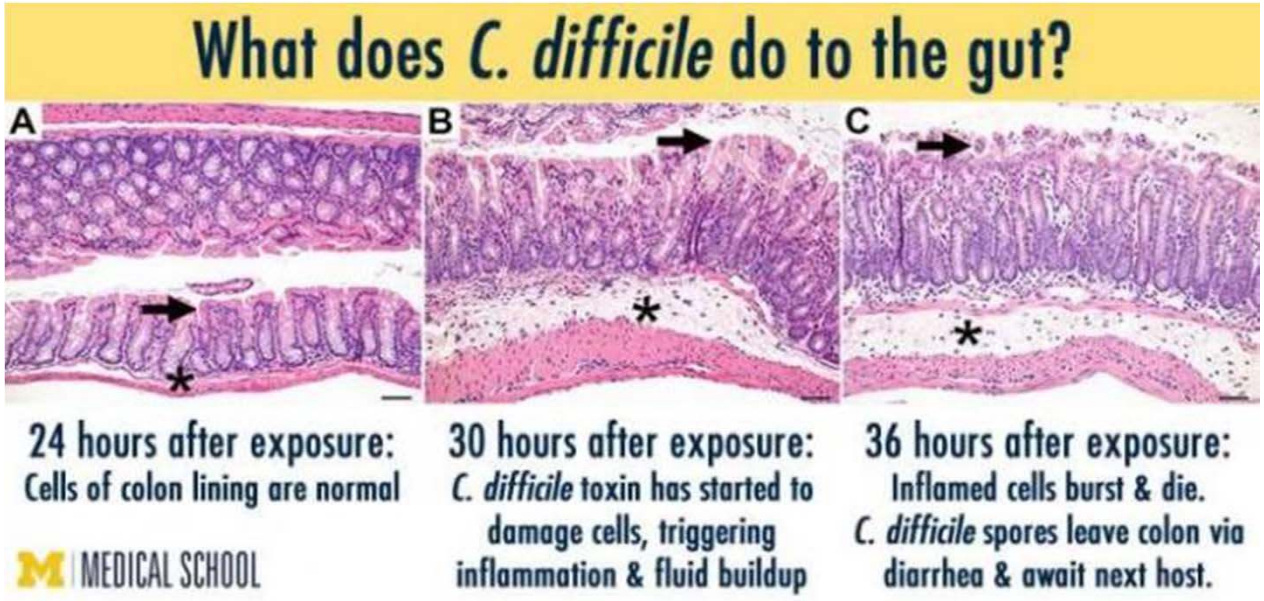
At the end of the training, user will be able to:

- Properly store and handle the Xpert *C.difficile* BT cartridge kit.
- Follow proper laboratory safety precautions.
- Identify appropriate specimen types and transport specimen.
- Prepare a cartridge and run the assay.
- Report and understand the various software generated-results.
- Understand the assay control strategy.

What is Clostridium difficile infection?

- The most common risk factor is exposure to antibiotics.





https://microbewiki.kenyon.edu/index.php/Clostridium_difficile-associated_disease
http://www.medicalnewstoday.com/articles/289817.php
http://www.cdc.gov/hai/organisms/cdiff/Cdiff-patient.html

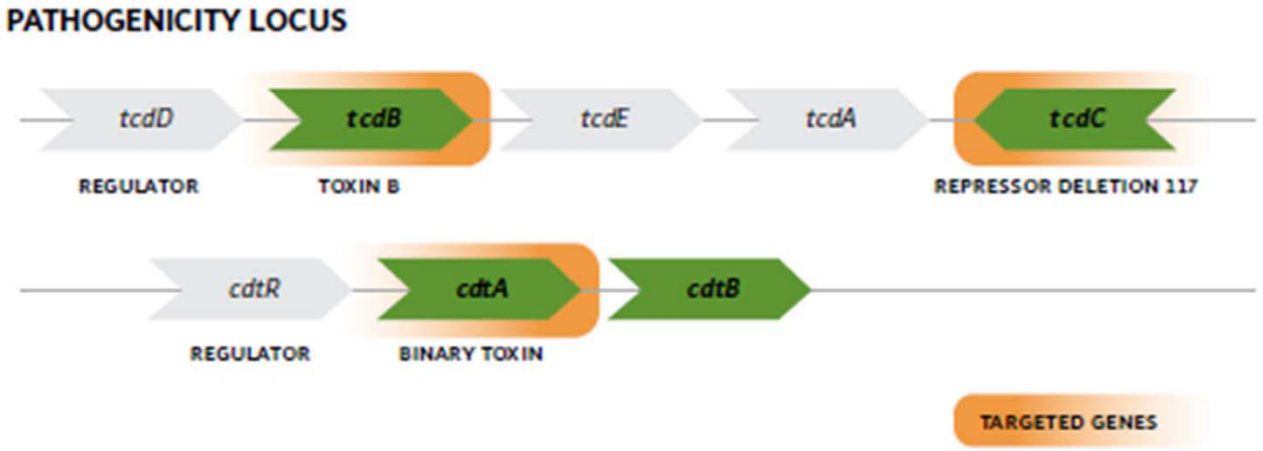
The Need

- **Numerous outbreaks caused by the epidemic strain of *C. difficile* (027/NAP1/BI strain) highlight the need for rapid and accurate differentiation of *C. difficile* 027/NAP1/BI strains in order to stay ahead of an outbreak.**
  - Higher risk of cross contamination with 027/NAP1/BI strain due to more efficient sporulation[1,2,3]
  - 027/NAP1/BI strain has been identified as a cause of outbreaks around the world[4,5,6,7,8]
  - Incremental costs due to *C. difficile* infection can be as high as $7,179[9] per patient
  - Binary toxin together with *tcdB* detection is often an indicator of more severe disease or recurrence of disease.

