# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY
ASSAY AND INSTRUMENT

## I Background Information:

### A 510(k) Number

K243730

### B Applicant

Cepheid

### C Proprietary and Established Names

Xpert *C. difficile/Epi*

### D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|---|---|---|---|
| OZN | Class II | 21 CFR §866.3130 – *Clostridium difficile* Toxin Gene Amplification Assay | MI – Microbiology |
| OOI | Class II | 21 CFR §862.2570 – Instrumentation for clinical multiplex test systems | CH – Clinical Chemistry |

## II Submission/Device Overview:

### A Purpose for Submission:

1. To obtain a substantial equivalence determination for Cepheid's Xpert *C. difficile/Epi* test for use with Cepheid-manufactured GeneXpert Instrument Systems family of instruments.

2. To update device labeling to reflect the inclusion of a member of the GeneXpert Instrument Systems family—namely, the *GeneXpert Infinity System*, which includes the instrument models GeneXpert Infinity-48s and GeneXpert Infinity-80, along with a new systems software (*GeneXpert Xpertise*)—in addition to the other members of the same family, namely, the *GeneXpert Dx System* (previously cleared for use with Xpert *C. difficile/Epi* Assay in K110203) and the *GeneXpert System with Touchscreen* (previously added to this test with CR240176).

### B Measurand:

Toxin B gene of *Clostridioides difficile*; binary toxin (CDT) gene and a single base pair deletion at nucleotide 117 in the *tcdC* gene (a.k.a. *tcdC*Δ117) present in strain 027/NAP1/BI of toxigenic *C. difficile*.

### C Type of Test:

Qualitative nucleic acid (DNA) amplification test using real time polymerase chain reaction (PCR) that utilizes primers and TaqMan probes specific for toxigenic *C. difficile* toxin genes with fluorogenic target-specific hybridization.

## III Intended Use/Indications for Use:

### A Intended Use(s):

See Indications for Use below.

### B Indication(s) for Use:

The Xpert *C. difficile/Epi* test is a qualitative *in vitro* diagnostic test for detection of toxin B gene sequences and for presumptive identification of 027/NAP1/BI strains of toxigenic *Clostridioides difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection (CDI). Presumptive identification of 027/NAP1/BI strains of *C. difficile* is by detection of binary toxin (CDT) gene sequences and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a negative regulator in *C. difficile* toxin production. The test is performed on the GeneXpert Instrument Systems and utilizes automated real-time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Xpert *C. difficile/Epi* test is intended as an aid in the diagnosis of CDI. Detection of 027/NAP1/BI strains of *C. difficile* by the Xpert *C. difficile/Epi* test is presumptive and is solely for epidemiological purposes and is not intended to guide or monitor treatment for *C. difficile* infections. Concomitant culture is necessary only if further typing or organism recovery is required.

### C Special Conditions for Use Statement(s):

Rx – For Prescription Use Only
IVD – For In Vitro Diagnostic Use Only

### D Special Instrument Requirements:

- Cepheid *GeneXpert Instrument Systems* family of instruments: GX-II, GX-IV, GX-XVI, GeneXpert Infinity-48s and GeneXpert Infinity-80.
- Systems software guiding the instrument functions, namely, *GeneXpert Dx* with GeneXpert Dx System or alternatively *Cepheid OS* with GeneXpert System with Touchscreen (for instruments GX-II, GX-IV, GX-XVI), and *GeneXpert Xpertise* for GeneXpert Infinity System (for instruments GeneXpert Infinity-48s and GeneXpert Infinity-80).

## IV Device/System Characteristics:

### A Device Description:

The candidate device, Xpert *C. difficile/Epi*, is an automated *in vitro* diagnostic test for qualitative detection of toxin producing *Clostridioides* (genus formerly known as "*Clostridium*") *difficile* directly from unformed (liquid or soft) stool specimens of patients suspected of having *C. difficile* infection (CDI). The Xpert *C. difficile/Epi* test reagents are provided in two (2) pack sizes, GXCDIFF/EPI-10 (pack size of 10 tests as previously cleared with K110203) and GXCDIFF/EPI-120 (for 120 tests, proposed with K243730).

