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

A. 510(k) Number:

K091109

B. Purpose for Submission:

Substantial equivalence for a new device.

C. Measurand:

C. difficile toxin B gene sequences

D. Type of Test:

Nucleic Acid Amplification Test

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Xpert® *C. difficile*

G. Regulatory Information:

1. Regulation section:

   866.2660

2. Classification:

   I

3. Product code:

   LLH

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The Cepheid Xpert® *C. difficile* Assay, performed on the Cepheid GeneXpert® Dx System, is a qualitative *in vitro* diagnostic test for rapid detection of toxin B gene sequences from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI). The test utilizes automated real-time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Xpert *C. difficile* Assay is intended as an aid in the diagnosis of CDI. Concomitant testing is necessary only if further typing or organism recovery is required.

2. Indication(s) for use

   The Cepheid Xpert® *C. difficile* Assay, performed on the Cepheid GeneXpert® Dx System, is a qualitative *in vitro* diagnostic test for rapid detection of toxin B gene sequences from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI). The test utilizes automated real-time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Xpert *C. difficile* Assay is intended as an aid in the diagnosis of CDI. Concomitant testing is necessary only if further typing or organism recovery is required.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Cepheid GeneXpert® Dx System

I. Device Description:

The GeneXpert Dx System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and RT-PCR assays. The system consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. For a full description of the system, see the GeneXpert Dx System Operator Manual.

The Xpert *C. difficile* Assay includes reagents for the detection of toxigenic *C. difficile*, as well as a Sample Processing Control (SPC). The SPC is present to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR

reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   BD GeneOhm™ Cdiff Assay
2. Predicate 510(k) number(s):
   K081920
3. Comparison with predicate:

	Device	**Predicate**
Item	**Xpert *C. difficile* Assay**	**BD GeneOhm Cdiff Assay (K081920)**
Intended Use	An automated test for the qualitative detection of toxigenic *C. difficile* in unformed (liquid or soft) stool specimens.	Same
Indication for Use	Identification of *C. difficile* from patients suspected of having *C. difficile* Infection (CDI).	Same
Technological Principles	Fully-automated nucleic acid amplification (DNA); real-time PCR	Same
Specimen Type	Unformed (liquid or soft) Stool	Same
Test Cartridge	Disposable single-use, multi-chambered fluidic cartridge.	Disposable single-use PCR tube
DNA Target Sequence(s)	*C. difficile* toxin B and binary toxin gene sequences and the single base pair deletion at nucleotide 117 in *tcdC* (*tcdC*Δ117).	*C. difficile* toxin B only
Instrument System	Cepheid GeneXpert Dx System	Cepheid SmartCycler Dx System
Sample Extraction	Self-contained and automated after swab elution and two single-dose reagent additions.	Manual
Probes	TaqMan® Probes	Molecular Beacons
Sample Extraction	Automated	Manual
Rapid test results	Less than 45 minutes to results.	Approximately 75-90 minutes to results.

Users	Operators with no clinical lab experience to experienced clinical laboratory technologists.	CLIA High Complexity Laboratory Users

K. Standard/Guidance Document Referenced (if applicable):

N/A

L. Test Principle:

The assay is a rapid, automated *in vitro* diagnostic test for the qualitative detection of *C. difficile* directly from unformed (liquid or soft) stool specimens of patients suspected of having *C. difficile* infection (CDI). The assay detects the following three elements: toxin B gene (*tcdB*), binary toxin gene (CDT), and the single-base-pair deletion at nucleotide 117 within the gene encoding a negative regulator of toxin production (*tcdC*Δ117). The assay is performed on the Cepheid GeneXpert Dx System. This is the same instrument system that is used with the Cepheid Xpert MRSA [510(k) #K070462], Xpert MRSA/SA SSTI [510(k) #K080837], and Xpert MRSA Blood Culture [510(k) #K082140] as well as the Cepheid Xpert GBS and Xpert EV Assays.

The assay uses real-time PCR amplification and fluorogenic target-specific hybridization detection. An important aspect of this submission is that the assay intends to detect toxigenic *C. difficile* and presumptive identification of an epidemic strain of *C. difficile* known as 027/NAP1/BI strain (Note: this strain designation terminology refers to designations given to this strain based on PCR ribotyping, Pulsed Field Gel Electrophoresis (PFGE) and restriction endonuclease analysis (REA), respectively).
This strain has been described as having a single-base-pair deletion at nucleotide 117 within the gene encoding a negative regulator of toxin production (*tcdC*Δ117). Cepheid selected primers and probes to identify the single-base- pair deletion at position 117 of the *tcdC* gene to detect and differentiate the 027/NAP1/BI hypervirulent *C. difficile* strains from other isolates.

