EVALUATION OF AUTOMATIC CLASS III DESIGNATION (DE NOVO) FOR PORTRAIT TOXIGENIC *C. DIFFICILE* ASSAY

REGULATORY INFORMATION

FDA identifies this generic type of device as:

A *Clostridium difficile* toxin gene amplification assay is a device that consists of reagents for the amplification and detection of target sequences in *Clostridium difficile* toxin genes in fecal specimens from patients suspected of having *Clostridium difficile* infection (CDI). The detection of clostridial toxin genes, in conjunction with other laboratory tests, aids in the clinical laboratory diagnosis of CDI caused by *Clostridium difficile.*

NEW REGULATION NUMBER: 21 CFR 866.3130

CLASSIFICATION: II

PRODUCT CODE: OZN

BACKGROUND

DEVICE NAME: PORTRAIT TOXIGENIC *C. DIFFICILE* ASSAY

510(K): K113358

DATE OF 510(K) NSE DECISION: FEBRUARY 3, 2012

DATE OF DE NOVO PETITION: MARCH 2, 2012

PETITIONER CONTACT: GREAT BASIN SCIENTIFIC, INC. – MR LARRY REA

PETITIONER'S RECOMMENDED CLASSIFICATION: II

PETITIONER'S RECOMMENDED CONTROLS:

- Class II Special Controls Guidance Document: Toxin Gene Amplification Assays for the detection of *Clostridium difficile.*
- General controls

INDICATIONS FOR USE

Portrait Toxigenic *C. difficile* Assay, a prescription device under 21 CFR Part 801.109 that is indicated for the detection of toxigenic *Clostridium difficile* in human fecal samples collected from patients suspected of having *Clostridium difficile* infection (CDI). The test utilizes automated blocked primer enabled helicase-dependent amplification (bpHDA) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Portrait Toxigenic *C. difficile* Assay is intended as an aid in the diagnosis of CDI.

LIMITATIONS

For prescription use only

PLEASE REFER TO THE LABELING FOR A MORE COMPLETE LIST OF WARNINGS, PRECAUTIONS AND CONTRAINDICATIONS.

DEVICE DESCRIPTION

The Portrait Toxigenic *C. difficile* Assay as run on the Portrait Analyzer is a bench top fully automated *in vitro* diagnostics system that includes the Portrait Analyzer, control Laptop PC and single-use Portrait Toxigenic *C. difficile* Test Cartridges and sample preparation apparatus. The Portrait Analyzer is designed to perform automated sample extraction; blocked primer mediated thermophilic helicase-dependent amplification (bpHDA); and chip-based detection with integrated data analysis in approximately 85 minutes.

The sample to be tested is inserted into the sample preparation apparatus that has been preloaded with buffer and briefly vortexed. The vortexed mixture is then loaded into the assay cartridge. The cartridge is loaded into the Portrait Analyzer which performs extraction (cell lysing), amplification, hybridization and signal formation on the detection chip. The resulting signal(s) are detected and interpreted by the automated Portrait Analyzer.

In addition to the necessary probes and primers to detect the presence of tcdB (toxin B gene) performance of the Portrait Toxigenic *C. difficile* Assay includes an integrated SPC to insure the adequate processing of the sample during preparation and subsequent extraction and amplification steps.

SUMMARY OF NONCLINICAL/BENCH STUDIES

a. The limit of detection (LoD) of the Portrait toxigenic *C. difficile* Assay for *C. difficile* was assessed and confirmed by using strains of two different toxinotypes: ATCC strain 43255 (CCUG 19126, VPI 10463), toxinotype 0 A+B+ and ATCC strain 43598 (1470), toxinotype VIII A-B+.

The following Table shows performance of the Portrait Toxigenic *C. difficile* assay on serial dilutions of two *C. difficile* strains for initial sensitivity study (CFU/test).

