

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

K110620

B. Purpose for Submission:

To seek clearance for the Premier™ *C. difficile* GDH Assay

C. Measurand:

Clostridium difficile antigen, glutamate dehydrogenase (GDH)

D. Type of Test:

Qualitative enzyme immunoassay

E. Applicant:

Meridian Bioscience, Inc.

F. Proprietary and Established Names:

Premier™ *C. difficile* GDH Assay

G. Regulatory Information:

1. Regulation section: 21 CFR 866.2660- Microorganism Differentiation and Identification Device
2. Classification: Class I
3. Product code: MCB- Antigen, *C. difficile*
4. Panel: 83, Microbiology

H. Intended Use:

1. Intended use(s):

Premier™ *C. difficile* GDH is a qualitative enzyme immunoassay screening test to detect *Clostridium difficile* antigen, glutamate dehydrogenase, in fecal specimens from symptomatic persons suspected of having *C. difficile* infection (CDI). This test does not distinguish between toxigenic and non-toxigenic strains of *C. difficile*. Samples from symptomatic patients that produce positive results with this test must be further tested with an assay designed to detect toxigenic *C. difficile* strains and assist with the diagnosis of CDI.

2. Indication(s) for use:

Premier™ *C. difficile* GDH is a qualitative enzyme immunoassay screening test to detect *Clostridium difficile* antigen, glutamate dehydrogenase, in fecal specimens from symptomatic persons suspected of having *C. difficile* infection (CDI). This test does not distinguish between toxigenic and non-toxigenic strains of *C. difficile*. Samples from symptomatic patients that produce positive results with this test must be further tested with an assay designed to detect toxigenic *C. difficile* strains and assist with the diagnosis of CDI.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Not Applicable

I. Device Description:

Premier™ *C. difficile* GDH is a qualitative enzyme immunoassay screening test to detect *Clostridium difficile* antigen, glutamate dehydrogenase, in fecal specimens from symptomatic persons suspected of having *C. difficile* infection (CDI). Breakaway microwells are coated with polyclonal rabbit anti-GDH antibodies. Diluted patient specimen and a horseradish peroxidase conjugated (HRP) anti-GDH antibody are added to the microwells and incubated to allow any antigen in the patient sample to bind the antibody conjugate and immobilized microwell antibody. Upon completion of the incubation, a wash step is performed to remove unbound material. If *C. difficile* GDH is present, an antibody-enzyme complex is formed. A chromogenic substrate is added to the microwells and incubated. A blue color develops in the presence of bound enzyme. Stop solution is added, changing the initial blue reaction to yellow. Test results are interpreted spectrophotometrically.

Reagents and Test Components provided:

- Premier™ *C. difficile* GDH Microwells: Polyclonal antibody-coated Microwells, specific to *C. difficile* GDH
- Premier™ *C. difficile* GDH Enzyme Conjugate: HRP-conjugated polyclonal antibodies specific to *C. difficile* GDH in a buffered protein solution containing 0.1% ProClin® and 0.03% gentamicin as preservatives
- Premier™ 20X Wash Buffer II: Concentrated wash buffer containing 0.2% thimerosal as a preservative. Reagent is diluted before use
- Premier™ Substrate I: Buffered solution containing urea peroxide and tetramethylbenzidine at pH 5.0
- Premier™ Stop Solution I: 1M Phosphoric acid
- Premier™ *C. difficile* GDH Sample Diluent/Negative Control: Buffered protein solution containing 0.1% ProClin® and 0.03% gentamicin as preservatives
- Premier™ *C. difficile* GDH Positive Control: *C. difficile* GDH in a buffered protein solution containing 0.1% ProClin® and 0.03% gentamicin as preservatives
- Transfer pipettes
- Microwell strip holder
- Microwell plate sealer
- General laboratory microplate washers and the StatFax™ incubator/shaker can be used with this assay. Use of this equipment is optional.