(1) Akerlund et al., Journal of Clinical Microbiology, 2008, p. 1530–1533; (2) Warny et al., Lancet 2005; 366: 1079–84; (3) Bartlett. Ann Intern Med. 2006;145:758-764; (4) Kalle et al., Infect Control Hosp Epidemiol 2009; 30:264-272; (5) Kuijer et al., Eurosurveillance Vol 12, Issues 3–6, Apr–Jun 2007; (6) Muto et al., Clinical Infectious Diseases 2007; 45:1266–73; (7) McDonald et al., New England Journal of Medicine 2005;353:2433-41; (8) Loo et al., New England Journal of Medicine 2005;353:2442-9; (9) Jarvis et al., American Journal of Infection Control, 2009;37:263-70

The Solution

- **Xpert *C. difficile* BT is the first commercially available test in the world to detect and differentiate the epidemic strain of *C. difficile* (027/NAP1/BI). With rapid and accurate identification of epidemic strain, Infection Control professional can stay ahead of potential outbreak situation.**
  - Innovative multiplex design enables detection of *C. difficile* and 027/NAP1/BI strain call-out in a single cartridge
- Cepheid's Xpert *C. difficile* BT test provides accurate, on-demand results to better manage patients.



The Cepheid Solution

- **Two controls for each individual sample**
  - Sample Processing Control (SPC)
  - Probe Check Control (PCC)
- **High sensitivity and specificity**
- **Simple and easy to use**
  - Closed cartridge system
- **On-demand results 24/7**
- **Random access**

CE-IVD. For in-vitro diagnostic use.

Intended Use

The Cepheid Xpert *C. difficile* BT Assay, 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 Assay 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 027/NAP1/BI strain.

Binary toxin is produced by a limited number of *C. difficile* strains, including the 027/NAP1/BI 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 (*cd*) 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.

System and Reagent Requirements

GeneXpert Systems

- GeneXpert Software, version 4.3 or higher

Test Kits (CE-IVD)

- GXCDIFFBT-CE-10

Materials Required but not Provided

- Dry swab:
  - Cepheid Sample Collection Device (Cepheid Catalog Number: 900-0370),
  - Cepheid Single-Use Disposable Swab (Cepheid Catalog Number SDPS-120)
- Disposable transfer pipettes
- Vortex mixer

Xpert *C.difficile* BT Kit Contents

Xpert C.difficile BT Assay	
Catalog Number	GXCDIFFBT-CE-10
Tests Per Kit	10
Cartridge Contents	Reagent beads
	Liquid Reagents
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Sample Reagent	Reagent Pouch
Reagent Samples per kit	10
Storage	2- 28 ˚C

CE-IVD. For in-vitro diagnostic use.

Cepheid

Good Laboratory Practice

PCR laboratory setup	• Cartridge/reagent preparation → Sample addition → Detection
Specimen and reagent storage	• Store specimens separately from reagents to prevent reagent contamination.
Equipment	• Use filtered pipette tips, when needed, for QC dilutions. • Follow the manufacturer's recommendation for calibration and maintenance of the lab equipment.

Good Laboratory Practice, cont'd

Housekeeping

- Clean work surfaces with a final concentration of 1:10 dilution of household bleach* and then 70% ethanol. Wipe work surfaces dry.
- If contamination occurs, thoroughly clean the contaminated area with a solution of a 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.

Personnel

- Wear clean lab coats and gloves.
- Change gloves between processing samples.

Lab bench area

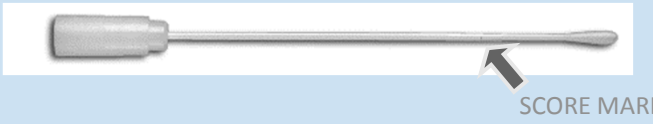
- Clean the lab bench area routinely.
- Keep the back of the instrument dust free.

** Final Active Chlorine concentration should be 0.5% regardless of the household bleach concentration in your country*

Xpert *C.difficile* BT Kit Storage and Handling

- **Store test kits at 2-28ºC. Do not use expired cartridges.**
- **Each single-use cartridge is used to process one test. Do not reuse processed cartridges.**
- **Do not open a cartridge until ready to use.**
  - Start the test within 30 minutes of adding the sample to the cartridge.
- **Avoid cross contamination during sample handling steps.**
  - Change gloves if they come in contact with specimen or appear to be wet.
  - Change gloves before leaving work area and upon entry into work area.
- **Do not use a cartridge that has been dropped or shaken after the sample has been transferred to 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 use a cartridge that has leaked.**

Cepheid Sample Collection

Collection Device	Part Number	
Cepheid Sample Collection Device	900-0370	
Copan Dual Swab and Transport System*	139CFM LQ STUART	
Cepheid Single-Use Disposable Swab	SDPS-120	

***This collection device does not have a score mark to indicate where to break the swab.**

Specimen Collection and Storage

1. Collect the unformed stool specimen in a clean container. Follow your institution's guidelines for collecting samples for *C.difficile* testing.

2. Label the sample with a Sample ID and send it to the laboratory.

Specimen	Transport and Storage Temperature (°C)	Storage Time
Unformed Stool	2-8 °C	5 days
	20-30 °C	24 hours

Stool Sample Preparation

1

Briefly place a swab in the unformed stool sample.

2

Obtain the correct amount of sample on the swab. See photos for sample amount to use for the test

Too little sample

Correct amount of sample

Too much sample

CE-IVD. For in-vitro diagnostic use.

Cepheid®

Xpert *C.difficile* BT Cartridge Preparation

Xpert® Cartridge Preparation

- Xpert MRSA
- Xpert MRSA/SA SSTI
- Xpert C. difficile
- Xpert C. difficile BT
- Xpert SA Nasal Complete
- Xpert vanA/vanB
- Xpert Norovirus

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

Cepheid Technical Support

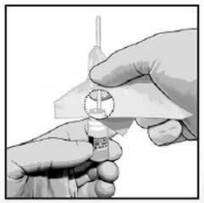
US office
(888) 838-3222, Option 2
techsupport@cepheid.com

European office
+33 563 82 53 19
support@cepheideurope.com

Cepheid
A better way.