The Xpert *C. difficile/Epi* test is performed on the GeneXpert Instrument Systems (comprised of the GeneXpert Dx System, GeneXpert System with Touchscreen, and GeneXpert Infinity System), as detailed in Section IV.C.(1) below.

### B Principle of Operation:

The test detects the toxin B gene (*tcdB*), the gene (*cdt*) encoding the binary toxin (CDT), and the single base pair deletion (a.k.a. *tcdC*Δ117) at nucleotide 117 within the gene *tcdC* encoding a negative regulator of toxin production in toxigenic *C. difficile*. The combined presence of the genes encoding

toxin B and the binary toxin and the *tcdC*Δ117 deletion has been associated with a hypervirulent *C. difficile* strain known as 027/NAP1/BI which has been associated with severe disease outbreaks in healthcare facilities worldwide.

The Xpert *C. difficile/Epi* test, performed on the GeneXpert Instrument Systems, carries out sample preparation (including an initial sample processing step) and real-time, multiplex polymerase chain reaction (PCR) for the detection of DNA specific for multiple *C. difficile* targets as indicated above. The Xpert *C. difficile/Epi* test utilizes primers and TaqMan probes specific for toxigenic *C. difficile* toxin genes with fluorogenic target-specific hybridization and includes reagents for the detection of toxigenic *C. difficile* and the presumptive detection of sequences found in 027/NAP1/BI strains. In addition, the test reagents include two (2) internal controls, a sample processing control (SPC) and a Probe Check Control (PCC). The SPC controls for adequate processing of the target bacteria, monitors the presence of inhibitor(s) in the PCR test, and also ensures that the PCR conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes (i.e., the PCC) to verify bead rehydration, PCR reaction tube filling in the cartridge, probe integrity, and dye stability.

For the performance of the Xpert *C. difficile/Epi* test, the kit labeling (i.e., package insert) includes an Assay Definition File (ADF) which defines assay parameters and instrument instructions (e.g., PCR parameters, Ct cutoffs, algorithm for determining assay results as well as reportable results, maximum allowable prepared cartridge hold time) for the members of the GeneXpert Instrument Systems family of instruments.

The Xpert *C. difficile/Epi* test provides test results for both detection of toxigenic *C. difficile* and presumptive identification of the 027/NAP1/BI strain of *C. difficile* according to the following algorithms presented in **Tables 1** and **2** below.

**Table 1. Toxigenic *C. difficile (C. diff)* Results Algorithm**

| Toxin B | Test Result |
|---|---|
| NEG | Toxigenic C. diff NEGATIVE* |
| POS | Toxigenic C. diff POSITIVE |
| *If SPC and/or any other target is INVALID, overall test result is INVALID | |

**Table 2. Results Algorithm for Presumptive 027 (i.e., strain 027/NAP1/BI)**

| Targets | | | Test Result |
|---|---|---|---|
| Toxin B | Binary Toxin | *tcdC*Δ117 | |
| NEG | NEG | NEG | 027 PRESUMPTIVE NEGATIVE* |
| NEG | POS | NEG | 027 PRESUMPTIVE NEGATIVE* |
| NEG | NEG | POS | 027 PRESUMPTIVE NEGATIVE* |
| POS | POS | POS | 027 PRESUMPTIVE POSITIVE |
| *If SPC and/or any other target is INVALID, overall test result is INVALID | | | |

## C Instrument Description Information:

1. Instrument Name:
   ***GeneXpert Infinity System*** is a relatively new member of the Cepheid-manufactured family of instruments, GeneXpert Instrument Systems. The currently marketed Xpert *C. difficile/Epi* test is intended for use on two (2) prior members of this family, i.e., the *GeneXpert Dx System* (as cleared under K110203) and the *GeneXpert System with Touchscreen* (as included under CR240176). The current submission (K243730) proposes to include yet another member of the same instrument family—i.e., the *GeneXpert Infinity System*—for conducting the Xpert *C. difficile/Epi* test.

