K091109

JUL - 9 2009

Cepheid

510(k) Summary

As required by 21 CFR Section 807.92(c).

Submitted by:	Cepheid 904 Caribbean Drive Sunnyvale, CA 90489 Phone number: (408) 400-8230 Fax number: (408) 541-6439
Contact:	Russel K. Enns, Ph.D.
Date of Preparation:	June 21, 2009
Device:	
Trade name:	Xpert® *C. difficile*
Common names:	*C. difficile* Assay and *Clostridrium difficile* identification and differentiation system
Type of Test:	Qualitative Nucleic Acid Amplification Test for *C. difficile* toxin B gene sequences from unformed stool specimens.
Classification:	I
Classification name:	Device reagents, *Clostridium difficile* toxin; microorganism differentiation and identification device.
Regulation number:	866.2660
Procode:	LLH
Classification Advisory Committee:	Microbiology
Panel:	83
Predicate Device:	BD GeneOhm™ Cdiff Assay [510(k) # K081920]

Device Description:

The Cepheid Xpert *C. difficile* Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection of *Clostridium difficile* directly from unformed (liquid or soft) stool specimens of patients suspected of having *Clostridium difficile* infection (CDI). The assay detects the toxin B gene (*tcdB*), and is performed on the Cepheid GeneXpert Dx System.

The Xpert *C. difficile* Assay system performs sample preparation and real-time, multiplex polymerase chain reaction (PCR) for detection of target-specific DNA.

The GeneXpert Dx System consists of a GeneXpert® instrument, personal computer, and disposable fluidic cartridges. Each instrument contains 1-16 randomly accessible modules that are each capable of performing separate sample preparation and real-time PCR tests for detection of *C. difficile* toxin B gene sequences in less than 45 minutes. Each module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing cells or spores, and I-CORE® thermocycler for performing real-time PCR and detection.

A swab is inserted into the stool specimen and then is placed in a tube containing elution reagent. Following brief vortexing, the eluted material and two single-use reagents (Reagent 1 and Reagent 2) that are provided with the Assay are transferred to different, uniquely-labeled chambers of the disposable fluidic cartridge (the Xpert *C. difficile* cartridge). The user initiates a test from the system user interface and places the cartridge into the GeneXpert® Dx System instrument platform, which performs hands-off real-time, multiplex polymerase chain reaction (PCR) for detection of DNA. In this platform, additional sample preparation, amplification, and real-time detection are all fully-automated and completely integrated.

The Xpert *C. difficile* Assay includes reagents for the detection of toxin B gene (*tcdB*). In addition, the assay reagents include an internal sample processing control (SPC) to ensure adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR Assay. The SPC also ensures that the PCR conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

Device Intended 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.

Substantial Equivalence:

The Xpert *C. difficile* Assay is substantially equivalent to the BD GeneOhm Cdiff Assay, manufactured by BD Diagnostics. Both Assays qualitatively detect *C. difficile* toxin B gene (*tcdB*) in unformed (liquid or soft) stool specimens. Both Assays use real-time PCR amplification and fluorogenic target-specific hybridization detection.

Table 5.1 shows the similarities and differences between the Xpert *C. difficile* Assay and the BD GeneOhm Cdiff Assay.

The Xpert *C. difficile* is also substantially equivalent to the *C. difficile* reference culture method followed with strain identification of all *C. difficile* isolates as shown in a multi-center clinical comparison study.

The multi-center clinical comparison study was conducted on 2296 patients to evaluate the performance of the Xpert *C. difficile* Assay relative to the reference culture method and cytotoxin B isolate testing.

The test results showed the Xpert *C. difficile* Assay to be substantially equivalent to the current standard of care, the *C. difficile* reference culture method.

Table 5.1
Similarities and Differences Between the Xpert *C. difficile* Assay and the BD GeneOhm Cdiff Assay

	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	Same
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

Item	Device Xpert *C. difficile* Assay	Predicate BD GeneOhm Cdiff Assay (K081920)
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

Non-Clinical Studies:

Analytical Inclusivity

The analytical inclusivity of the Xpert *C. difficile* Assay was determined using 13 *Clostridium difficile* strains of different toxinotypes selected to represent the range of genetic diversity found in *C. difficile*. Toxinotypes 0, I, III, IV, V, VI, VIII, IX, X, XII, XIV, XXI, and XXII were tested. All strains were tested in triplicate 9000 CFU per Assay. All tested toxinotypes were correctly reported as "Toxigenic C. diff POSITIVE".

Analytical Sensitivity (Limit of Detection)

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 CFU loadings. 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 5.2.