1 Obtain one Xpert cartridge and one Sample Reagent vial for each sample.

2 Insert the swab into the Sample Reagent vial.



3 Break the swab at the score mark near the opening of the vial.

4 Recap the Sample Reagent vial and vortex for 10 seconds.

5 Open the Xpert cartridge lid.

6 Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.

7 Empty the pipette into the sample chamber.

8 Close the Xpert cartridge lid.

9 Start the test within the timeframe specified in the package insert.

Note: Do not hold the swab below the score mark. Use gauze or its equivalent to minimize the risk of contamination.

301-1049, Rev. C July 2016

CE-IVD. For in-vitro diagnostic use.

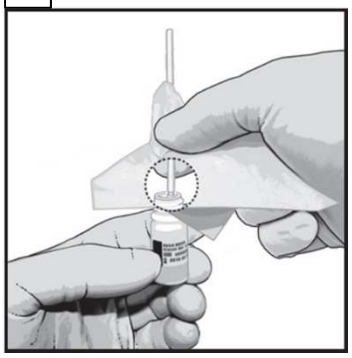
Cepheid

C.difficile BT Cartridge Preparation

1

Obtain one Xpert cartridge and one Sample Reagent vial for each sample.

2



Insert the swab into the Sample Reagent vial.

3

Break the swab at the score mark near the mouth of the vial.

4

Recap the Sample Reagent vial and vortex for 10 seconds.

5

Open the Xpert cartridge lid.

6

Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.

7

Empty the pipette into the sample chamber.

8

Close the Xpert cartridge lid.

9

Start the test within the timeframe specified in the package insert.

Note: Do not hold the swab below the score mark. Use gauze or its equivalent to minimize the risk of contamination.

CE-IVD. For in-vitro diagnostic use.

301-1049, Rev. C July 2016

Cepheid®

Automated Xpert *C. difficile* BT Load Test Steps

1. Add the sample to the cartridge.
2. Place the cartridge into the instrument.
3. Nucleic acids are purified.
4. Purified nucleic acids mix with PCR reagents.
5. Simultaneous amplification and detection occurs.
6. Results are ready to view.

CE-IVD. For in-vitro diagnostic use.

Cepheid

Quality Control

Refer to the Package Insert for complete details

Cepheid®

Instrument System Control – Check Status

- **System control checks the optics, temperature of the module, and mechanical integrity of each cartridge.**
  - If the system controls fail, an ERROR test result will be reported.

Cepheid Assay Control Strategy

- **Each Xpert cartridge is a self-contained test device.**
  - Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge.
    - Probe Check Control: PCC
    - Sample Processing Control: SPC

Probe Check Control - PCC

- **After sample preparation, bead reconstitution, and tube filling (prior to thermal cycling), multiple fluorescent readings are taken at different temperatures**
- **The readings are compared to default settings established by Cepheid**
- **The Probe Check feature controls for**
  - Missing Target Specific Reagent (TSR) beads, also controls for missing EZR beads, which contain all primers, probes, and internal control template
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Probe degradation
- **If the Probe Check fails, an ERROR test result will be reported.**

Sample Processing Controls - SPC

- **The Sample Processing Control (SPC) assesses the effectiveness of the sample preparation steps, including reaction tube filling.**
- **SPC is spores from *Bacillus globigii* bacteria.**
- **The SPC controls for:**
  - Missing primer/probe or enzyme beads
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Enzyme degradation
  - Sample lysis, nucleic acid extraction, and integrity of nucleic acid
  - Sample inhibition
- **The SPC can be negative or positive in an analyte-positive sample.**
- **If the SPC fails in an analyte-negative sample, an INVALID test result will be reported.**

Commercially Available External Controls

ZeptoMetrix Catalog Number	Control Type	Configuration	Storage Temp
NATCDI-6MC	C.difficile –NAP1 positive control, inactivated organisms	6 x 0.5mL	2-8°C
NATCSO-6MC	C.sordelli negative control, inactivated organisms	6 x 0.5mL	2-8°C
http://www.zeptometrix.com			

Procedure

1. Vortex NATtrol™ sample for 5-10s.
2. Add 50µL into sample reagent.
3. Mix well by vortexing for 5-10s.

Other options:

