AUG 2 0 2004

510(k) SUMMARY OF SAFETY & EFFECTIVENESS

IDENTIFICATION INFORMATION

SUBMITTER'S INFORMATION

This summary of 510(k) safety and effectiveness is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K041003

SUBMITTER'S NAME AND ADDRESS: Meridian Bioscience, Inc.
3471 River Hills Drive
Cincinnati, OH 45244

PHONE NUMBER: (513) 271-3700

FAX NUMBER: (513) 272-5213

CONTACT PERSON: Susan Rolih
Vice President, Regulatory Affairs and Quality Assurance
Official Correspondent

DATE SUMMARY PREPARED: August 18, 2004

NAME OF DEVICE: Immuno*Card*® Toxins A & B
(Immuno*Card* is a registered trademarks of Meridian Bioscience, Inc.)

COMMON NAME: Reagent, *C. difficile* toxins

CLASSIFICATION NAME: Reagents, *C. difficile* toxin [83(LLH)]

REGULATION: 866.2660

PREDICATE EQUIVALENT DEVICES: Premier Toxins A & B (Meridian Bioscience, Inc.), Immuno*Card* Toxin A (Meridian Bioscience, Inc.), *C. difficile* Tox A/B II (Techlab, Inc.), Oxoid Clearview *C. difficile* Toxin A (Unipath, Ltd.), ColorPAC Toxin A (Becton Dickinson)

INTENDED USE:
Immuno*Card*® 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.

BACKGROUND:
Toxigenic *Clostridium difficile* is the leading cause of nosocomial infectious diarrhea in developed countries. An estimated 300,000 cases of *C. difficile* associated disease (CDAD) are seen per year in U.S. hospitals alone. (1,2) *Clostridium difficile* is the etiologic agent in approximately 25% of all cases of antibiotic-associated diarrhea. Virtually any antibiotic can predispose a patient to CDAD. The clinical presentation for CDAD ranges from asymptomatic colonization to life-threatening pseudomembranous colitis and toxic megacolon. (2) Most pathogenic strains of *C. difficile* produce two biologically and immunologically distinct toxins: toxin A (enterotoxin) and toxin B (cytotoxin). Toxin A was once thought to be responsible for most of the pathology seen in human CDAD until reports of clinically relevant disease caused by strains of *C. difficile* that produce only toxin B began to appear in the late 1990's. (1)

The most accurate assay overall for the detection of *C. difficile* is the cytotoxin assay, however the method requires tissue culture facilities, 48-72 hour incubation and is not standardized. (2) The use of a rapid test such as Immuno*Card* Toxins A&B enables the physician to verify infection quickly, begin proper treatment and to initiate enteric isolation precautions in a hospital setting. (2)

INDICATIONS FOR USE

Immuno*Card*® 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.

Sypmtoms of the disease include:

1. Nosocomial infectious diarrhea
2. Antibiotic-associated diarrhea

Contraindications

There are no contraindications associated with the use of this product.

Special instrument requirements

No instruments are used with this product.

Combination with other medical devices

No other medical devices are used in combination with this device.

DEVICE DESCRIPTION AND TECHNOLOGICAL PRINCIPLES

Reagents

Immuno*Card* Toxins A & B is distributed as a test kit that includes the following reagents:

Immuno*Card* Toxins A & B Test Device: A chromatography strip membrane pad housed in a plastic frame and enclosed in a foil pouch with a desiccant. The membrane carries immobilized monoclonal anti-Toxin A and goat polyclonal anti-Toxin B at the TEST reaction port and crude *C. difficile* toxin at the CONTROL reaction port.

Sample Diluent: A buffered salt protein solution containing thimerosal (0.02%) as a preservative.

Positive Control: Inactivated crude *C. difficile* Toxin suspension in buffered solution containing thimerosal (0.02%) as a preservative.

Enzyme Conjugate: A blend of goat polyclonal antibodies to Toxins A and B conjugated to horseradish peroxidase and suspended in a buffered protein solution containing thimerosal (0.02%).

Wash Reagent: A buffered solution containing thimerosal (0.02%) as a preservative.

Substrate Reagent: A buffered solution containing tetramethyl-benzidine and peroxide.

Equipment needed to use the device

There is no equipment needed to use this device.

Cross reactivity, Interfering substances and Analytical Specificity

There are no known interfering substances that affect the performance of this device.