J. Substantial Equivalence Information:

1. Predicate device name(s):

TechLab *C. diff.* Chek-60 ELISA

2. Predicate 510(k) number(s):

K030992

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	K110620	K030992
Intended Use	Qualitative enzyme immunoassay screening test to detect <i>Clostridium difficile</i> glutamate dehydrogenase in fecal specimens from symptomatic persons suspected of having <i>C. difficile</i> infection (CDI). For professional use in a clinical laboratory setting. For use as an aid in diagnostic testing of samples from patients suspected of having <i>C. difficile</i> infection.	Same
Capture Reagents	Polyclonal antibodies specific to <i>C. difficile</i> GDH	Same
Specimen Preparation	<p>1. Add 200 uL of Sample Diluent/Negative Control to a test tube</p> <p>2. Add 50 uL of thoroughly mixed stool to the Sample Diluent/Negative Control test tube.</p> <p>3. Vortex tube. Add 100uL of diluted stool to microwell.</p>	Same
Test Procedure	<p>Add 1 drop (50 uL) Conjugate to each well</p> <p>Add 2 drops (100 uL) Substrate to each well.</p> <p>2 drops of Positive Control and 100 uL of Sample Diluent/Negative Control are added to the microwells</p>	Same
Interpretation of Results	Assay defines OD readings for positive and negative interpretations	Same

Differences		
Item	Device	Predicate
	K110620	K030992
Detection Reagents	Rabbit polyclonal antibodies specific to <i>C. difficile</i> GDH conjugated to horseradish peroxidase (HRP)	Antigen-specific mouse monoclonal antibody conjugated to HRP.
Test Procedure	<p>Microwells are washed 5-7 times with diluted Wash Buffer</p> <p>2 drops (100 uL) of Stop Solution are added to the Microwells</p> <p>Reaction must be read within 30 minutes of addition of Stop Solution</p>	<p>Microwells are washed 4 times with diluted Wash Buffer</p> <p>1 drop (50 uL) of Stop Solution is added to the Microwells</p> <p>Reaction must be read within 10 minutes of adding Stop Solution</p>
OD Reader	Automatic	Automatic with Visual Read option

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

1. Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff
<http://www.fda.gov/cdrh/oivd/guidance/1588.html>
2. Guidance for Industry and FDA Staff - Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests.
<http://www.fda.gov/cdrh/osb/guidance/1620.html>

L Test Principle:

Premier *C. difficile* GDH is a qualitative enzyme immunoassay screening test to detect *Clostridium difficile* antigen, glutamate dehydrogenase, in fecal specimens from symptomatic persons suspected of having *C. difficile* infection (CDI). Breakaway microwells are coated with polyclonal rabbit anti-GDH antibodies. Diluted patient specimen and a horseradish peroxidase conjugated anti-GDH antibody are added to the microwells and incubated to allow any antigen in the patient sample to bind the antibody conjugate and the immobilized microwell antibody. Upon completion of the incubation, a wash step is performed to remove unbound material. If *C. difficile* GDH is present, an antibody-enzyme complex is formed. A chromogenic substrate is added to the microwells and incubated. A blue color develops in the

presence of bound enzyme. Stop solution is added, changing the initial blue reaction to yellow. Test results are interpreted spectrophotometrically.

Premier *C. difficile* GDH is based on the well-established, widely used microplate enzyme immunoassay platform. The predicate device, TECHLAB C. DIFF CHEK-60, is also built on this platform.

Premier *C. difficile* GDH Positive Control: Non-toxigenic *C. difficile* strain 11186 is grown in liquid media, harvested, and filtered to remove organisms. Bulk manufacture of Positive Control includes dilution of *C. difficile* GDH antigen in a buffered protein solution containing Gentamicin and ProClin™ as preservatives.

