Meridian Bioscience, Inc.	Special 510(k) Application *illumigene C. difficile, Performance Characteristic Extension*	
	Description:	**510(k) Summary *illumigene C. difficile***
	Identification:	Attachment 002
	Date:	December 31, 2010

510(k) number: K100012 **Date of preparation:** December 31, 2010

Submitter: **Meridian Bioscience, Inc.**
Submitter's address: 3471 River Hills Drive
Cincinnati, Ohio 45244
Contact: Michelle Smith
Contact number: (513) 271-3700

FEB 2 4 2011

Device name: ***illumigene®* *C. difficile***

Common name: *C. difficile* DNA Amplification Assay

Classification name: *C. difficile* Nucleic Acids
OMN, CFR Section 866.2660

Predicate device: K100818: ***illumigene®*** Molecular Diagnostic Test System (***illumigene C. difficile*** DNA Amplification Assay, ***illumipro-10***)

Model 280050, 610172

Reference comparator: Cytotoxic bacterial culture

Description of the device:
The ***illumigene*** Molecular Diagnostic Test System is comprised of the ***illumigene C. difficile*** DNA Amplification Test Kit, the ***illumigene C. difficile*** External Control Kit and the *illumipro-10* Automated Isothermal Amplification and Detection System. The ***illumigene C. difficile*** DNA amplification assay utilizes loop-mediated isothermal amplification (LAMP) technology to detect the presence of toxigenic *C. difficile* in patients suspected of having *C. difficile* associated disease (CDAD). Each ***illumigene C. difficile*** assay is completed using an ***illumigene*** Sample Preparation Apparatus, ***illumigene*** Reaction Buffer, ***illumigene C. difficile*** Test Device, Sample Collection Brush, and ***illumigene*** Extraction Tube. Samples are prepared using the Sample Collection Brush and the ***illumigene*** Sample Collection Apparatus, target DNA is heat extracted in the Extraction Tube and DNA amplification occurs in the ***illumigene C. difficile*** Test Device.

The ***illumipro-10*** heats each ***illumigene C. difficile*** Test Device containing prepared samples, facilitating amplification of target DNA. When toxigenic *C. difficile* is present in the patient sample, a cytotoxin specific sequence is amplified and Magnesium pyrophosphate is formed. Magnesium pyrophosphate forms a precipitate in the reaction mixture. The ***illumipro-10*** detects the change in light transmission through the reaction mixture created by the precipitating Magnesium pyrophosphate. Sample results are reported as Positive or Negative based on the detected change in transmission.

The ***illumigene C. difficile*** External Control Kit consists of a Positive Control Reagent and a Negative Control Reagent. External Control reagents are provided to aid the user in detection of reagent deterioration, adverse environmental or test conditions, or variance in operator performance that may lead to test errors. The ***illumigene C. difficile*** External Control Kit is required for routine Quality Control.

Intended Use:

The ***illumigene*** *C. difficile* DNA amplification assay, performed on the ***illumipro-10,*** is a qualitative *in vitro* diagnostic test for the direct detection of toxigenic *C. difficile* in human stool specimens from pediatric and adult patients suspected of having *Clostridium difficile*-associated disease (CDAD).

The ***illumigene*** *C. difficile* assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect the pathogenicity locus (PaLoc) of toxigenic *Clostridium difficile*. The *Clostridium difficile* PaLoc is a gene segment present in all known toxigenic *C. difficile* strains. The *C. difficile* PaLoc codes for both the Toxin A gene (*tcdA*) and the Toxin B gene (*tcdB*), has conserved border regions, and is found at the same site on the *C. difficile* genome for all toxigenic strains. The ***illumigene*** *C. difficile* assay detects the PaLoc by targeting a partial DNA fragment on the Toxin A gene. The *tcdA* target region was selected as an intact region remaining in all known A+B+ and A-B+ toxinotypes.

illumigene *C. difficile* is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.