- Known patient positive and negative samples
- Microbiologics KWIK-STIK ™

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

Results Analysis

Refer to the Package Insert for complete details

Cepheid

Algorithm

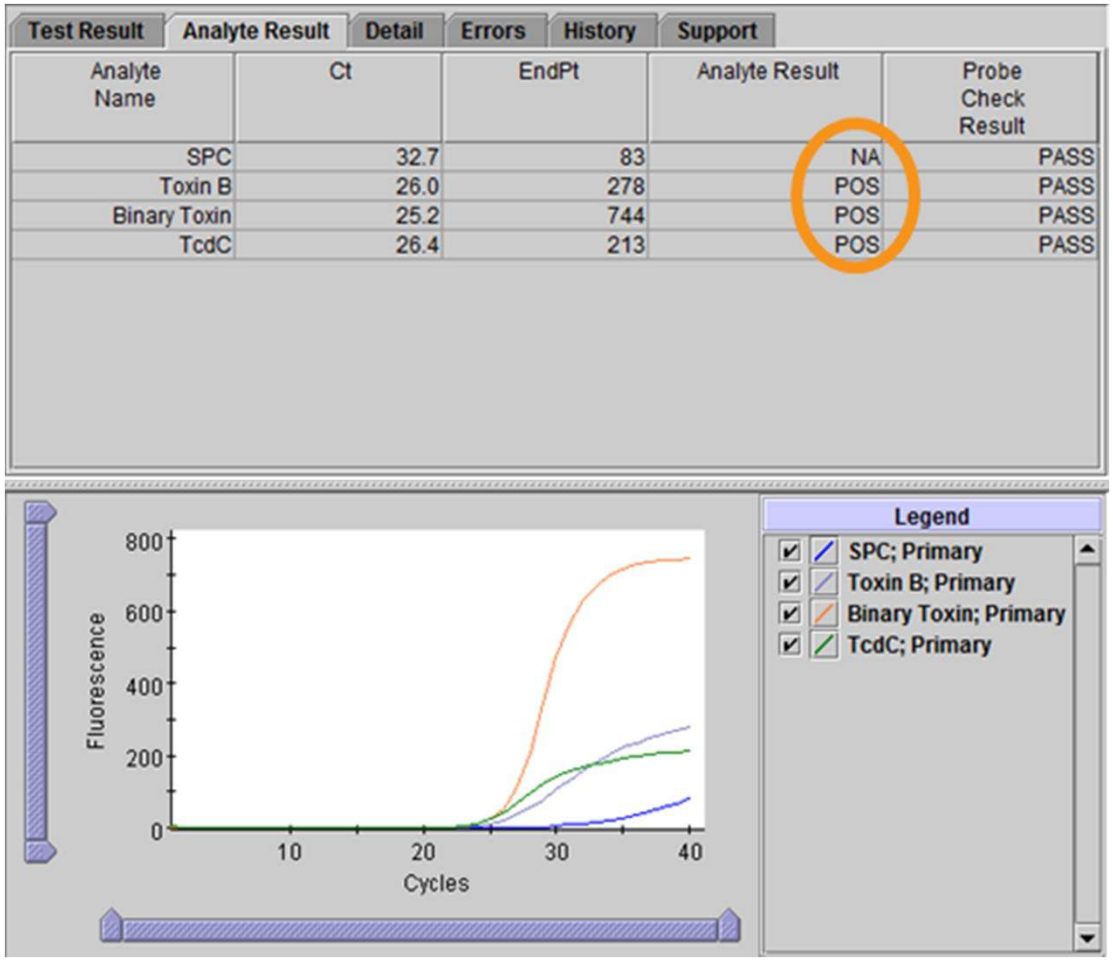
Result displayed	Toxin B	Binary Toxin	TcdC	SPC
Toxin B POS Binary Toxin POS 027 PRESUMPTIVE POS	+	+	+	+/-
Toxin B POS Binary Toxin NEG 027 NEG	+	-	-	+/-
Toxin B POS Binary Toxin POS 027 NEG	+	+	-	+/-
Toxin B NEG Binary Toxin POS 027 NEG	-	+	-	+/-
Toxin B NEG Binary Toxin NEG 027 NEG	-	-	-	+
Invalid	-	-	-	-

Toxigenic C.diff POS, Binary Toxin POS, 027 PRESUMPTIVE POS

Toxigenic C.diff POS;
Binary Toxin POS;
027 PRESUMPTIVE POS

Toxigenic *C. difficile* POSITIVE

- Toxin-producing *C. difficile* and presumptive 027/NAP1/BI target DNA sequences are detected.
- All toxin-producing toxin-producing *C. difficile*, presumptive 027/NAP1/BI targets (toxin B, binary toxin and *tcdC* deletion and nt 117) have Cts within the valid range and endpoint above the minimum setting.
- SPC—NA (not applicable); SPC is ignored because *C.difficile* target amplification may compete with this control.
- Probe Check—PASS; All probe check results pass.



CE-IVD. For in-vitro diagnostic use.