Drugs, Nonmicrobial Substances

To show that drugs or other nonmicrobial substances that might be present in stool (such as fecal fat, mucin, blood, bismuth sulfate, etc) do not effect Immuno*Card* results, tests were performed with five known positive and five known negative samples spiked with the potentially interfering material. Controls, consisting of the same stools spiked with the inert agent phosphate-buffered saline (PBS), were tested in parallel. The grades of each group of spiked samples were summed and averaged then compared to the relevant control average. A substance was considered to interfere when it changed the result of the group average by +/- three or more grades from the control average. As is demonstrated in Table 1, none of the following substances had a significant effect on the test results:

Fecal fat, metronidazole, whole blood, vancomycin, mucin, barium sulfate, Imodium AD®, Kaopectate® Caplets, Pepto Bismol®.

Microbial organisms (potentially cross-reactive species)

To prove that Immuno*Card* Toxins A & B is specific for *C. difficile* toxins, known positive and negative stool specimens were first spiked with bacterial, viral and yeast strains, then tested using Immuno*Card.* The bacteria, yeast and viruses selected were those that might be expected to be present in human stools either as part of normal flora or from a disease state. The final concentration of bacteria or yeast in each sample was $\geq 1 \times 10^8$ organisms/ml. Unspiked stool was tested in parallel to provide a reference against which the reactions with spiked stools could be compared. Reactions (the appearance of a blue color in the test port) were graded using a 10 point scale, where "0" equals no reaction and "10" equals the strongest color development and a positive reaction. In all test cases, the Control Port was expected to produce a positive reaction of ≥ 2. Organisms causing interference were those that diminished positive reactions by 2 or more grades, that caused a positive to become negative, or that caused the appearance of a positive reaction in a formerly negative sample.

As is shown in Table 2, only one of the microorganisms influenced test results. The organism, a related toxigenic form of *Clostridium sordellii*, caused a positive result in a negative stool.

Table 1. Affect of drugs and nonmicrobial substances on positive and negative test results.

Sample ID	PBS (control)		Steric Acid/Palmitic Acid (fecal fat) (4.8% w/v)		Metronidazole (0.25% w/v)		Whole blood (40% v/v)		Vancomycin (0.25% w/v)		Mucin (3.5% w/v)		Barium sulfate (5% w/v)		Imodium AD Loperamide HCl (5% w/v)		Kaopectate Attapulgite (5 mg/mL))		Pepto Bismol (5% v/v)	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
LP1	3	4	3	4	3	4/5	4	4	3	4	3	4	3	4/5	3	5	3	4	3	4
LP2	3	3	3	2	4	4	3	3	2	3	4	3	3	4	3	4	4	4	3	3
LP3	3	2	3	3	3	2	4	2	3	1/2	4	4	4	1	4	1	3	2	3	2
P4	3	4	3	4	3	5	3	4	1/2	4	3	4/5	3	4	3	4	3	3	2	4
LP5	3	2	3	1/2	3	2	3	2	4	2	4	3	2	2	3	3	3	3	3	2
Total + grade	15	15	15	14.5	16	17.5	17	15	13.5	14.5	18	18.5	15	15.5	16	17	16	16	14	15
Average + grade	3	3	3	2.9	3.2	3.5	3.4	3	2.7	2.9	3.6	3.7	3	3.1	3.2	3.4	3.2	3.2	2.8	3
N1	3	0	3	0	4	0	4	0	3	0	3	0	4	0	3	0	4	0	4	0
N2	3	0	4	0	4	0	4	0	3	0	4	0	2	0	2	0	3	0	4	0
N3	3	0	3	0	3	0	4	0	3	0	3	0	4	0	4	0	2	0	3	0
N4	3	0	3	0	2	0	4	0	3	0	3	0	2	0	2	0	3	0	3	0
N5	2	0	3	0	3	0	1/2	0	4	0	3	0	3	0	1	0	2	0	2	0
Total - grade	14	0	16	0	16	0	17.5	0	16	0	16	0	15	0	12	0	14	0	16	0
Average - grade	2.8	0	3.2	0	3.2	0	3.5	0	3.2	0	3.2	0	3	0	2.4	0	2.8	0	3.2	0

Control results

	Control Port	Test Port
ImmunoCard Positive Control	4	5
ImmunoCard Negative Control	3	0

Legend: LP = low positive (as defined by Premier Toxins A & B EIA), C = control port, T = test port, P = positive, N = negative, + = positive, - = negative

Table 2. Affect of microbial organisms on positive and negative test results.