M. Performance Characteristics (if/when applicable):

Performance characteristics of the Xpert *C. difficile* Assay were determined in a multi-site prospective investigation study at seven US and Canadian institutions by comparing the Xpert *C. difficile* Assay to reference culture followed by cell cytotoxicity testing on the isolates
Subjects included individuals whose routine care called for *C. difficile* testing. A portion of the leftover unformed stool specimens were obtained for testing by the Xpert *C. difficile* Assay. The remaining excess specimen was sent to a central laboratory for reference culture and cytotoxin B isolate testing. Each stool specimen was inoculated onto pre-reduced cycloserine-cefoxitin-fructose agar –direct plate (CCFA-D) and cycloserine cefoxitin mannitol broth with taurocholate lysozyme cysteine (CCMB-TAL). After 24 hours the

CCMBTAL was subcultured on to a second CCFA-E plate (CCFA- Enriched). This direct-enriched culture method is referred to hereafter as "reference culture". If *C. difficile* was isolated from the CCFA-D plate and the isolate was positive by cell cyotoxicity assay, the specimen was classified as "toxigenic *C. difficile* positive" and CCFA-E plate was not further analyzed. If no *C. difficile* was isolated from the CCFA-D plate or if the isolate was negative by cell cytotoxicity assay, the CCFA-E plate was further analyzed. If CCFA-E was positive for *C. difficile* and the isolate was positive for cell cytotoxicity assay, the specimen was classified as "toxigenic *C. difficile* positive". The specimen was reported as "negative" if CCFA-E is negative for *C. difficile* or the isolate was tested negative by cell cytotoxicity assay.

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

Overall Results

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

Performance vs. Direct Culture

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

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

Antibiotic Usage
Among the 2296 cases included in the main dataset, antibiotic use within the 2 months prior to sample collection was reported for 1633 and no antibiotic use was confirmed for 570; for 93 cases, antibiotic status was unknown. Antibiotic use did not cause a statistically significant difference in assay performance

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

Of the tested species, ten (10) non-toxigenic *C. difficile* strains and eleven (11) non *C. difficile Clostridium* species were included. The organisms tested were identified as either Gram-positive (37) or Gram negative (18). The organisms were further classified as aerobic (24), anaerobic (29) or microaerophilic (2).

Each strain was tested in triplicate at concentrations ranging from 1.1×10^{8} to 2.2×10^{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 NEGATIVE". The analytical specificity was 100%.

Analytical Sensitivity
Studies were consisted of human liquid feces (*C. difficile* negative by Xpert *C. difficile* Assay) diluted in PBS with 15% glycerol. The LoD is defined as the lowest number of colony forming units (CFU) per swab that can be reproducibly

Studies were performed to determine the 95% confidence intervals for the analytical limit of detection (LoD) of *C. difficile* diluted into a fecal matrix of human origin that can be detected by the Xpert *C. difficile* Assay. The fecal matrix consisted of human liquid feces (*C. difficile* negative by Xpert *C. difficile* Assay) diluted in PBS with 15% glycerol. The LoD is defined as the lowest number of colony forming units (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 Table 4.

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

Strain ID	Toxinotype	LoD95% (CFU/ swab)	Lower 95% CI	Upper 95% CI
VPI 10463 (CCUG19126)	0	255	190	632
90556-M6S (ATCC9689)	0	460	419	587
LUMC-1 (027/NAP1/BI)a	III	23	19	31
LUMC-5 (027/NAP1/BI)a	III	75	45	176
LUMC-7	V	45	34	104
LUMC-6	VIII	60	50	74
9101	XII	41	34	49

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

In addition to the LoD determination, eighteen *C. difficile* strains representing 12 variant toxinotypes, including four 027/NAP1/BI toxinotype III isolates, were tested using the Xpert *C. difficile* Assay. *C. difficile* strains were selected to broadly represent the majority of *C. difficile* toxinotypes encountered in practice. Stock cultures were prepared by suspending the bacterial growth from agar plates in PBS buffer containing 15% glycerol. The concentration of each stock was adjusted to 1.4-5.9 McFarland units. All strains were serially diluted to approximately 900 CFU/swab and tested in triplicate.