Comparison to predicated device:

Characteristic	***illumigene*™ C. *difficile*, Revised**	***illumigene*™ C. *difficile*, K100818**
Test Format	No Change	DNA Amplification Assay
Intended Use		
DNA Amplification Technology	No Change	Loop-Mediated Isothermal Amplification (LAMP)
Target Sequences Detected	No Change	Partial DNA fragment on the Toxin A gene of the pathogenicity locus (PaLoc) found in all known strains for toxigenic *C. difficile*.
Qualitative/Quantitative	No Change	Qualitative
Screening, Diagnostic or Identification Test	No Change	Diagnostic
Specimen Types		
Unformed Human Stool	No Change	Yes
Human Stool in Cary-Blair-based Media	No Change	Yes
Reagents/Components	***illumigene*** Sample Preparation Apparatus ***illumigene*** Reaction Buffer ***illumigene*** *C. difficile* Assay Device ***illumigene*** Heat Treatment Tubes Sample Collection Brushes	***illumigene*** Sample Preparation Apparatus ***illumigene*** Reaction Buffer ***illumigene*** *C. difficile* Assay Device ***illumigene*** Extraction Tubes Sample Collection Brushes
Extraction	Not Applicable. Sample preparation by heat treatment. DNA Extraction and purification not required.	Manual
Amplification	No Change	Self-contained and automated

Comparison to predicated device:

Characteristic	*illumigene™* C. *difficile, Revised*	*illumigene™* C. *difficile*
Detection	No Change	Self-contained and automated
Testing Time	No Change	Approximately 60 minutes
Calibration	No Change	Not required
Controls		
Inhibition, Assay	No Change	Provided *illumigene* Sample Preparation Apparatus: *Staphylococcus aureus* *illumigene C. difficile* Assay Device: *Staphylococcus aureus* LAMP Primers
External	No Change	Adjunct Reagents *illumigene C. difficile* External Control Kit Catalog 279920
Extraction	Not Applicable. Sample preparation, including heat treatment monitored by external thermometer and interval timer. Equipment is user supplied.	User Supplied
Equipment		
Instrumentation	No Change	*illumipro-10™* Automated Isothermal Amplification and Detection System
General Laboratory Equipment	Micropipette 50 µL, 200 µL Dry-bath with 12mm Heat Block, 95 C Interval Timer Vortex Mixer Digital Thermometer with Max/Min Temperature Memory	Micropipette 50 µL, 200 µL Dry-bath with 12mm Heat Block, 95 C Interval Timer Vortex Mixer
Reading Method	No Change	Visible Light Transmission
Results		
***C. difficile* Toxinotypes Tested**	No Change	**0 (A+/B+)** **III (A+/B+)** **V (A+/B+)** **VIII (A-/B+)** **X (A-/B+)** **XII (A+/B+)** **IX/XXIII (A+/B+)**
Results Interpretation	No Change	INVALID POSITIVE NEGATIVE

Performance Comparison, Non-clinical Tests:

Interference Testing *(Reference K100818)*

Selected drugs and other non-microbial substances that might be present in stool samples from healthy persons or patients suspected of having *C. difficile* associated disease were added to a natural negative and a contrived positive sample. The natural negative and contrived positive samples were prepared from donor samples and were confirmed negative by cytotoxic bacterial culture. The contrived positive sample was prepared by spiking a confirmed negative sample with toxinogenic *C. difficile* strain VPI 10463 to 18 CFU/test, slightly above the 16 CFU assay limit of detection for this organism. Potentially interfering substances were added at final concentrations of 5% V/V or greater. Dilution Controls for each sample were prepared by adding a phosphate-buffered saline solution in place of the potentially interfering substance. Each sample was tested in triplicate.

The following substances, at the specified saturated solvent/diluents concentrations, do not interfere with ***illumigene C. difficile*** test results in the final concentrations listed: Barium sulfate (5 mg/mL), fecal fat (equivalent to 2.65 mg stearic plus 1.3 mg palmitic acids per mL), hemoglobin (as methemoglobin) (3.2 mg/mL), IgA (5 mg/mL), Imodium AD® (0.00667 mg/mL), Kaopectate® (0.87 mg/mL), Metronidazole (12.5 mg/mL), mucin (3.33 mg/mL) Mylanta® (4.2 mg/mL), Pepto-Bismol® (0.87 mg/mL), Prilosec® (0.5 mg/mL), Tagamet® (0.5 mg/mL), TUMS® (0.5 mg/mL), Vancomycin (12.5 mg/mL), white blood cells (5%V/V), whole blood (5% V/V).