C. difficile strains	Toxinotype	Tested Serial Dilutions (CFU/test)	'*C. difficile* detected
ATCC 43255 (CCUG 19126, VPI 10463)	Type 0 A+B+	53.5	1/1
		5.35	3/4
ATCC 43598 (1470)	Type VIII A-B+	177	3/3
		88.5	3/3
		17.7	1/3

The following Table shows performance of the Portrait Toxigenic *C. difficile* Assay on 20 replicates of two *C. difficile* strains for establishing LoD (CFU/test).

C. difficile strains	Toxinotype	Tested CFU/test	*C. difficile detected*
ATCC 43255 (CCUG 19126, VPI 10463)	Type 0 A+B+	39	20/20
ATCC 43598 (1470)	Type VIII A-B+	39	20/20

b. In addition to the 2 strains used to determine the assay LOD, 44 additional strains of Toxigenic *C. difficile* were tested in replicates of 3 at concentrations just above the LOD (48 CFU/test), All strains were correctly identified as positive (including four NAP1/B1/027 strains: 2004013, 2004118, 2009292, and ATCC BAA-1805) by the Portrait Toxigenic *C. difficile* Assay. Toxinotypes and strains tested: Type 0 Strains: 2004111, 2004205, 2004206, 2005022, 2005283, 2006017, 2007070, 2007302, 2008222, 2009078, 2009087, 2009141, Type 0, A+B+: ATCC 9689(90556-M6S), Type A-B+: CCUG 37782 , Type A+B+: ATCC 17857 (870), ATCC43594 (1253), ATCC43594 (W1194), ATCC 43596 (545), ATCC43599 (2022), ATCC 43600 (2149), ATCC 51695 (BDMS 18 AN), ATCC 700792 (14797-2), ATCC BAA-1382 (630), CCUG 9004, CCUG 9018, CCUG 37766, CCUG 37770, CCUG 37774, CCUG 37776, CCUG 37783, CCUG 37784, ATCC BAA-1814, TYPE III: 2004013*, 2004118*, 2005359*, 2009292*, TYPE III A+B+: ATCC BAA-1805, TYPE V: 2005088*, 2005325*, 2007217*, 2007816*, 2007838*,TYPEVIII: 2006376*,TYPE X A-B+: CCUG 20309 (8864)

**C. difficile* isolates obtained from the CDC

c. These studies were done to assess the potential cross-reactivity of the Portrait Toxigenic *C. difficile* Assay with medically relevant levels of bacteria, viruses and fungi. Bacteria and fungi were tested at concentrations > 6×10^6 CFU/mL or 1×10^5 TCID50/mL. No cross reactivity was observed. Bacterial strains tested: *Aeromonas hydrophila, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Citrobacter freundii, Clostridium difficile (A-,B-), Clostridium perfringens, Clostridium sordellii, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium van A, Escherichia coli, Escherichia coli* O157:H7, *Escherichia fergusonii, Escherichia hermannii, Helicobacter pylori, Klebsiella pneumoniae, Lacotococcus lactis, Listeria monocytogenes, Peptostreptococcus anaerobius, Plesiomonas shigelloides, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enterica,* serovar Typhimurium (group B), *Salmonella enterica,* serovar Choleraesuis (group C1), *Salmonella enterica,* serovar Newport (group C2), *Salmonella enterica*, serovar Typhi (group D), *Salmonella enterica,* serovar Newington (group E), *Serratia liquefaciens, Serratia marcescens, Shigella boydii, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus* Cowan 1, *Staphylococcus epidermidis, Vibrio parahaemolyticus, Yersinia entercolitica.* Viruses: Adenovirus Type 40, Adenovirus Type 41, Coxsackievirus Type B4, Echovirus Type 11, Rotavirus. Fungi: *Candida albicans.* Parasites: *Cryptosporidium parvum (gDNA), Giardia intestinalis WB clone C6 gDNA.*

d. In addition to the Specificity/Cross reactivity testing, the Portrait toxigenic *C. difficile* Assay was tested against the same organisms that were used in the Analytical Specificity panel at the specified concentrations against two strains of toxigenic *C. difficile* spiked into the pooled human stool samples at two times the limit of detection (73 CFU/test). The strains tested were: ATCC 43255 (Toxinotype 0 A+B+) and ATCC 43598 (toxinotype VIII A-B+). All organisms were tested against each strain in triplicate and the Portrait results were positive for all microorganisms tested across both strains.