Under the conditions of this study, the Xpert *C. difficile* Assay correctly identified all 18 toxinotypes tested as "Toxigenic C. diff POSITIVE". Included in the panel were 8 toxinotypes reported to be positive for binary toxin (CDT) production as well. All were CDT positive using the Xpert *C. difficile* Assay. All four 027/NAP1/BI isolates representing toxinotype III were correctly identified as "Toxigenic C. diff POSITIVE".

Interfering Substances

Twenty-one (21) biological and chemical substances occasionally used or found in stool specimens were tested for interference with the Xpert *C. difficile* Assay. Potentially interfering substances include, but are not limited to, Vagisil cream and zinc oxide paste. The 19 substances listed in Table 5 showed no detectable interference with the Xpert *C. difficil*e Assay

Substance	Substance
Whole Blood Karolinska University Hospital	K-Y Jelly/Gelée® McNeil-PPC
Mucin (porcine) Sigma	Vaseline Unilever
Kaopectate® Chattem	Dulcolax® Boehringer Ingelheim Pharmaceuticals
Imodium® McNeil-PPC	Preparation H Portable Wipes Wyeth Consumer Healthcare
Pepto-Bismol® Procter & Gamble	Vaginal Contraceptive Film (VCF) Apothecus Pharmaceutical
Preparation H® Wyeth Consumer Healthcare	Vancomycin Fluka
Fleet® CB Fleet Company	Metronidazole Actavis
Fecal fats Karolinska University Hospital	Anusol® Plus TM Warner-Lambert Company
Monistat® McNeil-PPC	E-Z-HD™ High Density Barium Sulfate for suspension E-Z-EM Canada
Hydrocortisone Cream Longs Drugs	

Reproducibility

A panel of 7 specimens with varying concentrations of toxigenic *C. difficile* and *C. difficile*, 027/NAP1/BI were tested on 10 different days by two different operators at each of the three sites (7 specimens x 2 operators/ day x 10 days x 3 sites). One lot of Xpert *C. difficile* Assay was used at each of the 3 testing sites. Xpert *C. difficile* Assays were performed according to the Xpert *C. difficile* Assay procedure. Results are summarized below.

	% Agreement			
Specimen ID	**Site 1**	**Site 2**	**Site 3**	**% Total Agreement by Sample**
Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* High Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* Low Positive	100% (20/20)	85% (17/20)	85% (17/20)	90.0% (54/60)

Toxigenic *C. difficile* Moderate Positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
027/NAP1/BI High Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
027/NAP1/BI Low Positive	100% (20/20)	95% (19/20)	95% (19/20)	96.7% (58/60)
027/NAP1/BI Moderate Positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
% Total Agreement by Site	100% (140/140)	97.1% (136/140)	97.1% (136/140)	98.1% (412/420)

SPC			
Level	**Ave.**	**Std. Dev.**	**CV**
Toxigenic C. diff high neg	32.17	0.59	1.83%
Toxigenic C. diff low pos	32.14	0.53	1.66%
Toxigenic C. diff mod pos	31.98	0.47	1.47%
027/NAP1/BI high neg	32.11	0.65	2.03%
027/NAP1/BI low pos	31.93	0.72	2.26%
027/NAP1/BI mod pos	31.96	0.61	1.90%
Neg	32.26	0.72	2.22%
tcdB			
Level	**Ave.**	**Std. Dev.**	**CV**
Toxigenic *C. diff* high neg	39.59	0.70	1.77%
Toxigenic *C. diff* low pos	35.88	0.81	2.24%
Toxigenic *C. diff* mod pos	32.17	0.45	1.39%
027/NAP1/BI high neg	39.11	0.98	2.50%
027/NAP1/BI low pos	35.49	0.58	1.65%
027/NAP1/BI mod pos	32.10	0.63	1.97%

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

N. Instrument Name:

Cepheid GeneXpert® Dx System

O. System Descriptions:

1. Modes of Operation:

   Automated

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X_____ or No _____

3. Specimen Identification:

   Unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI).

4. Specimen Sampling and Handling:

   (1) Collect the unformed stool specimen in a clean container. Follow your institution's guidelines for collecting samples for *C. difficile* testing.
   (2) Label with Sample ID and send to the laboratory.
   (3) Store specimen at 2 – 8 °C. The specimen is stable for up to 5 days when stored at 2 – 8 °C. Alternatively, specimens can be kept at room temperature (20 – 30 °C) for up to 24 hours.

5. Calibration:

   NA

6. Quality Control:

   Each sample contains a Sample Processing Control and a Probe Check Control

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

N/A

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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