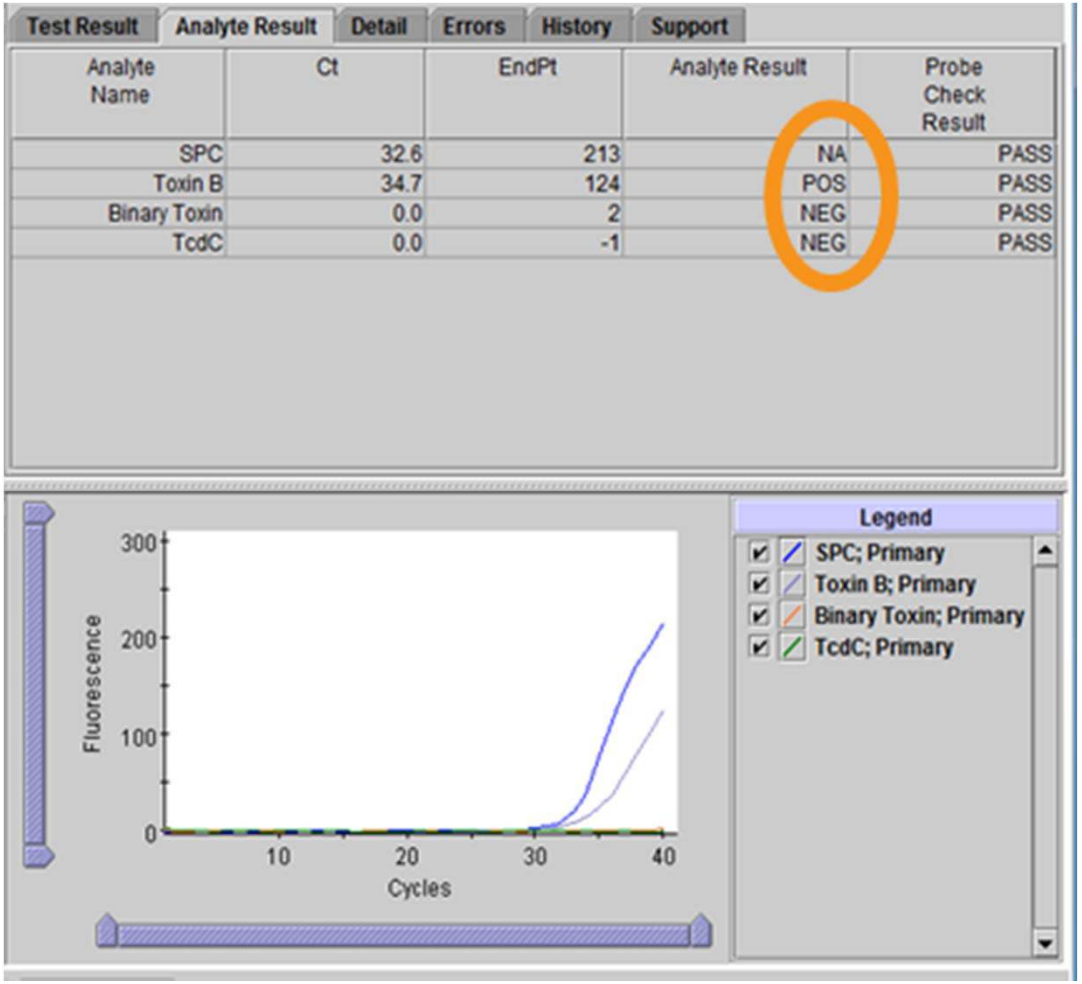
Cepheid

Toxigenic C.diff POS, Binary Toxin NEG, 027 NEG

Toxigenic C.diff POS;
Binary Toxin NEG;
027 NEG

Toxigenic *C.difficile* POSITIVE

- Toxin-producing *C.difficile* target DNA sequences are detected.
- The toxin-producing *C. difficile* target (toxin B) has a Ct within the valid range and an endpoint above the minimum setting.
- SPC—NA (not applicable); SPC is ignored because *C.difficile* target amplification may compete with this control.
- Probe Check—PASS; All probe check results pass.



CE-IVD. For in-vitro diagnostic use.

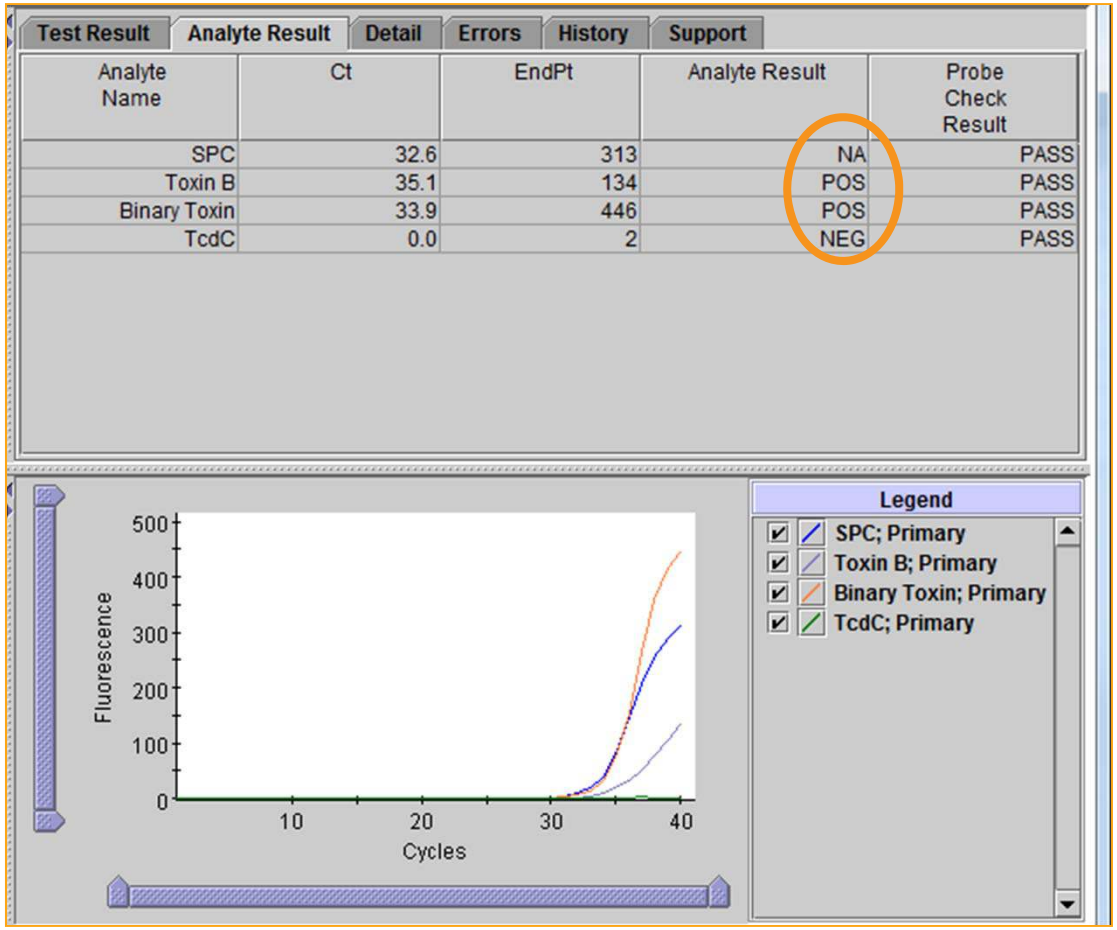
Cepheid

Toxigenic C.diff POS, Binary Toxin POS, 027 NEG

Toxigenic C.diff POS;
Binary Toxin POS;
027 NEG

Toxigenic *C.difficile* POSITIVE

- Toxin-producing *C.difficile* target DNA sequences are detected.
- Toxin-producing *C. difficile* targets (toxin B plus binary toxin) have Cts within the valid range and endpoints above the minimum setting
- SPC—NA (not applicable); SPC is ignored because *C.difficile* target amplification may compete with this control.
- Probe Check—PASS; All probe check results pass.