Cross-reactivity Study *(Reference K100818)*

Potentially cross-reactive microorganisms that might be present in stool samples from healthy persons or patients suspected of having *C. difficile* associated disease were added to a natural negative and a contrived positive sample. The natural negative and contrived positive samples were prepared from donor samples and were confirmed negative by cytotoxic bacterial culture. The contrived positive sample was prepared by spiking a confirmed negative sample with toxinogenic *C. difficile* strain VPI 10463 to 18 CFU/test, slightly above the 16 CFU assay limit of detection for this organism. Potentially cross-reactive microorganisms were added at concentrations of 1.2×10^8/mL (bacteria and fungi) or $1 \times 10^{5.29}$/mL $TCID_{50}$/mL (viruses). Dilution Controls for each sample were prepared by adding a phosphate-buffered saline solution in place of the potentially cross-reactive microorganisms. Each sample was tested in triplicate.

The following microorganisms, at the indicated concentrations, do not interfere with ***illumigene C. difficile*** test results:
Aeromonas hydrophila, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Citrobacter frendii, Clostridium sordellii, Clostridium perfringens, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Escherichia coli O157:H7, Escherichia fergusonii, Escherichia hermannii, Helicobacter pylori, Klebsiella pneumoniae, Lactococcus lactis, Listeria monocytogenes, Peptostreptococcus anaerobius, Plesiomonas shigelloides, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella Groups B-E, *Serratia liquefaciens, Serratia marcescens, Shigella boydii, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Vibrio parahaemolyticus, Yersinia enterocolitica,* Adenovirus Types 40 and 41, Coxsackievirus, Echovirus, Rotavirus.

Performance Comparison, Clinical Tests:

Clinical trials for the ***illumigene C. difficile*** assay, including the ***illumipro-10*** Automated Isothermal amplification and detection system, were conducted in 2010. Performance characteristics of the ***illumigene C. difficile*** assay were determined by comparison to cytotoxic bacterial culture in two separate studies: (1) Patients 2 years of age and above and (2) Patients less than 2 years of age.

(1) Patients 2 years of age and above: Independent clinical test sites located in the Midwestern and Southern regions of the United States and the manufacturer evaluated a total of 697 qualified patient samples. Samples were collected from 274 (39.3%) males and 419 (60.1%) females. In the case of 4 (0.6%) of the patients, sex was not known. The age groups of patients range from 2 years of age to 96 years. No differences in test performance were observed based on patient age, gender or geographical location. Overall Sensitivity was determined to be 95.2% (95% CI: 89.2% - 97.9%). Overall Specificity was determined to be 95.3% (95% CI: 93.2% - 96.7%). Subsequent tables show overall assay performance as well as performance by clinical site and patient age.

Table 1. Performance data (Patients 2 years of age and above)

Cytotoxic bacterial culture	*illumigene C. difficile*			
	Positive	**Negative**	**Invalid*** **	**Total**
Positive	99	5**	4	108
Negative	27*	546	16	589
Total	126	551	20	697
			95% CI	
Sensitivity	99/104	**95.2%**	89.2 - 97.9%	
Specificity	546/573	**95.3%**	93.2 - 96.7%	
Correlation	645/677	**95.3%**	93.4 - 96.6%	
Invalid Rate	20/697	**2.9%**	N/A	

* 15/27 false positive results were positive by another FDA cleared molecular assay. Of the remaining 12 false positive results, 8 were positive by a FDA cleared assay for the detection of GDH.

** 2/5 false negative results were negative by another FDA cleared molecular assay.

*** Invalid results were obtained for 20/697 (2.9%) samples tested. Eleven (1.6%) of the invalids observed were categorized as Assay Invalids, indicative of improper sample preparation, reagent failure, instrument failure or internal control failure. One of the eleven specimens remained invalid after repeat testing from the original sample.

