

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

A. 510(k) Number:

K041003

B. Purpose of Submission:

For the detection of *C. difficile* toxins A & B in human stool

C. Analyte:

Clostridium difficile toxins A & B

D. Type of Test:

Horizontal-flow enzyme immunoassay

E. Applicant:

Meridian Bioscience, Inc.

F. Proprietary and Established Names:

ImmunoCard® Toxins A & B

G. Regulatory Information:

1. Regulation section:
21 CFR Part 866.2660 Microorganism Differentiation and Identification
2. Classification:
I
3. Product Code:
LLH – Reagents, *Clostridium difficile* toxin
4. Panel:
83 Microbiology

H. Intended Use:

1. Intended use(s):
ImmunoCard® Toxins A & B is a rapid, qualitative, horizontal-flow enzyme immunoassay (EIA) for detecting *Clostridium difficile* toxins A and B in human stool. This assay is used as an aid in the diagnosis of *C. difficile*-associated disease.
2. Indication(s) for use:
ImmunoCard® Toxins A & B is an in-vitro diagnostic qualitative enzyme immunoassay to detect the presence of *Clostridium difficile* toxins A and B in human stool. The assay is used as an aid in the diagnosis of *C. difficile*-associated disease.

3. Special condition for use statement(s):
For Prescription Use Only
4. Special instrument Requirements:
Not applicable

I. Device Description:

The device consists of a chromatography strip membrane housed in a plastic frame with two sample ports and two reaction ports. The membrane carries immobilized antibodies to toxins A and B at the test reaction port and crude *C. difficile* toxin at the control reaction port. The enzyme conjugate consists of a blend of goat polyclonal antibodies to toxins A & B. The group specific antibodies are bound to horseradish peroxidase as an indicator in the test and control regions of the strip.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Premier Toxins A & B, ImmunoCard Toxin A, ColorPac Toxin A, *C. difficile* Toxin A, *C. difficile* Tox A/B II.
2. Predicate K number(s):
K993914, K55859, K980185, K955067, K003306
3. Comparison with predicate(s):

Similarities		
Item	Device	Predicates
Intended use	an <i>in vitro</i> diagnostic product for the detection of <i>C. difficile</i> toxins in human stool	same
Specimen type	Human stool	same
technology	Enzyme Immunoassay	same
Level of skill	Moderately complex	same
Differences		
Item	Device	Predicates
Limit of detection	3.0 ng/ml of toxins A & B	≥0.8-2.5 ng/ml
Clinical sensitivity	95.2% CI (90.9-99.1%)	81-94.7% CI (72.4-97.5%)
Clinical specificity	98.5% CI (98.0-100%)	96.7-100% CI (95.4-100%)

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

CDRH Draft Guidance Document: “Review Criteria for Assessment of Laboratory Tests Directed at Assisting in the Diagnosis of *C. difficile* Associated Disease” for Industry and FDA Staff.

L. Test Principle:

A chromatographic membrane strip held in a plastic frame and is coated with immobilized antibodies to toxin A and B. The enzyme conjugate reagent consists of group specific antibodies to toxins A and B bound to horseradish

peroxidase. The sample is diluted with specimen diluent and enzyme conjugate and the mixture is incubated for 5 minutes. During the incubation, molecules of toxin, if present, are bound to the anti-toxin antibodies of the conjugate. Following incubation, an aliquot of the mixture is added to each of the two sample ports and the test is incubated for an additional 5 minutes. During the second incubation the toxin-conjugate complex is separated from particulate matter as the fluid portion of the sample flows through the membrane to the test and control reaction ports. The toxin-conjugate complexes are then captured at the test reaction port by immobilized antitoxin in the reaction membrane. The second of the two reaction ports serves as an internal control. Both reaction ports are subsequently washed with wash reagent to reduce interference by contaminating proteins before substrate reagent is added. The reaction ports are incubated for an additional 5 minutes during which time the enzyme conjugate modifies the substrate reagent. If A & B toxins are present, the development of a blue color in the test reaction port indicates a positive test. In the control port, the anti-toxin antibodies of the conjugate bind directly to the immobilized toxin. The appearance of a blue color in the control reaction port indicates that sample was added, that reagents were active at the time of use and that proper sample migration occurred. Reactions are read visually.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The reproducibility of ImmunoCard® Toxins A & B was determined with known negative (n=2) and positive (n=6) samples that were coded and randomly sorted to prevent their identification during testing. Two of the positive samples were near the limit of detection for the assay. Three laboratories tested the samples on three consecutive days. The samples produced the expected results 100% of the time.