		Spiked into Positive Stool		Spiked into Negative Stool		
Organism	**ATCC # or ID #**	**Control Port**	**Test Port**	**Control Port**	**Test Port**	**Organism effects outcome?**
Unspiked stool control	Not applicable	3-4	2-3	2-4	0	Not applicable
Adenovirus 40 (1:125) (exp. 30Jul04)	01080032H	3	2	4	0	No
Adenovirus 41 (exp. 15Jan05)	4017441.008	4	3	3	0	No
Aeromonas hydrophila	35654	3	3	3	0	No
Bacillus cereus	11778	3	2	3	0	No
Bacillus subtilis	6051	4	3	4	0	No
Bacteroides fragilis	23745	4	2	3	0	No
Campylobacter coli	49941	3	3	3	0	No
Campylobacter jejuni	29428	3	2	3	0	No
Candida albicans	BHI 15004	3	3	4	0	No
Clostridium butyricum	4855	4	2	3	0	No
Clostridium difficile (non-toxogenic) Q11	620-3/28	3	2	2	0	No
Clostridium difficile (non-toxogenic) Q12	UNC19904	2	2	3	0	No
Clostridium difficile (non-toxogenic) Q13	X-15076-590-1/20	3	3	4	0	No
Clostridium difficile (non-toxogenic) Q15	611	3	2	3	0	No
Clostridium difficile (non-toxogenic) Q17	234-25039	3	2	3	0	No
Clostridium difficile (non-toxogenic) Q18	2C62-1/31	4	1	3	0	No
Clostridium difficile (non-toxogenic) Q19	11186	3	2	3	0	No
Clostridium difficile (non-toxogenic) Q20	2C165	2	1	3	0	No
Clostridium difficile (non-toxogenic) Q32	TCH3	3	2	4	0	No
Clostridium difficile (non-toxogenic) Q31	C124	2	2	4	0	No
Clostridium perfringens	3624	3	2	2	0	No
Clostridium septicum	12464	3	1/2	3	0	No
Clostridium sordellii	VPI 9048	3	2	3	1	Yes
Clostridium sordellii	9714	4	3	4	0	No
Clostridium sporogenes	3584	3	2/3	4	0	No
Enterobacter cloacae	3251	3	2	4	0	No

Table 2 continued.

Organism	ATCC # or ID #	Control Port	Test Port	Control Port	Test Port	Organism effects outcome?
Escherichia coli 0157:H7	43895	3	3	3	0	No
Eschericia coli 0157:H7	EMDI-012	3	2	3	0	No
Escherichia coli	8739	4	2	4	0	No
Escherichia coli	9637	3	3	3	0	No
Helicobacter pylori	43504	3	3	4	0	No
Klebsiella pneumoniae	13883	3	2	3	0	No
Peptostreptococcus anaerobius	27337	3	2	3	0	No
Porphyromonas asaccharolytica	25260	4	3	3	0	No
Proteus vulgaris	6380	3	2	3	0	No
Pseudomonas aeruginosa	Meridian Microbank # 18	2	3	3	0	No
Rotavirus (20Aug08)	TRV061	3	2	4	0	No
Salmonella typhimurium	14028	3	3	4	0	No
Serratia liquefaciens	35551	4	3	3	0	No
Shigella dysenteriae	Meridian Microbank # 14	3	3	3	0	No
Shigella flexneri	Meridian Microbank # 15	3	3	3	0	No
Shigella sonnei	Meridian Microbank # 6	3	3	4	0	No
Staphylococcus aureus	6538	4	3	4	0	No
Staphylococcus aureus (Cowans I)	12598	4	4	4	0	No
Staphylococcus epidermidis	12228	3	2	3	0	No
Streptococcus faecalis	Meridian Microbank # 7	4	2	4	0	No
Vibrio parahaemolyticus	17802	3	3	3	0	No
Yersinia enterocolitica	Meridian Microbank # 10	4	3	3	0	No

Calibrators

There are no calibrators used with this device.

Controls

The assay includes an internal control (control port) that is used to demonstrate that sample has been applied, that it has flowed correctly and that the Enzyme Conjugate is active at the time of testing. In addition, a Positive Control Reagent and Sample Diluent (used for a negative control reagent) are supplied as external controls. Proper results obtained with the Control Port, Positive Control and negative control Diluent serve as indicators that the test was performed correctly, that the antibodies embedded in the membrane and the Enzyme Conjugate are active at the time of testing, and that the membrane supports proper sample flow. Failure of the internal and external control to produce the expected results suggests the test was not performed correctly (ie, incorrect volume of reagents added; incorrect incubation temperature or times used or that reagents were not brought to room temperature prior to testing).

Technological principles

ImmunoCard Toxins A & B consists of a membrane held in a plastic frame with two sample ports and two reaction ports. The membrane carries immobilized antibodies to toxins A and B. The Enzyme Conjugate Reagent consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, patient stool 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 at 20-26 C. 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. The result is the appearance of a blue color. Reactions are read visually. 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 blue 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.