Table 2. Performance characteristics by site (Patients 2 years of age and above)

Site	Positive Samples			Negative Samples		
	illumigene/ Cytotoxic bacterial culture	Sensitivity %	95% CI	*illumigene*/ Cytotoxic bacterial culture	Specificity %	95% CI
Total	**99/104**	**95.2%**	**89.2 – 97.9%**	**546/573**	**95.3%**	**93.2 – 96.7%**
Site 1	4/5	80.0%	37.6 – 96.4%	58/60	97.6%	88.6 – 99.1%
Site 2	12/12	100%	75.7 – 100%	62/67	92.5%	83.7 – 96.8%
Site 3	20/20	100%	83.9 – 100%	87/92	94.6%	87.9 – 97.7%
Site 4	8/8	100%	67.6 – 100%	36/39	92.3%	79.7 – 97.3%
Site 5	55/59	93.2%	83.8 – 97.3%	303/315	96.2%	93.5 – 97.8%

(2) Patients less than 2 years of age: Independent clinical test sites located in the Midwestern and Southern regions of the United States and the manufacturer evaluated a total of 193 qualified patient samples. Samples were collected from 103 (53.4%) males and 90 (46.6%) females. The age groups of patients tested ranged from 0 months to 24 months. No differences in test performance were observed based on patient age, gender or geographical location. Overall Sensitivity was determined to be 93.3% (95% CI: 78.7 - 98.2%). Overall Specificity was determined to be 96.3% (95% CI: 92.2% - 98.3%). Subsequent tables show overall assay performance as well as performance by clinical site and patient age.

Table 3. Performance data (Patients less than 2 years of age)

Cytotoxic bacterial culture	*illumigene C. difficile*			
	Positive	**Negative**	**Invalid*** **	**Total**
Positive	28	2**	1	31
Negative	6*	156	0	162
Total	34	158	1	193
			95% CI	
Sensitivity	28/30	**93.3%**	78.7 - 98.2%	
Specificity	156/162	**96.3%**	92.2 - 98.3%	
Correlation	184/192	**95.8%**	92.0 - 97.9%	
Invalid Rate	1/193	**0.5%**	N/A	

* 3/6 false positive results were positive by another FDA cleared molecular assay. Of the remaining 3 false positive results, all were positive by a FDA cleared assay for the detection of GDH.

** 1/2 false negative results were negative by another FDA cleared molecular assay.

*** Invalid results were obtained for 1/193 (0.5%) samples tested. The invalid observed was categorized as an Assay Invalid, indicative of improper sample preparation, reagent failure, instrument failure or internal control failure. The specimen remained invalid after repeat testing from the original sample.

Table 4. Performance characteristics by site (Patients less than 2 years of age)

Site	Positive Samples			Negative Samples		
	illumigene / Cytotoxic bacterial culture	Sensitivity %	95% CI	*illumigene* / Cytotoxic bacterial culture	Specificity %	95% CI
Total	**28/30**	**93.3%**	**78.7 - 98.2%**	**156/162**	**96.3%**	**92.2 - 98.3%**
Site 1	8/8	100%	67.6 - 100%	48/49	98.0%	89.3 - 99.6%
Site 2	20/22	90.9%	72.2 - 97.5%	105/109	96.3%	90.9 - 98.6%
Site 4	0/0	N/A	N/A	2/3	66.7%	20.8 - 93.9%
Site 5	0/0	N/A	N/A	1/1	100%	20.7 - 100%

Table 5. Overall results by patient age

Patient age	Positive Samples			Negative Samples		
	illumigene / Toxigenic culture	Sensitivity %	95% CI	*illumigene* / Toxigenic culture	Specificity %	95% CI
< 2 years	28/30	93.3%	78.7 - 98.2%	156/162	96.3%	92.2 - 98.3%
≥ 2 to 12 years	10/11	90.9%	62.3 - 98.4%	75/79	94.9%	87.7 - 98.0%
> 12 to 21 years	5/5	100%	56.6 - 100%	53/56	94.6%	85.4 - 98.2%
> 21 years	83/87	95.4%	88.8 - 98.2%	417/437	95.4%	93.0 - 97.0%
Age Unknown	1/1	100%	20.7 - 100%	1/1	100%	20.7 - 100%

Analytical Sensitivity *(Reference K100818)*

The analytical sensitivity of this assay for *C. difficile* was based on 20 replicates for each measurand and with a stated probability (e.g., 95% or 19/20 positive replicates) of obtaining positive responses at the following levels of the measurands:

Strain ID	Toxinotype	Phenotype	LoD/Test
VPI 10463	0	A+/B+	4 CFU/test
2007431	III (NAP1)	A+/B+	32 CFU/test
CF1	VIII	A-/B+	64 CFU/test
2006240	V (NAP7)	A+/B+	32 CFU/test
BI8	III	A+/B+	64 CFU/test
2007858	IX/XXIII	A+/B+	32 CFU/test
8864	X	A-/B+	64 CFU/test