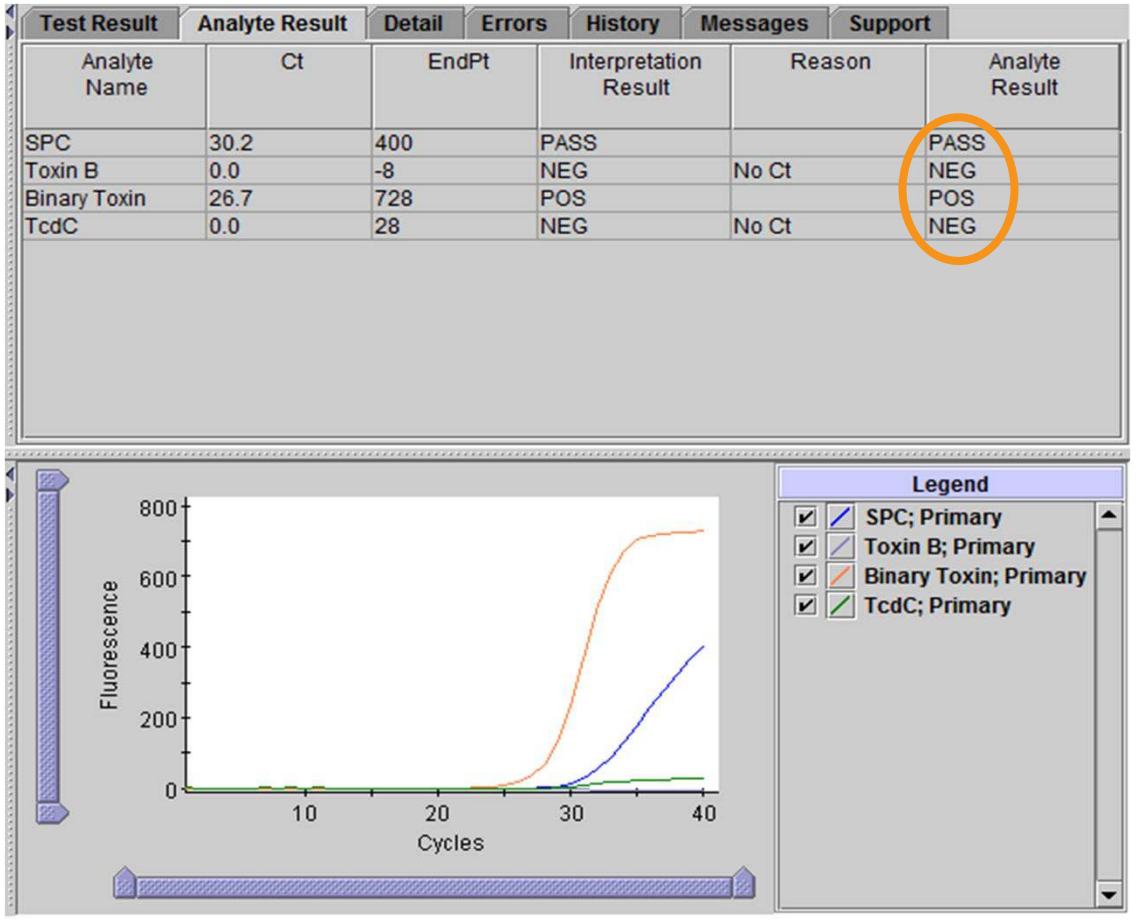
Cepheid

Toxigenic C.diff NEG, Binary Toxin POS, 027 NEG

Toxigenic C.diff NEG;
Binary Toxin POS;
027 NEG

Toxigenic *C.difficile* NEGATIVE

- *C. difficile* toxin B sequences are NEG; however, another DNA target (binary toxin) is detected and has a Ct within the valid range and an endpoint above the minimum setting.
- SPC—NA (not applicable); SPC is ignored because *C.difficile* target amplification may compete with this control.
- Probe Check—PASS; All probe check results pass.

