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

A. 510(k) Number:
K033479

B. Analyte:
Clostridium difficile Toxin A & B

C. Type of Test:
Enzyme immunoassay,Qualitative

D. Applicant:
Remel Inc.

E. Proprietary and Established Names:
ProSpect® Clostridium difficile toxin A/B Microplate Assay

F. Regulatory Information:

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

G. Intended Use:

1. Intended use(s):
   Remel's ProSpecT® Clostridium difficile Toxin A/B Microplate Assay is a qualitative enzyme immunoassay (EIA) for the detection of C. difficile Toxin A and B in human fecal specimens from patients suspected of having Clostridium difficile disease. The test is intended for use as an aid in diagnosis of Clostridium difficile associated disease (CDAD).
2. Indication(s) for use:
   Remel's ProSpecT® Clostridium difficile Toxin A/B Microplate Assay is a qualitative enzyme immunoassay (EIA) for the detection of C. difficile Toxin A and B in human fecal specimens from patients suspected of having Clostridium difficile disease. The test is intended for use as an aid in diagnosis of Clostridium difficile associated disease (CDAD)

3. Special condition for use statement(s):
   The product is for prescription use
4. Special instrument Requirements:
   Not applicable

H. Device Description:
The kit consists of a 96 well microplate coated with mouse anti-Toxin A and rabbit anti-Toxin B; conjugate consisting of peroxidase labeled goat anti-Toxin A and rabbit

anti-Toxin B; positive and negative controls; sample diluent; wash buffer; color substrate, stop solution; transfer pipettes, procedure card; instructions for use and a plate cover.

I. Substantial Equivalence Information:

1. Predicate device name(s):

Meridian Premier Toxins A&B
Wampole *C. difficile* TOX A/B II

2. Predicate K number(s):

K993914
K003306/K971182

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate (s)**
Intended Use	Detection of C. difficile Toxins A and B in fecal specimens	Detection of C. difficile Toxins A and B in fecal specimens
Technology	Enzyme immunoassay	Enzyme immunoassay
Material : device	Microwell	Microwell
Material: conjugate	Horseradish peroxidase conjugated to anti-toxins	Horse radish peroxidase conjugated to anti-toxins
Specimen type	Fresh human stool specimens or specimens in modified Cary-Blair	Fresh human stool specimens
Differences		
Item	**Device**	**Predicate (s)**
Capture antibodies or molecules:device	Mouse monoclonal anti-Toxin A and rabbit anti-Toxin B	Wampole: Polyclonal goat antibody against Toxins A and B Meridian: Mouse monoclonal anti-Toxin A and polyclonal goat anti-Toxin B
Antibodies: conjugate	Goat anti-Toxin A and rabbit anti-Toxin B	Wampole: Toxin A monoclonal mouse antibody and Toxin B polyclonal goat antibody Meridian: Polyclonal goat anti-Toxin A and anti-Toxin B
Sample volume	200µl	50µl

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

CDRH Guidance Document for Industry and FDA Staff : "Review Criteria for assessment of laboratory tests directed at assisting in the diagnosis of C.difficile associated disease"

K. Test Principle:

The ProSpecT® Clostridium difficile Toxin A/B test detects the presence of Toxin A and Toxin B in clinical stool specimens through the use of specific antibodies. Microwell strips are coated with mouse monoclonal anti-Toxin A and rabbit anti-Toxin B antibodies. A stool specimen is diluted in Sample Diluent or used directly if pre-diluted in modified Cary-Blair medium. The sample is added to a microwell allowing the toxins, if present, to bind to the immobilized antibodies. After washing to remove unbound components, a conjugate reagent containing goat anti-Toxin A-HRP and rabbit anti-Toxin B-HRP is added to each well. Unbound conjugate is removed by washing and a chromagenic substrate solution is added to detect the presence of bound toxin. A stop reagent is added and the test results are read visually or spectrophotometrically. The presence of a yellow color indicates the presence of toxin.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility testing was conducted at three sites on three separate days with four blinded samples. Each site tested eight replicate wells of each specimen on each day of testing (n=288). The specimens included one negative specimen and three positive specimens with varying levels of reactivity. The average intra-assay coefficient of variation (CV) for a mid-range sample was 7.7%. The average inter-assay CV for a mid-range sample was 18.9%.