Interfering Substances:

The following potentially inhibiting substances were tested without showing Assay Interference:

Substance	Active Ingredient	Substance	Active Ingredient
Anusol® Plus (TUCKS)	Mineral oil, pramoxine HCl, Zinc oxide	Moist Towelettes	water, aloe, glycerin, polysorbate 20, disodium cocoamphodiacetate, tocopheryl acetate, methylchloroisothiazolinone, methylisothiazolinone, quaternium-15, potassium sorbate, disodium EDTA, Citric acid, fragrance
Barium sulfate	Barium sulfate	Miconazole 2% cream (Rite Aid)	Miconazole nitrate 2%
Calcium carbonate (TUMS)	Calcium carbonate	Mucin (porcine)	
Cimetidine (Tagamet HB 200)	Cimetidine	Naproxen	Naproxen
Fecal fats	Stearic acid	(Prilosec OTC)	Omeprazole magnesium
Fleet® CB (liquid glycerin laxative)	Glycerin	Pepto-Bismol® Proctor & Gamble	Bismuth subsalicylate
Hydrocortisone Cream (Cortizone-10 max strength)	Hydrocortisone 1%	Preparation H® Wyeth	Glycerin, Phenylephrine HCl, Pramoxine, White petrolatum
Imodium® McNeil-PPC	Loperamide HCl	Senna laxative (Rite Aid)	Sennosides
Kaopectate® Chattem	Bismuth subsalicylate	Vancomycin Fluka	Vancomycin
K-Y Jelly® McNeil-PPC	water, glycerin, hydroxyethylcellulose, chlorhexidine gluconate, gluconolactone, methylparaben, sodium hydroxide	Vaseline Unilever	Petroleum jelly
Metranidazole Actavis (0.75%)	Metranidazole	Whole Blood	

Reproducibility:

Reproducibility studies were performed in-house and at two external clinical sites which were provided with masked coded panels of low, moderate and high concentrations of *C. difficile*. Over the course of five days, two runs were performed with three replicates of each sample per run on each day. A minimum of two operators performed the runs at each site and two different lots of disposable Test Cartridges were used. Results of the Reproducibility studies are summarized in the table below.

Reproducibility Study
Results

Sample Type	% agreement*							
	In-house site		Site 1 (external)		Site 4 (external)		All Sites	
Moderate Positive	30/30	100%	30/30	100%	28/30	93.3%	88/90	97.8%
Low Positive	30/30	100%	30/30	100%	27/30	90.0%	87/90	96.7%
High Negative	27/30	90.0%	29/30	96.7%	30/30	100%	86/90	95.6%

* For Moderate Positive and Low Positive samples, % agreement = '*C. difficile* positive' calls/total runs. For High Negative samples, % agreement = '*C. difficile* ' negative calls/total runs.

† Site 1, Clarion Health Indianapolis, IN (Dr. Gerald Denys, PI)

‡ Site 4, Medical College of Wisconsin, Milwaukee, WI (Dr. Nate Ledeboer , PI)

A precision study was also performed in-house over the course of 12 days. Each day two runs were performed using different operators and two replicates of each sample per run.

Results of the in-house Precision Study were as follows:

Sample Type	% agreement*	
Moderate Positive	58/58	100%
Low Positive	57/58	98.3%
High Negative	54/58	93.1%

* For Moderate Positive and Low Positive samples, % agreement = '*C. difficile* positive' /total runs.

For High Negative samples, % agreement = '*C. difficile* ' negative/total runs.

The results for the reproducibility and precision studies of the Portrait Toxigenic *C. difficile* Assay were within the expected percent agreement across all three sites.

BIOCOMPATIBILITY/MATERIALS

N/A

SHELF LIFE/STERILITY

The Portrait Analyzer has no sterility requirements.