Rabbit polyclonal anti-*C. difficile* Common Antigen antibody is used in the manufacture of Premier *C. difficile* GDH Enzyme Conjugate and Premier *C. difficile* GDH Microwells. The antibody is specific for *C. difficile* Common Antigen (GDH) and is internally manufactured at Meridian Bioscience. The antibody is purified using Protein-A, and is qualified for use by Meridian using internally qualified enzyme immunoassay (EIA) methods.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Reproducibility:*

Reproducibility studies were performed by three clinical laboratories using blind coded panels. Samples were randomly sorted within each panel to mask identities. Each panel consisted of three contrived moderately positive specimens, three contrived low-positive specimens, three contrived high-negative specimens, and one natural negative specimen. The moderately-positive, low-positive, and high-negative samples were prepared from negative stool spiked with *C. difficile* GDH antigen. Panels were tested at three independent laboratories by two operators at each laboratory, twice each day over 5 non-consecutive days. Results were read at single (OD₄₅₀) and dual (OD_{450/630}) wavelengths. The expected results were obtained with all samples by all technologists at all test sites at each time interval. Reproducibility of the assay is 100% for moderate positives, low-positives, high-negatives, and weak negatives.

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

No calibrators are supplied or used with this assay.

External Controls:

Two External Control reagents are included with the Premier *C. difficile* GDH assay. External Controls aid the user in detection of reagent deterioration, adverse environmental or test conditions, washing failures, or variance in operator performance that can cause test errors. External Controls are required for routine Quality Control and should be included with each test run.

Positive Control:

and *C. difficile* GDH antigen is in a buffered protein solution containing 0.1% ProClin® and 0.03% gentamicin as preservatives. Non-toxicogenic *C. difficile* 11186 is grown in liquid media, harvested, and filtered to remove organisms. Bulk manufacture of Positive Control includes dilution of *C. difficile* antigen in a buffered protein solution containing Gentamicin and ProClin® as preservatives. Negative Control: Buffered protein solution containing 0.1% ProClin® and 0.03% Gentamicin as preservatives.

Storage Conditions:

A Sample Storage study was designed to verify the adequacy of the sample age, preservation, handling, and storage criteria included in the Premier *C. difficile* GDH package insert. Aliquots of unpreserved contrived positive and negative samples were stored at the following temperatures to determine the worst case storage time for each temperature range: room (19-30 C), refrigerated (2-8 C), conventional freezer (-16 to -28 C), and ultralow freezer (-66 to -84 C). A total of 10 samples were tested, with the Limit of Detect (LoD), Low Positive (LP), and High Negative (HN) samples consisting of manufactured specimens. Results suggest that the Control reagents should optimally be stored at 2-8 C when not in use.

Stability:

Dating is provisionally set with data derived from accelerated stability studies. Results are confirmed with real-time shelf-life studies. Accelerated Stability performed with three lots of Positive and Negative Controls has verified the stability for at least 18 months under normal conditions of use and storage. Real-time studies are ongoing.

Expected Values:

Expected Values for OD₄₅₀ :

Positive Control: ≥ 0.600

Negative Control: <0.150

Expected Values for OD_{450/630} :

Positive Control: ≥ 0.600

Negative Control: < 0.100

Validation:

The performance of the Positive and Negative Controls was validated as part of clinical trials.

d. *Detection limit:*

In order to determine the analytical limit of detection (LoD) of *C. difficile* GDH antigen diluted in a human stool matrix, purified GDH antigen was spiked into negative stool matrix and diluted serially two-fold. A minimum of 45 replicates of each concentration were tested to determine the concentration that represents the Limit of Blank, or the dilution that produces approximately 50% positive and 50% negative results. The next higher two-fold concentration from the Limit of Blank that produced a minimum of 95% positive results was determined to be the Limit of Detection. The next lower two-fold concentration from the Limit of Blank that produced a minimum of 95% negative results was determined to be the High Negative value.