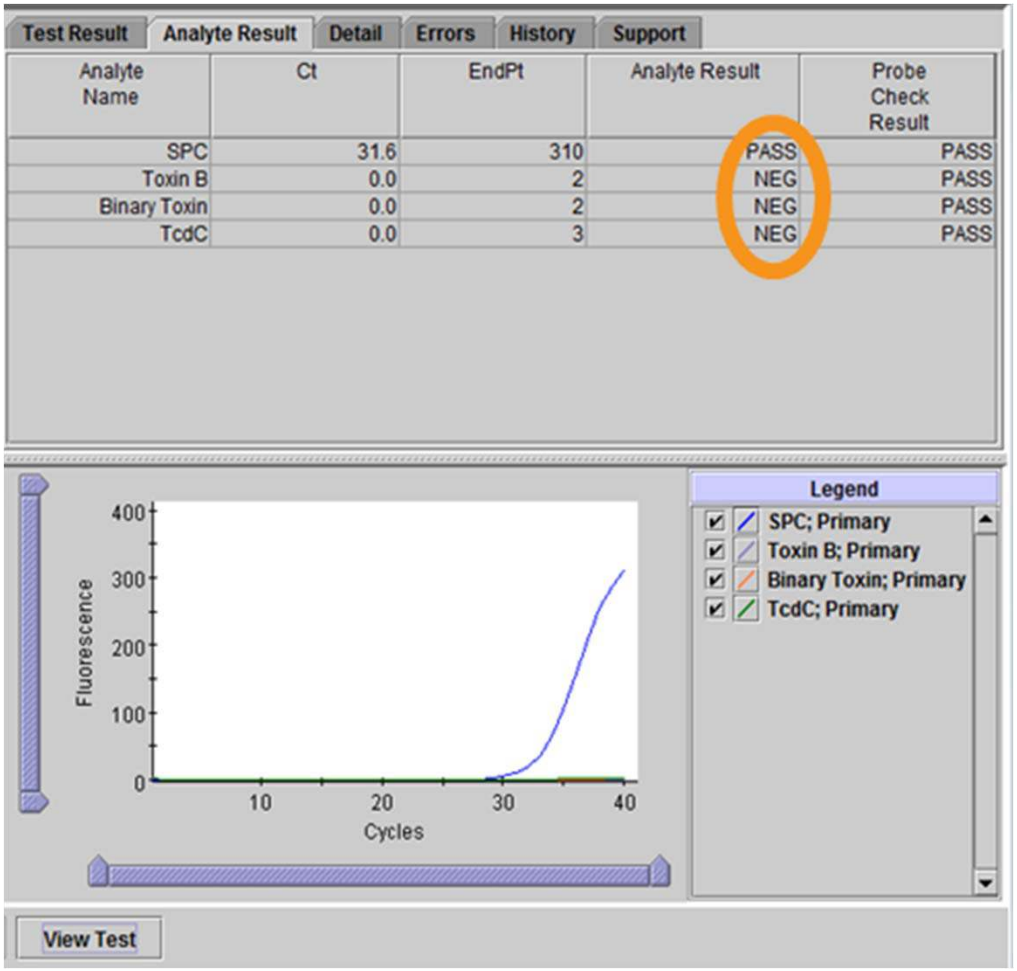


Toxigenic C.diff NEG, Binary Toxin NEG, 027 NEG

Toxigenic C.diff NEG;
Binary Toxin NEG;
027 NEG

Toxigenic *C.difficile* Negative

- Toxin-producing *C. difficile* sequences (toxin B and binary toxin) are not detected; other DNA targets for toxigenic *C.difficile* (*tcdC* deletion at nt 117) are not detected.
- SPC—NA (not applicable);
  SPC is ignored because *C.difficile* target amplification may compete with this control.
- Probe Check—PASS;
  All probe check results pass.



CE-IVD. For in-vitro diagnostic use.

Cepheid

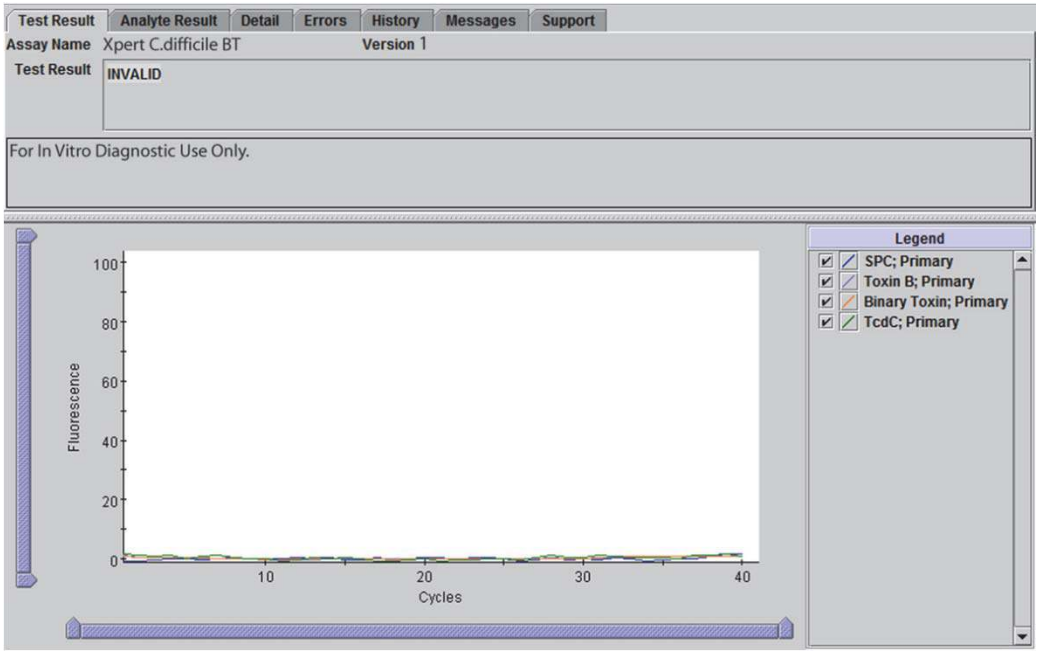
Reasons to Repeat the Assay

- **An INVALID result indicates that the sample was not properly processed, PCR was inhibited, or the sample was inadequate.**
- **An ERROR result indicates that the Probe Check Control failed, maximum pressure limits were exceeded, or a hardware failure.**
- **A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress a load error occurred, or the software was closed prematurely.**

Invalid

INVALID

- Presence or absence of *C.difficile* target DNA cannot be determined. Repeat the test according to the instructions in the Retest Procedure section of the package insert. SPC does not meet acceptance criteria, the sample was not properly processed or PCR is inhibited.



- SPC—FAIL:
  The SPC target result is negative, and the SPC Ct is not valid.

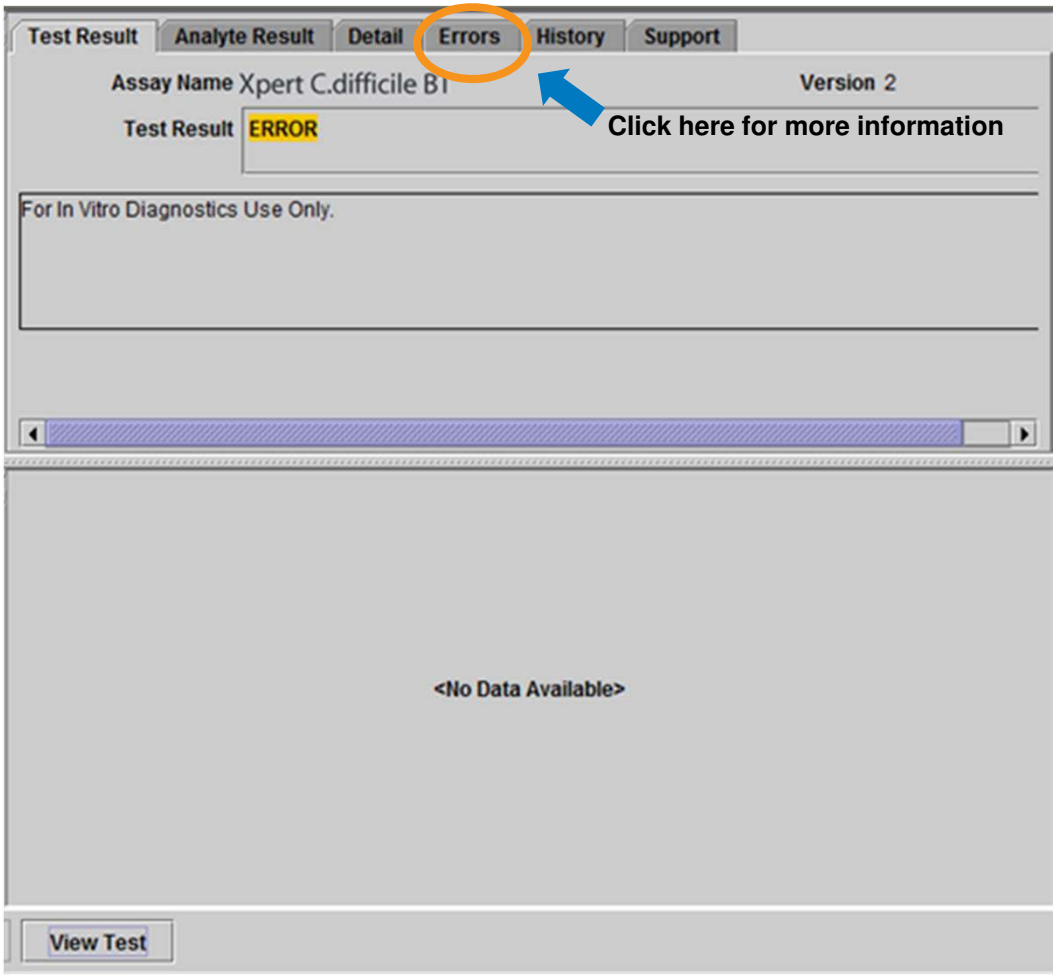
- Probe Check—PASS:
  All probe check results pass.