The Portrait Toxigenic *C. difficile* Assay cartridge will be tested to achieve a minimum of 26 weeks stability.

ANIMAL STUDIES

N/A

ELECTROMAGNETIC COMPATIBILITY AND ELECTRICAL SAFETY

The Portrait Analyzer was tested and complied with the requirements for electromagnetic compatibility.

MAGNETIC RESONANCE (MR) COMPATIBILITY

N/A

MECHANICAL SAFETY

N/A

SOFTWARE

Version: Portrait Analyzer software version 1.5.23.19		
Level of Concern: Moderate		
	Yes	**No**
Software description:	X	
Device Hazard Analysis:	X	
Software Requirements Specifications:	X	
Architecture Design Chart:	X	
Design Specifications:	X	
Traceability Analysis/Matrix:	X	
Development:	X	
Verification & Validation Testing:	X	
Revision level history:	X	
Unresolved anomalies:	X	

The information provided is adequate for each of the items listed above.
Links and relationships between software requirements, specifications, testing and software related hazards are summarized in a Traceability Matrix. In summary, the Analyzer function is controlled by software communicating with hardware drivers and responding to signals from various sensors that monitor fluid, thermal and optical conditions on the assay cartridge. Performance of all individual modules (e.g. Thermal PID Module, Optical Sense Module, etc.) has undergone verification testing at the module level (Intra-Module and Regression Testing). All system related functions have undergone system level verification testing to confirm that all interrelated systems meet all performance specifications.

SUMMARY OF CLINICAL INFORMATION

Clinical performance of the Portrait toxigenic *C. difficile* Assay was determined in a multi-site clinical evaluation at 4 US institutions by comparison of the Portrait Assay results to reference culture/cell cytotoxicity (TBC/CCNA) testing. A total of 540 eligible specimens were tested with the Portrait toxigenic *C. difficile* Assay vs.

TBC/CCNA. Relative to TBC/CCNA, Portrait toxigenic *C. difficile* Assay demonstrated a sensitivity of 98.0% and a specificity of 90.9% for toxigenic C. *difficile* when compared to the reference method. The resulting Negative predictive value NPV is 99.5 % and a Positive predictive value of 71.4%.

	Cytotoxic bacterial		
Portrait *C.*	Positive	Negative	Total
Positive	100	40	140
Negative	2	398	400
Totals	102	438	540

		CI_{95}
Sensitivity	100/102 98.0%	92.4-99.6%
Specificity	398/438 90.9%	87.7-93.3%
PPV	71.4%	63.1-78.6%
NPV	99.5%	98.0-99.9%

CI_{95}: confidence intervals at 95%

PPV: Positive predictive value NPV: Negative predictive value

LABELING

Labeling has been provided which includes instructions for use and an appropriate prescription statement as required by 21 CFR 801.109(b)

RISKS TO HEALTH

The table below identifies the risks to health that may be associated with use of the *Clostridium difficile* toxin gene amplification assay and the measures recommended to mitigate these risks.

Identified Risk	**Mitigation Method**
A false positive test result for an individual may lead to inappropriate use of antibiotics for treatment	Kit includes quality control material and instructions for use.
A false negative test result for an individual may lead to a potential delay in treatment	Kit includes quality control material and instructions for use
Failure of the test to perform properly	Product labeling provides instructions for use and limitations of the assay
Failure to properly interpret the test results	Product labeling describes interpretation of results

SPECIAL CONTROLS:

In combination with the general controls of the FD&C Act, the Portrait Toxigenic *C. difficile* Assay is subject to the following special controls:

1. The special controls for the *Clostridium difficile* toxin gene amplification assay are contained in the guidance document: "Class II Special Controls Guidance Document: Toxin Gene Amplification Assays for the Detection of *Clostridium difficile*."

CONCLUSION

The De Novo petition for the Portrait Toxigenic *C. difficile* assay is granted and the device is classified under the following:

Product Code: OZN
Device Type: *Clostridium difficile* toxin gene amplification assay
Class: II
Regulation: 21 CFR 866.3130