Encompassing all three (3) instruments, the family name *GeneXpert Instrument Systems* is incorporated in the candidate device's Intended Use/Indications for Use statement.

Each member of the GeneXpert Instrument Systems family (as presented in **Table 3** below) consists of a GeneXpert/GX instrument along with personal computers preloaded with software for systems operations (e.g., running tests by following instructions from an Assay Definition File) and analysis (e.g., analysis and conversion of raw PCR data into reportable test results). During the Xpert *C. difficile/Epi* test, the Windows 10 IoT Enterprise Operating System (OS)-based personal computers (PC)—a standalone PC and an embedded PC—run the following resident Systems software applications, namely, (a) GeneXpert Dx (v2.1 or higher), (b) Cepheid OS (v2.1 or higher), and (c) GeneXpert Xpertise (v6.8 or higher; currently at v7.1), that are specific to instrument models, as shown in **Table 3**.

**Table 3. GeneXpert Instrument Systems family (instruments and systems software)**

| Instrument Family Members | Instrument Systems | Systems Software | Instrument models with the number of accessible modules in parentheses |
|---|---|---|---|
| GeneXpert Instrument Systems | GeneXpert Dx System | *GeneXpert Dx* | • GX-I (1)†<br>• GX-II (1–2)<br>• GX-IV (1–4)<br>• GX-XVI (1–16) |
| | GeneXpert System with Touchscreen | *Cepheid OS* | • GX-II (1–2)<br>• GX-IV (1–4)<br>• GX-XVI (1–16) |
| | GeneXpert Infinity System | *GeneXpert Xpertise* | • GeneXpert Infinity-48s (1–48)<br>• GeneXpert Infinity-80 (1–80) |

† No longer manufactured or distributed in the US but is in circulation in the market

2. Specimen Identification:
   Unformed (liquid or soft) stool specimens from patients suspected of having *C. difficile* infection (CDI).

3. Specimen Sampling and Handling:
   A double swab is inserted into the stool specimen and then placed in a tube containing Sample Reagent. Following brief vortexing, the content of the Sample Reagent is transferred to a different, uniquely labeled Sample Chamber of the disposable fluidic cartridge (i.e., the Xpert *C. difficile/Epi* cartridge).

   In the GeneXpert Instrument Systems platform, sample preparation, amplification, and real-time detection are all fully automated and completely integrated. The single-use disposable cartridges (required for use on the platform) hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. In the device workflow, the user initiates a test from the system user interface of the GeneXpert Instrument Systems platform and places the cartridge into the GeneXpert instrument which performs hands-off real-time, multiplex PCR for the detection of *C. difficile* DNA.

   Depending on the specific instrument, the GeneXpert instrument system may contain 1–80 modules, each of which are randomly accessible and capable of performing separate sample preparation and real-time PCR tests for the detection of *C. difficile* toxin B and binary toxin gene sequences and the *tcdC*Δ117 deletion in <45 minutes. Each module contains a syringe drive for dispensing fluids (i.e., the syringe drive activates the plunger that works in concert with the rotary

valve in the cartridge to move fluids between chambers), an ultrasonic horn for lysing cells or spores, and a proprietary I-CORE thermocycler for performing real-time PCR and detection.