Error

ERROR

- Presence or absence of *C.difficile* cannot be determined. Repeat the test using the steps in the package insert.
- The Probe Check control failed probably because the reaction tube was filled improperly, a probe integrity problem was detected, or because the maximum pressure limits were exceeded.
- Toxin producing *C.difficile* targets yield NO RESULT
- Probe Check—FAIL*:
  One or more of the probe check results fail

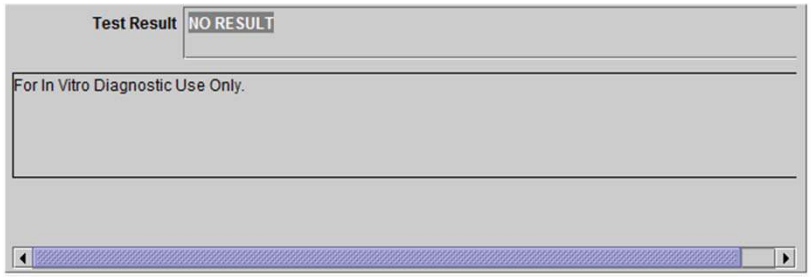
* If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range.



No Result

NO RESULT

- Presence or absence of *C.difficile* cannot be determined. Repeat the test using the steps in the package insert.
- 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)

Retest Procedure

Xpert Retest Procedure

- Xpert C. difficile
- Xpert C. difficile BT
- Xpert MRSA/SA SSTI
- Xpert van*A*/vanB

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit
www.cepheid.com or
www.cepheidinternational.com

Cepheid Technical Support

US office
(888) 838-3222, Option 2
techsupport@cepheid.com

European office
+33 563 82 53 19
support@cepheideurope.com

Follow this retest procedure within 3 hours of an **ERROR**, **INVALID**, or **NO RESULT**.

Otherwise:
Retest with the remaining sample if the volume is sufficient
or
Collect a new sample and process the sample per the package insert.

Cepheid
A better way.

1 Retain the used cartridge. Obtain a new Xpert cartridge and a new Sample Reagent vial.

2 Transfer all the remaining contents from the Sample chamber of the used cartridge to a new Sample Reagent vial.

3 Recap the Sample Reagent vial and vortex for 10 seconds.

4 Open the new Xpert cartridge lid.

5 Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.

6 Empty the pipette into the sample chamber.

7 Close the Xpert cartridge lid and start the test within the timeframe specified in the package insert.

301-6925 Rev. A July 2016

CE-IVD. For in-vitro diagnostic use.

Cepheid

Retest Procedure

1

Retain the used cartridge. Obtain a new Xpert cartridge and a new Sample Reagent vial.

2

Transfer all the remaining contents from the Sample chamber of the used cartridge to a new Sample Reagent vial.

3

Recap the Sample Reagent vial and vortex for 10 seconds.

4

Open the new Xpert cartridge lid.

5

Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.

6

Empty the pipette into the sample chamber.

7

Close the Xpert cartridge lid and start the test within the timeframe specified in the package insert.

Follow the retest procedure within 3 hours of an **ERROR, INVALID,** or **NO RESULT**.

Otherwise:
Retest with the remaining sample if the volume is sufficient
or
Collect a new sample and process the sample per the package insert.

CE-IVD. For in-vitro diagnostic use.

Cepheid®

Factors That Negatively Affect Results

- **Improper specimen collection**
  - Performance with other collection devices and specimen types has not been assessed.
- **Improper transport or storage of collected specimen**
  - Storage and transport conditions are specimen specific.
  - Refer to the Package Insert for the appropriate handling instructions.
- **Improper testing procedure**
  - Modification to the testing procedures may alter the performance of the test.
  - Technical error or sample mix-up can impact test results.
  - Careful compliance with the package insert is necessary to avoid erroneous results.
- **Interfering substances**
  - False negative test results or invalid results may be observed in the presence of interfering substances.
- **The number of organisms in the specimen is below the detection limit of the test**
- **See package insert for comprehensive information regarding the factors that affect the assay performance**

Technical Support

Cepheid provides technical support in the field, on the phone, by fax, and by email.

- **Contact information for other Cepheid offices is available on our website at www.cepheid.com or www.cepheidinternational.com under the SUPPORT tab. Select the Contact Us option.**

Discussion and Q&A


GeneXpert
Thank You.
Visit us at www.cepheid.com
Cepheid®
A better way.