SUBSTANTIAL EQUIVALENCE TO PREDICATE DEVICES

Characteristics	**IC Toxins A & B**	**Cytotoxin/ Neutralization (Std)**	**Premier Toxins A & B**	**BD ColorPAC Toxin A**	**Oxoid C. difficile Toxin Detection**	**Wampole C. difficile Tox A/B II**
Device Type						
In vitro diagnostic device	Yes	Yes	Yes	Yes	Yes	Yes
Control	No	No	No	No	No	No
Calibrator	No	No	No	No	No	No
Intended Use						
Detection of Toxins A and B in human stool	Yes	Yes	Yes	Only A	Only A	Yes
Detection of Toxins A and B in culture	No	Yes	Yes	Only A	No	Yes
Screening test	Yes	No	Yes	Yes	Yes	Yes
Diagnostic test	No	Yes	No	No	No	No
Monitoring therapy	No	Yes	No	No	No	No
Acceptable Sample						
Formed stool	Yes	Yes	Yes	Yes	Yes	Yes
Semi-solid stool	Yes	Yes	Yes	Yes	Yes	Yes
Liquid stool	Yes	Yes	Yes	Yes	Yes	Yes
Stool collected in transport media	No	No	No	No	No	No
Broth culture	No	Yes	Yes	Yes	No	Yes

Performance Characteristics (rounded) in Direct Comparison to Clinical Status or Condition	IC Toxins A & B	Cytotoxin (Std)	Premier Toxins A & B	BD ColorPAC Toxin A	Oxoid C. difficile Toxin Detection	Wampole C. difficile Tox A/B II
Clinical Sensitivity	95.2%		94.7%	81%	83.3%	92.2%
Clinical Specificity	98.5%		97.3%	97%	96.7%	100%
Predictive Value of a Positive Test (incidence = 1%)	93.5%		87.4%	ND	91.7%	100%
Predictive Value of a Negative Test	99%		98.9%	ND	93.2%	98.6%
Correlation	98%		96.9%	ND	ND	98.8%
Laboratory Equivalence in Stool tests with (Predicate Device)			(cell cytotoxicity)	(cytotoxin B)	(cell cytotoxicity and neutralization	Tissue culture
Concordance of positive tests with predicate device	95%		90/95 (95%)	96/119 (81%)	110/132 (83%)	165/179 (92%)
Discordance of positive tests with predicate device	5%		5/95 (5%)	23/119 (19%)	22/132 (17%)	14/179 (8%)
Concordance of negative tests with predicate device	98%		465/478 (97%)	622/641 (90%)	301/311 (97%)	973/973 (100%)
Discordance of negative tests with predicate device	2%		13/478 (3%)	19/641 (10%)	10/311 (3%)	0/973 (0%)
Performance characteristics						
Precision/Reproducibility (intra-assay)	100%		See insert	100%	ND	ND
Precision/Reproducibility (inter-assay)	100%		See insert	100%	ND	ND
Linearity/reportable range	NA		See insert	N/A	ND	ND
Limit of detection	3 ng/mL		1.2 ng/mL A 2.4 ng/mL B			≥0.8 ng/mL A ≥2.5 ng/ML B
Liquid stool			ND	1.38-5.21	ND	ND
Semi-solid stool			ND	1.61 – 18.71	ND	ND
Solid stool			ND	3.19 – 22.58	ND	ND
Assay cutoff	NA		See insert	N/A	N/A	See insert