Additional *C. difficile* stock cultures from different sources were tested and produced positive reactions at 64 CFU/test with ***illumigene C. difficile***. Strains and toxinotypes tested were as follows: **Type 0 Strains:** 10463, 2004111, 2004205, 2005070, 2005257, 2008029, 2008162, 2008341, 2008351, 2009066, 2009099, B1, G1, J7, K12, Y1; **Type III Strains:** 2004052, 2004118, 2007431, BI17, BI8; **Type V Strains:** 2005325, 2006240, 2008188, 2009018, 2009065, BK6; **Type VIII Strains:** 43598, 2008016, CF1; **Type X Strains:** 8864; **Type XII Strains:** 2007435; **Type IX/XXIII Strains:** 2007858; **Unknown Strains:** 2009132, 2009155, 2009277.

Reproducibility *(Reference K100818)*

Blind coded panels of 10 samples were supplied to three independent laboratories for precision studies. Samples were randomly sorted within each panel to mask sample identities. The panels included contrived samples manufactured at the assay limit of detection (n = 3) and just below the limit of blank (i.e., high negative sample, n = 3). The panels also included uncharacterized positive (n = 2) and negative (n = 2) samples. Testing was performed by different operators at each site on the same day (intra-assay variability) for five days (inter-assay variability). Three lots of ***illumigene C difficile*** were used in this study. The results are given in the table below:

	Site 1		Site 2		Site 3		Total	
Sample Type	**Percent agreement**		**Percent agreement**		**Percent agreement**		**Percent agreement**	
Negative	20/20	100%	20/20	100%	19/19****	100%	59/59	100%
High Negative	25/30	83%	29/30	97%	28/30	93%	82/90	91%
Low Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%
Positive	20/20	100%	20/20	100%	20/20	100%	60/60	100%

***** 1 specimen generated an instrument invalid test result.*

Conclusions

The ***illumigene C. difficile*** assay used in conjunction with the ***illumipro-10*** can be used to detect toxigenic *C. difficile* in human stool samples from pediatric and adult patients. The test is diagnostic for toxigenic *C. difficile* infection.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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

Meridian Bioscience, Inc.
c/o Ms. Michelle L. Smith
Director Quality Systems
3471 River Hills Drive
Cincinnati, OH 54244

FEB 2 4 2011

Re: K110012
Trade/Device Name: illumigene™ *C. difficile* DNA Amplification Assay
Regulation Number: 21 CFR § 866.2660
Regulation Name: Microorganism differentiation and identification device
Regulatory Class: Class I
Product Codes: OMN
Dated: December 31, 2010
Received: January 3, 2011

Dear Ms. Smith:

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 class II (Special Controls), 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.

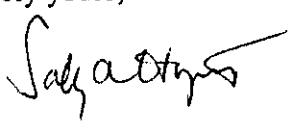
Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); 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 Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

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 (301) 796-7100 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

Indication(s) for Use Form

510(k) Number (if known): K110012

Device Name: *illumigene* Molecular Diagnostic Test System (***illumigene®*** *C. difficile* DNA Amplification Assay, ***illumipro-10™***)

Indications for Use:

The ***illumigene*** *C. difficile* DNA amplification assay, performed on the ***illumipro-10,*** is a qualitative *in vitro* diagnostic test for the direct detection of toxigenic *C. difficile* in human stool specimens from pediatric and adult patients suspected of having *Clostridium difficile*-associated disease (CDAD).

The ***illumigene*** *C. difficile* assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect the pathogenicity locus (PaLoc) of toxigenic *Clostridium difficile*. The *Clostridium difficile* PaLoc is a gene segment present in all known toxigenic *C. difficile* strains. The *C. difficile* PaLoc codes for both the Toxin A gene (*tcdA*) and the Toxin B gene (*tcdB*), has conserved border regions, and is found at the same site on the *C. difficile* genome for all toxigenic strains. The ***illumigene*** *C. difficile* assay detects the PaLoc by targeting a partial DNA fragment on the Toxin A gene. The *tcdA* target region was selected as an intact region remaining in all known A+B+ and A-B+ toxinotypes.

illumigene *C. difficile* is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.

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 OF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Freddie K. Poole

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) K110012