Table 5.2 - 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/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

[a]By PCR-ribotyping/pulse-field gel electrophoresis/restriction endonuclease analysis

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".

Linearity

A study was conducted to define the reportable range of the Xpert *C. difficile* Assay and demonstrated a linear relationship.

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 microaerophillic (2).

Each strain was tested in triplicate from cultures adjusted to 0.5 – 4.7 McFarland units. 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%.

Interfering Substances

Twenty-one (21) biological and chemical substances occasionally used or round 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 5.3 showed no detectable interference with the Xpert *C. difficile* Assay.

Table 5.3 – Substances Tested and Showing No Assay 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
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	[redacted]

Clinical Studies

Clinical Comparison Study

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 CCFA-D (cycloserine-cefoxitin-fructose agar –direct plate) 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 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 REA strain typing, the Xpert *C. difficile* Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 98.79% and 90.82%, respectively (Table 5.4).

Table 5.4 - Xpert *C. difficile* Assay Performance vs. Direct Culture

		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%	
		PPV[a]:	56.58%	
		NPV[b]:	99.83%	
		Prevalence:	10.80%	

()Xpert *C. difficile* results on first attempt
[a]Positive predictive value
[b]Negative predictive value

Performance vs. Reference Culture

Reference (enriched) culture is a more sensitive method for detection of *C. difficile* in symptomatic patients, for example it allows detection of low number of organism due to prior antibiotic treatment and potential lost of viability due to specimen transport.

Relative to reference culture, the Xpert *C. difficile* Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.49% and 94.02%, respectively (Table 5.5).

Table 5.5 - Xpert *C. difficile* Assay Performance vs. Reference Culture

		Reference Culture		
		C. diff	NEG	Total
Xpert *C. difficile*	Toxin B+	316 (310)	117 (113)	433 (423)
	NEG	22 (22)	1841 (1776)	1863 (1798)
	Total	338 (332)	1958 (1889)	2296 (2221)
		Sensitivity:	93.49%	
		Specificity:	94.02%	
		Accuracy:	93.95%	
		PPV[a]:	72.98%	
		NPV[b]:	98.82%	
		Prevalence	14.72%	

()Xpert *C. difficile* results on first attempt
[a]Positive predictive value
[b]Negative predictive value

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.

Reproducibility

Reproducibility of the Xpert *C. difficile* Assay was demonstrated using a panel of 7 specimens with varying concentrations of a toxigenic *C. difficile* strain, a toxigenic *C. difficile* 027/NAP1/BI strain and a negative that were tested in duplicate on 10 different days at each of the three sites (7 specimens x 2 times/ day x 10 days x 3 sites). One lot of Xpert *C. difficile* kit was used at each of the 3 testing sites. Xpert *C. difficile* Assays were performed according to the Xpert *C. difficile* procedure.

A panel of 7 specimens with varying concentrations of *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 in Table 5.6.

Table 5.6 – Summary of Reproducibility Results (all)

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)

Table 5.7 - 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/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**	**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/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%

An additional panel of 6 specimens, three negative and three toxigenic *C. difficile* high negative, were tested on 5 different days by two different operators at each of the three 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* 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 in Table 5.8.

Table 5.8 – Summary of Additional Reproducibility Specimen Results

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[a]	60.0% (18/30)[b]	60.0% (18/30)[b]	53.3% (16/30)[b]	57.8% (52/90)[b]

[a]20-80% agreement expected for high negative sample
[b](# negative results / total high negative samples run)

Conclusions

The results of the nonclinical analytical and clinical performance studies summarized above demonstrate that the Xpert *C. difficile* Assay is as safe, as effective, and performs as well as or better than the predicate device.

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

JUL - 9 2009

Cepheid
Russel K. Enns, Ph.D.
Senior Vice President
904 Caribbean Drive
Sunnyvale, CA 94089-1189

Re: K091109
Trade/Device Name: Xpert® *C. difficile*
Regulation Number: 21 CFR 866.2660
Regulation Name: Microorganism differentiation and detection device
Regulatory Class: Class I
Product Code: LLH
Dated: June 22, 2009
Received: June 23, 2009

Dear Dr. Enns:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at 240-276-0450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at 240-276-3474. For questions regarding the reporting of device adverse events (Medical Device Reporting (MDR)), please contact the Division of Surveillance Systems at 240-276-3464. You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

4.0 Indications for Use Statement

510(k) Number (if known): K091109

Device Name: Xpert® *C. difficile*

Indications 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 culture is necessary only if further typing or organism recovery is required.

Prescription Use ___X___ (Part 21 CFR 801 Subpart D)	AND/OR	Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE

Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) K091109