Comparison of Assay Methods

Characteristic	***ICTAB***	***Premier Toxins A & B***	***BD ColorPAC***	***Oxoid C. difficile Toxin A***	***Wampole Tox A/B II***
Intended use	Detection of toxins in human stool	Detection of toxins in human stool and broth culture	Detection of Toxin A in human stool or broth culture	Detection of Toxin A in human stool	Detection of Toxins A and B in fecal specimens and broth culture
Specimen Required	1. Human stool	Human stool	1. Human Stool 2. Broth culture	Human stool	Human stool Human stool in prep soln
Technology	Horizontal-flow EIA	Microplate-based EIA	Rapid chromatographic assay	Rapid chromatographic assay	Microwell based EIA
Level of skill required	Moderate complexity	Moderate complexity	Moderate complexity	Moderate complexity	Moderate complexity
Assay steps	1. Add 200 uL Sample Diluent to a tube 2. Add 3 drops Enzyme Conjugate. 3. Add 25 uL stool to tube, vortex and let stand 5 min. 4. Vortex sample mixture. 5. Add 150 uL diluted sample to test and control ports. 6. Incubate 5 min., 20-26 C. 7. Add 3 drops Wash Reagent to each port. 8. Add Substrate. 9. Incubate 5 min, 20-26 C. 10. Read at end of incubation.	1. Add 200 uL Sample Diluent to a tube. 2. Add 50 uL stool and vortex. 3. Add 100uL diluted sample to test well. 4. Add 1 drop Enzyme Conjugate to well. 5. Incubate 50 min, 35-39 C. 6. Wash wells with Wash Buffer. 7. Add 2 drops Substrate and shake plate and wait 30 seconds. 8. At 2 drops Stop Solution. 9. Wait 2 minutes then read at OD at 450 nm or 450/630nm.	1. Add 1 mL Sample Buffer to tube. 2. Add 0.5 mL or 0.5 g stool and vortex. 3. Add 3 drops diluted sample to test device 4. Add 1 drop Wash Reagent and absorb. 5. Add 1 drop Detector A and wait 3 minutes. 6. Add 1 drop Wash Reagent and absorb. 7. Add 1 drop Detector B and wait 3 minutes. 8. Add 1 drop Wash Reagent and absorb. 9. Read after 1 minute.	1. Add 1 mL Sample Diluent to tube. 2. Add 100 uL or stool bead to tube and vortex. 3. Centrifuge sample to separate solids from supernate. 4. Add 125 uL supernate to Test Unit. 5. Incubate 30 minutes. 6. Read test at end of 30 minutes.	1. Add 200 uL Diluent to tube. 2. Add 50 uL of stool to tube and vortex. 3. Add 1 drop Conjugate to test well. 4. Add100 uL of diluted specimen. 5. Incubate, 37 C, 50 minutes. 6. Wash wells with Wash Solution 7. Add 100 uL Substrate 8. Incubate for 10 minutes. 9. Add 1 drop Stop Solution and wait 2 minutes. 10. Read at OD 450 nm.
End point	Appearance of blue color	Appearance of yellow color	Appearance of pink line	Appearance of blue line	Appearance of yellow color
Interpretation of test result	Positive = appearance of blue color in test and control ports (indicates presence of toxin) Negative = no color in test port with blue color in reaction port (indicates absence of toxin)	Positive = appearance of yellow color in test and positive control wells (indicates presence of toxin) Negative = no color in test well with yellow color in positive control (indicates absence of toxin)	Positive = appearance of a pink line at test and control points (indicates presence of toxin) Negative = no color at test point with pink color at control point	Positive = appearance of a blue line at test and control points (indicates presence of toxin) Negative = no color at test point with blue color at control point	Positive = appearance of yellow color in test and positive control wells (indicates presence of toxin) Negative = no color in test well with yellow color in positive control (indicates absence of toxin)

CLINICAL TRIALS

Two independent laboratories and Meridian's Development Laboratory performed testing on archival (retrospective) or fresh (prospective) samples collected from symptomatic patients that had been submitted for toxin testing. Each laboratory tested the samples by its own reference method (if applicable), a predicate device and ImmunoCard Toxins A and B. The status of all samples (whether positive or negative by ImmunoCard) was confirmed by cytotoxin and neutralization. Because of their experience, and to ensure assay consistency, Site 1 was assigned the responsibility of performing all confirmation cytotoxin assays for Site 1 and Site 3.

Investigators were required to record the age and sex of the patient, the consistency, appearance and age of each stool specimen at the time of testing. Samples were tested fresh (stored at 2-8 C for no more than 72 hours) or after frozen storage at ≤ -20 C.

Patient characteristics (patient age, sex)

The age of the patients included in the clinical trial ranged from 1 to 99 years. Males and females were equally represented. Less than 2% of the 591 samples tested were from pediatric patients (≤15 years) therefore the IFU carries the limitation that the performance of specimens from pediatric patients has not been evaluated. It is expected however, there will be a higher incidence of positive tests in asymptomatic pediatric patients because of the carrier state associated with this population. There were no significant differences in test results attributable to age or sex in tests performed with samples from adult patients.

Table 3. Patient age, sample storage statistics and mean positive reaction strengths