4. Calibration:
   (a) As indicated in the GeneXpert Infinity system operator's manual, Cepheid performs all necessary calibrations before the system is shipped, and calibration of the GeneXpert instrument is not required during the initial system setup. Multiple calibration processes are undertaken during manufacturing, e.g.,
      (i) The thermal reaction chamber thermistors are calibrated to ±1.0°C using National Institute of Standards and Technology (NIST) traceable standards.
      (ii) Temperature of the heating system is measured at 60°C and 95°C to generate calibration coefficients which correct for small errors in the raw thermistor readings of the heaters and are stored in the memory of each I-CORE module.
      (iii) Using standard concentrations of individual unquenched fluorescent dye-oligos, the optical system is calibrated per channel against the blank (a tube alone) signal. The blank is subtracted from the raw signal produced by the dye-oligo standard to determine the spectral characteristics. Using the individual spectral characteristics of the pure dye-oligos, signals from an unknown mixture of dye-oligos can be resolved into corrected signals for the individual dye-oligos in the mixture.

   The system is designed to measure module performance with internal assay controls. In the event of a module replacement, the replacement module to be provided is calibrated prior to shipment.

   (b) However, Cepheid recommends that from the point of initial use, the system should be checked for proper calibration on an annual basis or more frequently, if warranted by the usage and care of each system. As a part of system maintenance tasks for the GeneXpert Infinity system instruments, the Systems software GeneXpert Xpertise may be manually run to perform a GeneXpert module self-test to check calibration test counts. A GeneXpert operator or Field Service Engineer with Administrator user permissions can perform calibration checks during annual maintenance.

   (c) *Within run:* For multi-color detector modules within the Xpert *C. difficile/Epi* test cartridge's optical system, the system uses appropriate calibration and data analysis algorithms to determine the concentrations of each reporter dye for compensation of the spectral overlap during assay run.

5. Quality Control:
   As indicated in the GeneXpert Infinity system operator's manual, Quality Control (QC) measures are undertaken to ensure accurate performance of the Xpert *C. difficile/Epi* test and proper functioning of the GeneXpert Infinity instruments. There are two (2) types of QC measures in instrument operation:
   (a) *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.
   (b) *Internal Test Controls*. For every assay run within a GeneXpert cartridge, the cartridge performs internal QC using the internal control reagents available for the Xpert *C. difficile/Epi* test, i.e.,
      (i) The sample processing control (SPC), as noted in Section IV.B above. The SPC should be positive in a negative sample and may be negative/positive in a positive sample. The SPC passes if it meets the assigned acceptance criteria.

(ii) The Probe Check Control (PCC), as noted in Section IV.B above. The PCC passes if it meets the assigned acceptance criteria.

(c) *External QC (Optional)*. In addition, the test labeling (i.e., package insert/Instructions for Use and Operator Manual for the GeneXpert Infinity System instruments) indicates that external controls may be optionally used by customers—in accordance with local, state or federal accrediting organizations, as applicable—to fulfill various needs including, but not limited to, verification of new assays, verification of new assay kit lots, standard quality control (QC) processes, or for training of new operators. Depending upon the assay, these external controls may be purchased from outside vendors or may be prepared from the leftover samples or by following institutional procedures.

## V Substantial Equivalence Information:

### A Predicate Device Name(s):
cobas Cdiff nucleic acid test for use on the cobas Liat System

### B Predicate 510(k) Number(s):
K212427

### C Comparison with Predicate(s):

| **Device & Predicate Device(s):** | **Candidate: K243730** | **Predicated: K212427** |
|---|---|---|
| ***Device Trade Name*** | **Xpert *C. difficile/Epi*** | **cobas Cdiff Nucleic acid test for use on the cobas Liat System** |
| **General Device Characteristic Similarities** | | |
| ***Device Class*** | Class II | -same- |
| ***Product Code*** | OZN (*C. difficile* toxin gene amplification assay); OOI (Real Time Nucleic Acid Amplification System) | -same- |
| ***Regulation*** | 21 CFR §866.3130 | -same- |
| ***Intended Use/Indications For Use*** | The Xpert *C. difficile/Epi* test is a qualitative *in vitro* diagnostic test for detection of toxin B gene sequences and for presumptive identification of 027/NAP1/BI strains of toxigenic *Clostridioides difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection (CDI). Presumptive identification of 027/NAP1/BI strains of *C. difficile* is by detection of binary toxin (CDT) gene sequences and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a | The cobas Cdiff Nucleic acid test for use on the cobas Liat System is an automated, qualitative *in vitro* diagnostic test that uses real-time polymerase chain reaction (PCR) for the detection of the toxin B (*tcdB*) gene of toxigenic *Clostridioides difficile* (*C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The cobas Cdiff Nucleic acid test for use on the cobas Liat System is intended for use as an aid in the diagnosis of CDI in humans in conjunction with clinical and |