Results: Limit of Blank = 4 ng/mL
Limit of Detection = 8 ng/mL
High Negative = 2 ng/mL

e. *Analytical specificity:*

Interference Studies:

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 three negative stool samples and three positive stool samples. The contrived positive specimens were prepared from a pool of donor stools that were confirmed as negative. The samples were inoculated with *C. difficile* strain 11186 at 8 ng/mL, which is the limit of detection for this assay. 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/diluent concentrations, do not interfere with Premier *C. difficile* GDH test results in the final concentrations listed: Barium sulfate (5 mg/mL), Fecal fat (2.65 mg stearic acid and 1.3 mg palmitic acid/mL), Hemoglobin (3.2 mg/mL), Imodium AD® (Loperamide HCl) (6.67 x 10⁻³ mg/mL), Kaopectate® (Bismuth subsalicylate) (0.87 mg/mL), Metronidazole (12.5 mg/mL), Mucin (3.33 mg/mL), Mylanta® (Aluminum hydroxide w/ magnesium hydroxide) (4.2 mg/mL), Pepto-Bismol® (Bismuth subsalicylate) (0.87 mg/mL), Polyethylene glycol (79.05 mg/mL), Prilosec® (Omeprazole) (0.5 mg/mL), Simethicone (0.625 mg/mL), Tagamet® (Cimetidine) (0.5 mg/mL), Tums® (Calcium carbonate) (0.5 mg/mL), Vancomycin HCl (2.5 mg/mL), Whole blood (25%), White

blood cells (5%).

Cross-reactivity Studies:

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 contrived positive sample. The contrived positive specimens were prepared from a pool of donor stools that was confirmed negative. The contrived positive sample was prepared by spiking a confirmed negative sample with *C. difficile* strain 11186 at 8 ng/mL, which is the limit of detection for this assay. Potentially cross-reactive microorganisms were added at final concentrations of 1.2×10^8 CFU/mL (bacteria or fungi) or final concentrations greater than 1×10^5 TCID₅₀/mL (viruses). Dilution controls for each sample were prepared by adding a 0.85% saline solution in place of the potentially cross-reactive organisms.

The following microorganisms, at the indicated concentrations, do not interfere with Premier *C. difficile* GDH test results: *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Bacteroides fragilis*, *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, *Candida albicans*, *Citrobacter freundii*, *Clostridium butyricum*, *Clostridium bifermentans*, *Clostridium histolyticum*, *Clostridium novyi*, *Clostridium perfringens*, *Clostridium septicum*, *Clostridium sordellii*, *Clostridium tetani*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli O157:H7*, *Escherichia hermannii*, *Escherichia fergusonii*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Lactococcus lactis*, *Listeria monocytogenes*, *Peptostreptococcus anaerobius*, *Plesiomonas shigelloides*, *Porphyromonas asaccharolytica*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella Group B*, *Salmonella Group C*, *Salmonella Group D*, *Salmonella Group E*, *Serratia liquefaciens*, *Serratia marcescens*, *Shigella boydii*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, Adenovirus Type 40, Adenovirus Type 41, Coxsackievirus Strain B4, Echovirus Strain 11, Rotavirus Strain WA.

Stools spiked with *Staphylococcus aureus* (Cowan strain I) and *Clostridium sporogenes* were found to be cross-reactive with Premier *C. difficile* GDH.

Strain Reactivity Studies:

C. difficile stock cultures from different sources were tested and produced positive reactions at a concentration of 5.7×10^7 cells/mL with the Premier *C. difficile* GDH assay. Strains tested were as follows: 8864, 10463, 43598, 2004052, 2004111, 2004118, 2004205, 2004206, 2005070, 2005257, 2005325, 2006240, 2007431, 2007435, 2007858, 2008016, 2008029, 2008162, 2008188, 2008341, 2008351, 2009018, 2009065, 2009066, 2009099, 2009132, 2009155, 2009277, 11186, B1, B18, BI17, BK6, CF1, G1, J7, K12, Y1, 234, 586, 611, 620, 2C62, 2C165, C122, UNC 19904, X15076.

f. *Assay cut-off:*

Premier *C. difficile* GDH is manufactured with a fixed cut-off with cut-off values of 0.200 at A450 or 0.150 at A450/630. This is accomplished on a lot to lot basis with specific procedures for optimizing solid phase and conjugate reagents. The validity of this cutoff was proven in verification studies on three lots of fully manufactured product and clinical trials performed on three lots of fully manufactured product.