Patient Age and Sample Storage	<1 Yr.	1-5 Yrs	6-15 Yrs	> 15 Yrs	Not defined
Clinical site 1					
Total tested Fresh	0	0	2	140	N/A
Mean positive reaction strength	N/A	N/A	N/A	6.6	N/A
Positive reaction range	N/A	N/A	N/A	1-10	N/A
Total tested Frozen	0	1	0	51	N/A
Mean positive reaction strength	N/A	N/A	N/A	5.7	N/A
Positive reaction range	N/A	N/A	N/A	1-10	N/A
Clinical site 2					
Total tested Fresh	0	0	0	0	N/A
Mean positive reaction strength	N/A	N/A	N/A	N/A	N/A
Positive reaction range	N/A	N/A	N/A	N/A	N/A
Total tested Frozen	0	3	1	51	N/A
Mean positive reaction strength	N/A	8.0	N/A	8.0	N/A
Positive reaction range	N/A	1-10	N/A	1-10	N/A
Clinical site 3					
Total tested Fresh	N/A	N/A	N/A	N/A	N/A
Mean positive reaction strength	N/A	N/A	N/A	N/A	N/A
Positive reaction range	N/A	N/A	N/A	N/A	N/A
Total tested Frozen	N/A	N/A	N/A	N/A	342
Mean positive reaction strength	N/A	N/A	N/A	N/A	7.1
Positive reaction range	N/A	N/A	N/A	N/A	1-10
Clinical site Totals					
Total tested Fresh	0	1	2	140	N/A
Mean positive reaction strength	N/A	N/A	N/A	7.0	N/A
Positive reaction range	N/A	N/A	N/A	1-10	N/A
Total tested Frozen	0	3	1	102	342
Mean positive reaction strength	N/A	8.0	N/A	8.0	7.1
Positive reaction range	N/A	1-10	N/A	1-10	1-10

Table 4. Patient gender statistics and mean positive reaction strengths

	Male	Female	Not defined
Clinical site 1			
Total tested	70	124	0
Mean positive reaction strength	5.6	6.7	N/A
Positive reaction range	1-10	1-10	N/A
Clinical site 2			
Total tested	28	27	0
Mean positive reaction strength	8.2	7.9	N/A
Positive reaction range	1-10	1-10	N/A
Clinical site 3			
Total tested	N/A	N/A	400
Mean positive reaction strength	N/A	N/A	7
Positive reaction range	N/A	N/A	1-10
Clinical site Totals			
Total tested	98	151	400
Mean positive reaction strength	6.6	7.1	7
Positive reaction range	1-10	1-10	1-10

Sample comparison

Semi-solid or liquid (diarrheal) stools are more frequently encountered in patients with *C. difficile*-associated disease (CDAD). However, it may be necessary to test solid stool for the presence of toxin. Instructions for collecting, preparing and storing the three stool types are given in the instructions for use (package insert). The majority of specimens used in the clinical trial were semi-solid. Appropriate results were obtained with all three types.

Table 5. Stool sample type and mean positive reaction strengths

	Stool Type			
	Solid	Semi-solid	Liquid	Not defined
Total tested -- Clinical Site 1				
Total tested	23	108	63	0
Mean positive reaction strength	7.0	5.6	6.7	N/A
Positive reaction range	1-10	1-10	1-10	N/A
Total tested -- Clinical Site 2				
Total tested	20	19	16	0
Mean positive reaction strength	7.2	7.8	9.7	N/A
Positive reaction range	1-10	1-10	1-10	N/A
Total tested -- Clinical Site 3				
Total tested	12	124	206	0
Mean positive reaction strength	3	7.5	7.1	N/A
Positive reaction range	1-3	1-9	1-10	N/A
Total tested -- All Sites				
Total tested	55	251	285	0
Mean positive reaction strength	6.9	6.9	7.1	N/A
Positive reaction range	1-10	1-10	1-10	N/A

Table 6. Stool sample storage parameters and mean positive reaction strengths

	Stool Type		
	Fresh	Frozen	Not recorded
Total tested -- Clinical Site 1			
Total tested	142	52	0
Mean positive reaction strength	6.6	5.7	N/A
Positive reaction range	1-10	1-10	N/A
Total tested -- Clinical Site 2			
Total tested	0	55	0
Mean positive reaction strength	N/A	8.0	N/A
Positive reaction range	N/A	1-10	N/A
Total tested -- Clinical Site 3			
Total tested	0	342	0
Mean positive reaction strength	N/A	7.1	N/A
Positive reaction range	N/A	N/A	N/A
Total tested -- All Sites			
Total tested	142	449	0
Mean positive reaction strength	6.6	7.2	N/A
Positive reaction range	1-10	1-10	N/A

Clinical trial data summarized

The data collected during clinical trials is shown in the spreadsheets provided at the end of these sections. The results can be summarized as follows:

Table 7. Results of clinical evaluations

Clinical site 1	**ICTAB**			**Reference BD ColorPAC**			**Reference Wampole**		
	Pos	**Neg**	**Total**	**Pos**	**Neg**	**Total**	**Pos**	**Neg**	**Total**
Cytotoxin Pos (Std)	41	2	43	35	8	43	38	5	43
Cytotoxin Neg (Std)	1	150	151	2	149	151	6	145	151
Total	42	152	194	37	157	194	44	150	194
			95% CI						
Clinical sensitivity	41/43 (95.3%)		89.1-100%	35/43 (81.4%)			38/43 (88.4%)		
Clinical specificity	150/151 (99.3%)		97.4-100%	149/151 (98.7%)			145/151 (96.0%)		
Predictive value positive test	41/42 (97.6%)		94.1-100%	35/37 (94.6%)			38/44 (86.4%)		
Predictive value negative test	150/152 (98.5%)		97.4-100%	149/157 (94.9%)			145/150 (96.7%)		

Clinical site 2	**ICTAB**			**Reference Oxoid**			**Reference PTAB**		
	Pos	**Neg**	**Total**	**Pos**	**Neg**	**Total**	**Pos**	**Neg**	**Total**
Cytotoxin Pos (Std)	25	3	28	25	3	28	26	2	28
Cytotoxin Neg (Std)	1	26	27	1	26	27	3	24	27
Total	26	29	55	26	29	55	29	26	55
			95% CI						
Clinical sensitivity	25/28 (89.3%)		77.2-100%	25/28 (89.3%)			26/28 (92.9%)		
Clinical specificity	26/27 (96.3%)		88.2-100%	26/27 (96.3%)			24/27 (88.9%)		
Predictive value positive test	25/26 (96.2%)		88.2-100%	25/26 (96.2%)			26/29 (89.7%)		
Predictive value negative test	26/29 (89.7%)		78.2-100%	26/29 (89.7%)			24/26 (92.3%)		

Clinical site 3	ICTAB			Reference BD		
	Pos	Neg	Total	Pos	Neg	Total
Cytotoxin Pos (Std)	34	0	34	25	9	34
Cytotoxin Neg (Std)	5	303	308	11	297	308
Total	39	303	342	36	306	342
			95% CI			
Clinical sensitivity	34/34 (100%)		NA	25/34 (71.4%)		
Clinical specificity	303/308 (98.3%)		96.4-99.6%	297/308 (96.4%)		
Predictive value positive test	34/39 (87.2%)		75.2-98.8%	25/36 (69.4%)		
Predictive value negative test	303/303 (100%)		NA	297/306 (97.1%)		

Total Sites Combined Data – Comparison to Standard	ICTAB			
	Pos	Neg	Total	
Cytotoxin Pos (Std)	100	5	105	
Cytotoxin Neg (Std)	7	479	486	
Total	107	484	591	
				95% CI
Clinical sensitivity	100/105 (95.2%)			90.9-99.1%
Clinical specificity	479/486 (98.5%)			98.0-100%
Predictive value positive test	100/107 (93.5%)			88.1-97.9%
Predictive value negative test	479/484 (99.0%)			98.2-99.8%

Combined data differentiated based on sample type

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

Characterization of samples producing discordant results

Samples producing discordant test results between ImmunoCard Toxins A& B and cytotoxin are listed below. Three of the five samples classified as ImmunoCard false-positive represented true positive samples. These samples contained high levels of toxins that were not neutralized in the cytotoxin assay until the specimens were diluted 1 in 10.

Table 8. Samples producing discrepant results.

Sample Number	ICTAB Result	Cytotoxin Result	Counted as ICTAB	Comment
1-32	Neg	Pos	FN	No repeat testing performed
1-119	Neg	Pos	FN	No repeat testing performed
1-270	Pos	Neg	FP	No repeat testing performed
2-1	Neg	Pos	FN	No repeat testing performed
2-2	Neg	Pos	FN	No repeat testing performed
2-25	Neg	Pos	FN	No repeat testing performed
2-41	Pos	Neg	FP	No repeat testing performed
3-103	Pos	Neg	FP	Strong positive sample. Cytotoxin assay converted to positive on repeat testing at 1:10 dilution
3-118	Pos	Neg	FP	Strong positive sample. Cytotoxin assay converted to positive on repeat testing at 1:10 dilution
3-200	Pos	Neg	FP	Cytotoxin assay remained negative on repeated testing
3-233	Pos	Neg	FP	No repeat testing performed
3-241	Pos	Neg	FP	Strong positive sample. Cytotoxin assay converted to positive on repeat testing at 1:10 dilution

Legend: ICTAB = ImmunoCard Toxins A & B, FN = false negative, FP = false positive

Reproducibility

Reproducibility panels, consisting of eight coded specimens were sent to the three clinical sites. Five of these samples were classified by the predicate device Premier Toxins A & B as positive and one was at the limit of detect of this assay. The samples were expected to produce a positive or negative result. Even though the trial sites were instructed to grade reactions, there were no criteria regarding the strength of a positive reaction that was expected. As the data shows, there was 100% reproducibility/precision within a test site and between the test sites.