| Device & Predicate Device(s): | Candidate: K243730 | Predicated: K212427 |
|---|---|---|
| | negative regulator in *C. difficile* toxin production. The test is performed on the GeneXpert Instrument Systems and utilizes automated real-time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Xpert *C. difficile/Epi* test is intended as an aid in the diagnosis of CDI. Detection of 027/NAP1/BI strains of *C. difficile* by the Xpert *C. difficile/Epi* test is presumptive and is solely for epidemiological purposes and is not intended to guide or monitor treatment for *C. difficile* infections. Concomitant culture is necessary only if further typing or organism recovery is required. | epidemiological risk factors. |
| ***Microbial Target*** | Toxigenic *C. difficile* | -same- |
| ***Specimen*** | Unformed (liquid or soft) stool | -same- |
| ***Test Technology*** | Real-time PCR | -same- |
| ***Detection Technique*** | Multiplex assay using different reporter dyes for each target | -same- |
| ***Test Automation*** | Fully automated DNA extraction, detection, results interpretation | -same- |
| ***Test Format*** | Disposable single-use, multi-chambered, fluidic cartridge | -same- |
| ***Intended Use Environment*** | Professional Use, by trained users | -same- |
| ***Test Access*** | By prescription use only | -same- |
| **General Device Characteristic Differences** | | |
| ***Test targets (DNA sequence)*** | • Toxin B gene<br>• Binary toxin gene<br>• *tcdC* gene with deletion at nucleotide 117 (*tcdC*Δ117) | Toxin B gene |
| ***Assay Internal Controls*** | • Sample Processing Control (SPC)<br>• Probe Check Control (PCC) | A gram-positive *Bacillus thuringiensis israelensis* bacterial organism to monitor the full process of cobas Liat Analyzer. Native sequence in the bacteria is used as the Internal Control Target. |
| ***Time-to-Result*** | <45 minutes | ~20 minutes |

| Device & Predicate Device(s): | Candidate: K243730 | Predicated: K212427 |
|---|---|---|
| ***Instrument systems*** | • GeneXpert Infinity System<br>• GeneXpert Dx System<br>• GeneXpert System with Touchpad | cobas Liat analyzer |
| ***Systems software for instrument family*** | • GeneXpert Xpertise<br>• GeneXpert Dx<br>• Cepheid OS | • cobas Liat Analyzer Core Software 3.3<br>• CDFA 1.1 |
| ***Collection device*** | Cepheid Sample Collection Device | cobas PCR Media Swab Sample Kit |

## VI Standards/Guidance Documents Referenced:

FDA's Class II Special Controls Guideline Document: Toxin Gene Amplification Assays for the Detection of Clostridium difficile | FDA.

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

1. Analytical Sensitivity/Limit of Detection:
   Analytical sensitivity/Limit of Detection (LoD) for the Xpert *C. difficile/Epi* Assay is defined as the lowest number of colony-forming units (CFU) of *C. difficile* per swab that can be reproducibly distinguished from negative samples with 95% confidence. Studies to determine the LoD of the Xpert *C. difficile/Epi* test when performed on an instrument of the GeneXpert Instrument Systems family were previously conducted in support of K110203, assessed and deemed acceptable. Refer to the K110203 Decision Summary for additional details.