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity:*

Clinical trials for the Premier *C. difficile* GDH assay were conducted from November 2010 to February 2011. Performance characteristics of the Premier *C. difficile* assay were determined by comparison to bacterial *C. difficile* culture. Independent clinical test sites located in the Midwestern, Southeastern and Southwestern regions of the United States evaluated a total of 733 qualified patient samples. Samples were prospectively collected from 337 (46.0%) males and 390 (53.2%) females. Gender was not defined for 6 (1%) patients. The age groups of patients ranged from 22 days to 99 years. No differences in test performance were observed based on patient age, gender, or geographic location. Overall sensitivity was determined to be 92.3% (95% CI: 86.0 - 95.9%). Overall specificity was determined to be 95.8% (95% CI: 93.9 – 97.1%).

Performance Characteristics for Premier *C. difficile* GDH (by Site)

Site	Positive Samples			Negative Samples		
	Premier GDH/ Culture	Sensitivity %	95% CI	Premier GDH/ Culture	Specificity %	95% CI
Total Sites	108/117	92.3%	86.0 – 95.9%	590/616	95.8%	93.9 – 97.1%
Site 1	13/16	81.3%	57.0 – 93.4%	84/87	96.6%	90.3 – 98.8%
Site 2	28/30	93.3%	78.7 – 98.2%	132/140	94.3%	89.1 – 97.1%
Site 3	44/46	95.7%	85.5 – 98.8%	147/153	96.1%	91.7 – 98.2%

Site 4	15/15	100.0%	79.6 – 100.0%	169/175	96.6%	92.7 – 98.4%
Site 5	8/10	80.0%	49.0 – 94.3%	58/61	95.1%	86.5 – 98.4%

- Discrepant specimens were evaluated using an FDA-cleared ELISA test for the detection of *C. difficile* GDH. This evaluation was not used to change the initial study results.
- Sixteen of the 26 false positive specimens were positive when tested with another FDA cleared GDH assay.
- Eight of the nine false negative specimens were negative when tested with another FDA cleared GDH assay

Performance Characteristics for Premier *C. difficile* GDH (by Patient Age)

Patient Age	Positive Samples			Negative Samples		
	Premier GDH/ Culture	Sensitivity %	95% CI	Premier GDH/ Culture	Specificity %	95% CI
0-28 days	0/0	N/A	N/A	1/1	100.0%	20.7 – 100.0%
29 days to 2 years	22/24	91.7%	74.2 – 97.7%	56/60	93.3%	84.1 – 97.4%
> 2 years to < 12 years	24/25	96.0%	80.5 – 99.3%	106/109	97.2%	92.2 – 99.1%
12 years to < 18 years	9/11	81.8%	52.3 – 94.9%	59/61	96.7%	88.8 – 99.1%
18 years to 21 years	3/4	75.0%	30.1 – 95.4%	22/23	95.7%	79.0 – 99.2%
> 21 years	50/53	94.3%	84.6 – 98.1%	345/361	95.6%	92.9 – 97.3%
Not Defined	0/0	N/A	N/A	1/1	100.0%	20.7 – 100.0%

b. *Clinical specificity:*

See 3a) above

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The frequency of antibiotic-associated diarrhea caused by *C. difficile* is dependent on several factors including: patient population, type of institution and epidemiology. The reported incidence of *C. difficile* infection in patients suspected of having antibiotic-associated diarrhea is 15-25% although different facilities may find positivity rates outside this range.

N. Proposed Labeling:

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

O. Conclusion:

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