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability (controls, calibrators, or method):*

Not applicable

d. *Detection limit:*

The ImmunoCard® Toxins A&B test kit detects toxins A & B at levels of 3.0 ng/ml.

e. *Analytical specificity:*

Stool specimens inoculated with the following microbial agents (to a final sample concentration of $\geq 10^8$ organisms/mL) do not react with ImmunoCard® Toxins A and B: *Adenovirus 40*, *Adenovirus 41*, *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*,

Bacteroides fragilis, *Campylobacter coli*, *Campylobacter jejuni*, *Candida albicans*, *Clostridium butyricum*, nontoxigenic *C. difficile* (10 strains), *Clostridium perfringens*, *Clostridium septicum*, *Clostridium sordellii* strain VPI9048, *Clostridium sporogenes*, *Enterobacter cloacae*, *Escherichia coli* (four strains including two O157:H7 strains), *Helicobacter pylori*, *Klebsiella pneumoniae*, *Peptostreptococcus anaerobius*, *Porphyromonas asaccharolytica*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Rotavirus*, *Salmonella typhimurium*, *Serratia liquefaciens*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus aureus* Cowan I, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*. The only non-*C. difficile* microorganism reactive with ImmunoCard® Toxins A & B was *Clostridium sordellii* VPI 9714. This strain produces toxin A and B homologs HT and LT.

An interference study was conducted and the following substances were determined to have no effect on test results when present in stool in the concentrations indicated: fecal fat (4.8% w/v), whole blood (40% v/v), mucin (3.5% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v), barium sulfate (5% w/v), Imodium AD® (5% w/v), Kaopectate® Caplets (5 mg/mL) Pepto Bismol® (5% v/v).

f. Assay cut-off

The assay was determined to detect Toxin A and Toxin B at 3.0 ng/ml.

2. Comparison studies:

a. Method comparison with gold standard:

Three independent laboratories tested specimens with ImmunoCard® toxins A&B and the standard cell cytotoxicity assay with neutralization. The results of the parallel tests are given below.

Prospective Samples	ICTAB		
	Pos	Neg	Total
Cytotoxin Pos (Std)	67	5	72
Cytotoxin Neg (Std)	2	176	178
Total	69	181	250

		95% CI
Clinical sensitivity	67/72 (93.1%)	87.1-98.9%
Clinical specificity	176/178 (98.9%)	97.6-100%
Predictive value positive test	67/69 (97.1%)	92.9-100%
Predictive value negative test	176/181 (97.2%)	94.5-99.5%

Retrospective Samples	ICTAB		
	Pos	Neg	Total
Cytotoxin Pos (Std)	33	0	33
Cytotoxin Neg (Std)	5	303	308

Total	38	303	341
			95% CI
Clinical sensitivity	33/33 (100%)		N/A
Clinical specificity	303/308 (98.4%)		96.4-99.6%
Predictive value positive test	33/38 (86.8%)		76.2-97.8%
Predictive value negative test	303/303 (100%)		N/A

b. Matrix comparison:
Not applicable

3. Clinical studies:

a. Clinical sensitivity:

Two independent laboratories and Meridian's Development Laboratory performed testing on archival (retrospective) or fresh (prospective) samples collected from symptomatic patient samples that had been submitted for toxin testing. The study included a minimum of 100 cytotoxin positive specimens and 75 cytotoxin negative specimens. The status of all samples (whether positive or negative by *ImmunoCard*) was confirmed by cytotoxin and neutralization. Less than 2% of the 591 samples tested were from pediatric patient (≤ 15 years). (See table above)

b. Clinical specificity:
Refer to (a.) above

c. Other clinical supportive data (when a and b is not applicable):
Not applicable

4. Clinical cut-off:

The lower limit of detection of this assay is approximately 3 ng of toxin A and 3 ng of Toxin B per mL of stool. This limit does not vary from solid to liquid/semi-solid stool.

5. Expected values/Reference range: (Interpretive Criteria)

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*-associated disease in patients suspected of having antibiotic-associated diseases is 15-20%, although different facilities may find positive rates above or below this range. A positive result in an uninfected patient is not expected.

N. Conclusion:

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