2. Precision/Reproducibility:
   Precision/reproducibility of the Xpert *C. difficile/Epi* test when performed on an instrument of the GeneXpert Instrument Systems family was previously assessed and deemed acceptable in K110203. Refer to the K110203 Decision Summary for additional details.

3. Precision/Repeatability:
   Precision/repeatability of the Xpert *C. difficile/Epi* test when performed on an instrument of the GeneXpert Instrument Systems family was previously assessed and deemed acceptable in K110203. The repeatability study was performed and analyzed in conjunction with the reproducibility study. Refer to the K110203 Decision Summary for additional details.

4. Linearity/Assay Reportable Range:
   Not Applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
   Validated internal and external controls for the Xpert *C. difficile/Epi* test when performed on an instrument of the GeneXpert Instrument Systems family were previously assessed and deemed acceptable in K110203. Refer to the K110203 Decision Summary for additional details.

6. Analytical Reactivity/Inclusivity:

Studies to determine the Analytical Reactivity (Inclusivity) of the Xpert *C. difficile/Epi* test, when performed on an instrument of the GeneXpert Instrument Systems family, were previously conducted in support of K110203, assessed and deemed acceptable. Briefly, the inclusivity study included eighteen (18) *C. difficile* strains representing the toxinotype 0 and twelve (12) variant toxinotypes, including four (4) 027/NAP1/BI toxinotype III isolates. *C. difficile* strains were selected to broadly represent the majority of *C. difficile* toxinotypes encountered in practice. Under the conditions of this study, the Xpert *C. difficile/Epi* test device correctly identified all eighteen (18) toxigenic *C. difficile* strains and indicated all four (4) 027/NAP1/BI toxinotype III strains to be presumptively positive for "027" (i.e., the 027/NAP1/BI strain).

Of note, the Xpert *C. difficile/Epi* test reported toxinotype XIV as presumptively positive for "027" even though it is not a ribotype 027 strain. This is expected since toxinotype XIV is binary positive and bears the *tcdC*Δ117 deletion. Further, the Xpert *C. difficile/Epi* test reported two (2) replicates of toxinotype IV and one (1) of toxinotype X as presumptively positive for "027." Both toxinotypes have been sequenced and shown to be intermediate between non-027/NAP1/BI strain and the 027/NAP1/BI strain*, i.e.,* they lack the *tcdC* gene, but both have the same base at nucleotide 120 as for the 027/NAP1/BI strain, which is the second base utilized to distinguish between 027/NAP1/BI and other strains.

The Class II Special Controls Guideline requires inclusivity studies to include at least twenty (20) strains of toxigenic *C. difficile* in addition to the strains used in the LoD study. The number of strains previously tested in the inclusivity study in support of K110203 (indicated above) was not sufficient to satisfy this requirement since that study included six (6) strains which were also tested in the LoD study. Therefore, to comply with the Class II Special Controls guideline, a supplemental inclusivity study was conducted with Xpert *C. difficile/Epi* test using another eight (8) *C. difficile* strains of PCR ribotype 033. Cultured *C. difficile* stocks were diluted in a negative fecal matrix (10% stool in PBS/15% glycerol) and tested in triplicate at 2–3X LoD (CFU/per swab) with the Xpert *C. difficile/Epi* test.

Under the conditions of this supplemental study, the Xpert *C. difficile/Epi* test device correctly reported the negative status of negative samples (negative fecal matrix only) and identified all eight (8) toxigenic *C. difficile* strains. Of these, the strain ATCC BAA-1805 (which carries the Binary Toxin gene *cdtB*) was correctly indicated as presumptively positive for "027" whereas the remaining seven (7) strains (all identified as Toxin B positive and Binary Toxin negative) were correctly reported as positive for toxigenic *C. difficile* and negative for "027" as expected. **Table 4** shows the supplemental inclusivity study results for each strain/toxinotype tested. No non-determinate GeneXpert result was obtained.