Table 9. Results with reproducibility test panels.

	Clinical Site 1			Clinical Site 2			Clinical Site 3		
Sample ID	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1 LP	2	1	2	5	4	4	2	2	3
2 LP	2	2	3	7	5	6	6	6	5
3 MP	5	6	6	4	6	6	5	5	6
4 MP	7	7	8	9	9	9	6	7	8
5 HP	10	9	10	8	9	10	10	10	8
6 N	0	0	0	0	0	0	0	0	0
7 N	0	0	0	0	0	0	0	0	0
8 Limit of detect	3	3	3	4	5	6	3	4	4
Total positive score	29	28	32	37	38	41	32	34	34
Average positive score	4.8	4.6	5.3	6.2	6.3	6.8	5.3	5.7	5.7
Percent correlation	100	100	100	100	100	100	100	100	100

Legend: LP = low positive, MP = moderate positive, HP = high positive, N = negative,

Analytical sensitivity

The analytical sensitivity of the assay was determined in tests using negative stool samples that had been spiked with varying concentrations of pure toxin A or pure toxin B. The assay limit of detection is one less than the highest dilution that produced a positive reaction of grade 1 or more across three lots of ImmunoCard. The results of these studies showed the assay limit of detection is 3 ng/mL for both toxin A and toxin B.

Table 10. Limit of detection testing.

	ICTAB Lot 712050.002			ICTAB Lot 712050.003			ICTAB Lot 712050.004		
Toxin A ng/mL	Control	Test	Interp	Control	Test	Interp	Control	Test	Interp
5.00	4	1/2	Pos	7	1/2	Pos	9	2	Pos
4.00	4	1/2	Pos	7	1	Pos	9	2	Pos
3.00	**4**	**1**	**Pos**	**7**	**1**	**Pos**	**9**	**1**	**Pos**
2.00	4	1	Pos	7	1	Pos	9	1	Pos
1.00	4	0	Neg	7	1	Pos	9	0/1	Pos
0.50	4	0	Neg	7	0	Neg	9	0/1	Pos
0.25	3	0	Neg	7	0	Neg	9	0	Neg
0.00	2	0	Neg	7	0	Neg	9	0	Neg
PC	Pass								
NC	Pass								
Endpoint			2.00			1.00			2.00
Toxin B ng/mL									
3.00	**2**	**1**	**Pos**	**7**	**2**	**Pos**	**9**	**2**	**Pos**
2.00	2	1	Pos	7	2	Pos	9	2	Pos
1.00	3	0/1	Pos	7	1/2	Pos	9	1/2	Pos
0.50	3	0/1	Pos	7	0/1	Pos	9	1	Pos
0.25	3	0	Neg	7	0	Neg	9	0/1	Pos
0.125	3	0	Neg	7	0	Neg	9	0	Neg
0.0625	3	0	Neg	7	0	Neg	9	0	Neg
0.00	3	0	Neg	7	0	Neg	9	0	Neg
PC	Pass								
NC	Pass								
Endpoint			2.00			1.00			0.50

High dose hook effect

There was no high dose hook effect observed in verification or clinical testing performed with this assay.

Clinical trial data shows that ImmunoCard Toxins A & B is substantially equivalent to the standard method (cytotoxicity) and to predicate devices currently approved to market in the United States.

CONCLUSIONS

ImmunoCard Toxins A & B meets all performance claims when used to test human stool specimens from the general population.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

AUG 2 0 2004

Ms. Susan Rolih
Vice President, Regulatory Affairs/Quality Assurance
Meridian Bioscience, Inc.
3471 River Hills Drive
Cincinnati, OH 45244

Re: k041003
Trade/Device Name: Immuno *Card*® Toxins A & B
Regulation Number: 21 CFR 866.2660
Regulation Name: Microorganism differentiation and identification device
Regulatory Class: Class I
Product Code: LLH
Dated: July 21, 2004
Received: July 22, 2004

Dear Ms. Rolih:

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.

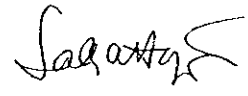
If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish 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 information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 594-3084. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). 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) 443-6597 or at its Internet address http://www.fda.gov/cdrh/dsma/dsmamain.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

INDICATIONS FOR USE
(Amended 8/14/04)

510(K) Number (if known): K041003

Device Name: Immuno*Card* Toxins A& B

Indications For Use: Immuno*Card* 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.

Prescription Use x **AND/OR** **Over-The-counter Use** _____
(Part 21 CFR 801 Subpart D) **(21 CFR 807 Subpart C)**

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

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

Division Sign-Off

Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) K041003