**Table 4. Supplemental Analytical Inclusivity Results**

| Strain ID | Toxino-type and/or toxin | Test Result | Mean SPC Ct | Mean *tcdB* Ct | Mean CDT Ct | Mean *tcdC* Ct |
|---|---|---|---|---|---|---|
| Negative | N/A | Toxigenic C. diff NEGATIVE; 027 PRESUMPTIVE NEGATIVE | 30.3 | 0 | 0 | 0 |

| Strain ID | Toxino-type and/or toxin | Test Result | Mean SPC Ct | Mean *tcdB* Ct | Mean CDT Ct | Mean *tcdC* Ct |
|---|---|---|---|---|---|---|
| ATCC 700792 (14797-2)[a] | A+B+ | Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE | 31.7 | 36.4 | 0 | 0 |
| ATCC BAA-1805[b] | III, A+B+ | Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE POSITIVE | 31.1 | 36.4 | 35.6 | 36.5 |
| ATCC 51695 (BDMS 18 AN)[a] | A+B+ | Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE | 31 | 35.3 | 0 | 0 |
| ATCC 43600 (2149)[a] | A+B+ | Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE | 31.5 | 36 | 0 | 0 |
| ATCC 43599 (2022)[a] | A+B+ | Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE | 31.2 | 36.4 | 0 | 0 |
| ATCC 43596 (545)[a] | A+B+ | Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE | 31.4 | 36.6 | 0 | 0 |
| ATCC 43594 (W1194)[a] | A+B+ | Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE | 31.5 | 36.4 | 0 | 0 |
| ATCC 43598 (1470)[a] | VIII, A-B+ | Toxigenic C. diff POSITIVE; 027 PRESUMPTIVE NEGATIVE | 30.6 | 35 | 0 | 0 |

a. Strains identified by vendor as Binary Toxin negative and Toxin B positive.
b. Strains identified by vendor as carrying the Binary Toxin gene *cdtB*, therefore Binary Toxin Positive

The results of the analytical reactivity/inclusivity study described above along with the prior studies conducted in support of K110203 demonstrated that the Xpert *C. difficile/Epi* accurately identified all strains and toxinotypes of *C. difficile* tested in the study and that the inclusivity rate for the Xpert *C. difficile/Epi* test is 100%. Collectively, this and other analytical performance data provided in K110203 and K243730 may be considered sufficient and demonstrative of general compliance with the requirements of the applicable Class II Special Controls.

7. Analytical Specificity/Cross-reactivity:
Studies to determine the analytical specificity/cross reactivity of the Xpert *C. difficile/Epi* test, when performed on an instrument of the GeneXpert Instrument Systems family, were previously conducted in support of K110203 using bacterial strains that are phylogenetically related to toxigenic *C. difficile* (including non-toxigenic *C. difficile* and non-*C. difficile Clostridioides* species) or potentially encountered in the patient's bowel flora. These studies and results were assessed and deemed acceptable. Refer to the K110203 Decision Summary for additional details.

8. Interference Studies:
Studies to determine potentially inhibitory effects (if any) of substances that may be encountered in patient specimens (unformed stools) upon the Xpert *C. difficile/Epi* test, when performed on an instrument of the GeneXpert Instrument Systems family, were previously conducted in support of K110203, assessed and deemed acceptable. Refer to the K110203 Decision Summary for additional details.

9. Assay Cut-Off:
Studies to determine Lot-specific parameters and assay settings, as well as Lot-independent general assay settings for the Xpert *C. difficile/Epi* test, when performed on an instrument of the GeneXpert Instrument Systems family, were previously conducted in support of K110203, assessed and deemed acceptable. Refer to the K110203 Decision Summary for additional details.

10. Carry-Over/Cross-contamination:
Studies were previously conducted in support of K110203 to determine any potential carry-over contamination of Xpert *C. difficile/Epi* cartridges run within the same module of an instrument of the GeneXpert Instrument Systems family. Upon review, the study results were deemed acceptable. Refer to the K110203 Decision Summary for additional details.

**B Comparison Studies:**

1. Method Comparison with Reference Methods:
Comparison of the Xpert *C. difficile/Epi* test with reference methods was performed and presented in support of K110203 and was assessed and found acceptable upon review. Briefly, clinical performance of the candidate device was evaluated against reference culture, followed by cell cytotoxicity testing on the isolates, and strain typing on the toxigenic strains by PCR-ribotyping, pulsed-field gel electrophoresis (PFGE) and restriction endonuclease analysis (REA) methods. Refer to the K110203 Decision Summary for additional details.

2. Matrix Comparison:
Not applicable.

**C Clinical Studies:**

Clinical Sensitivity, Specificity, and Other Performance Characteristics:
Clinical performance characteristics of the Xpert *C. difficile/Epi* test were evaluated and deemed acceptable in support of K110203. Briefly, the device was validated in a prospective clinical study at seven (7) North American (i.e., US and Canadian) institutions, which tested a total of 2,293 leftover unformed stool specimens from subject whose routine care called for *C. difficile* testing. The study—which compared the Xpert *C. difficile/Epi* test results to reference methods (i.e., culture, followed by cell cytotoxicity testing on the isolates, and strain typing on the toxigenic strains by PCR-ribotyping, pulsed-field gel electrophoresis (PFGE), and restriction endonuclease analysis (REA)—was presented in support of K110203, assessed and found acceptable upon review. Performance estimates of the Xpert *C. difficile/Epi* test were calculated relative to both the results of direct (agar) culture and reference (broth/agar enhanced) culture followed by strain typing by each of PCR-ribotyping, PFGE, and REA. Refer to the K110203 Decision Summary for additional details.

**D Clinical Cut-Off:**
Not Applicable.

**E Expected Values/Reference Range:**
From the clinical studies with Xpert *C. difficile/Epi* assay (performed with an instrument of the GeneXpert Instrument Systems family) that were previously conducted in support of K110203, the percent prevalences of overall toxigenic *C. difficile* and *C. difficile* strain 027/NAP1/BI were estimated based on assay results. Upon review, the study results were deemed acceptable. Refer to the K110203 Decision Summary for additional details.

**F Other Supportive Instrument Performance Characteristics Data:**
To support the performance of the Xpert *C. difficile/Epi* test on the GeneXpert Infinity System instruments, two (2) additional analytical verification studies were performed.

1. Prepared Cartridge Hold Time Study:

*Hold time* refers to the duration between the preparation of the cartridge (i.e., addition of processed sample to the cartridge) and the initiation of the test. The hold time was investigated at hold temperatures of ambient (22°C), elevated (25°C) with high (75%) relative humidity, and high (35°C), using replicates of contrived positive (*C. difficile* cells added to negative human stool matrix) and negative (negative matrix only) specimens that were tested with the Xpert *C. diff/Epi* test performed on the GeneXpert Infinity Systems. The maximum acceptable hold time for a prepared cartridge was verified to be 4 hours under all conditions.

2. Functional Testing Study:
   A comparative functional testing study was conducted to evaluate the performance of the Xpert *C. difficile/Epi* test on the current instrument under review, the GeneXpert Infinity System (running under systems software GeneXpert Xpertise v6.8 or v7.1). The study evaluated performance against the previous cleared GeneXpert Dx System (running under systems software GeneXpert Dx v6.5) and used replicates of contrived positive (*C. difficile* cells added to negative human stool matrix) and negative (negative matrix only) samples and showed 100% agreement with the expected results, with no statistically significant differences in Ct values observed between test runs in GeneXpert Dx System and GeneXpert Infinity System instruments. This study demonstrated equivalent performance of the Xpert *C. difficile/Epi* test when performed on any family member of the GeneXpert Instrument Systems.

## VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

## IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.