b. Linearity/assay reportable range:

Not applicable

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

Not applicable

d. Detection limit:

The ProSpecT® Clostridium difficile Toxin A/B Microplate Assay detects Toxin A at levels of $\geq$0.20 ng/ml and Toxin B at levels of $\geq$0.61 ng/ml.

e. Analytical specificity:

Forty (40) microorganisms were evaluated with the ProSpecT® Clostridium difficile Toxin A/B Microplate Assay. Bacteria and yeast isolates were tested at $\geq 10^8$ colony-forming units per ml (cfu/ml). Viral isolates were tested at concentrations of 10^4 $TCID_{50}$/ml (tissue culture infectious dose per milliliter). No cross-reactivity was observed. There was no cross-reactivity observed with *Clostridium sordellii* ATCC® 9714. However, published literature indicates that certain strains of *C. sordellii* can produce toxins which may be cross-reactive with antibodies to *C. difficile* Toxins A and B. The following organisms were tested in the ProSpecT® Clostridium difficile Toxin A/B Microplate Assay.

Organism	ATCC® designation
Aeromonas hydrophila	35654
Bacillus cereus	11778
Bacillus subtilis	6633
Bacteriodes fragilis	25285
Campylobacter coli	33559

Campylobacter jejuni	33291
Candida albicans	10231

Organism	ATCC® designation
Clostridium beijerinckii (butyricum)	8260
Clostridium difficile (non-toxigenic)	700057
Clostridium haemolyticum	9650
Clostridium histolyticum	19401
Clostridium novyi (toxin A)	19402
Clostridium perfringens (Type A)	13124
Clostridium septicum	12464
Clostridium sordellii	9714
Clostridium sporogenes	19404
Clostridium tetani	19406
Enterobacter aerogenes	35028
Enterobacter cloacae	13047
Enterococcus faecalis	19433
Escherichia coli	11229
Klebsiella pneumoniae	13882
Peptostreptococcus anaerobius	27337
Porphyromonas asaccharolytica	25260
Proteus vulgaris	49132
Pseudomonas aeruginosa	27853
Salmonella choleraesuis (typhimurium)	23852
Serratia liquefaciens	27592
Shigella dysenteriae	11835
Shigella flexneri	12022
Shigella sonnei	25931
Staphylococcus aureus	25923
Staphylococcus aureus (Cowan)	12598
Staphylococcus epidermidis	12228
Vibrio cholerae	9459
Vibrio parahaemolyticus	17802
Yersinia enterocolitica	23715
Adenovirus type 40	VR-930
Adenovirus type 41	VR-931
Rotavirus (Complement fixation antigen)	

Interfering Substances

The following substances were tested with the ProSpecT® Clostridium difficile Toxin A/B Microplate Assay: Vancomycin (12.5 mg/ml), Metronidazole (12.5 mg/ml), blood, mucous, fecal fat, and the following over-the-counter anti-diarrheal products: Pepto-Bismol®, Imodium® A-D, and Kaopectate® (active ingredients: bismuth subsalicylate, loperamide HCl, and attapugite respectively). No interference with positive or negative specimens was observed.

f. Assay cut-off:
The assay was determined to detect Toxin A at levels of ≥ 0.20ng/ml and Toxin B at ≥ 0.61 ng/ml. Concentrations of purified toxins were assigned from titration results obtained using a predicate device. Concentration of toxin testing close to the assay cut off was determined. The toxins were also titrated beyond the assay cut off on the test device. The last dilution remaining at or above the assay cut off was defined as the endpoint dilution. Toxin concentrations were plotted versus OD values. Then an equation that best represented the curve was used to calculate the concentration at the OD cut off assay value

2. Comparison studies:
   a. Method comparison with predicate device:
   Not applicable
   b. Matrix comparison:
   Not applicable
3. Clinical studies:
   a. Clinical sensitivity:

Sensitivity/Specificity

Compared to Tissue Culture Cytotoxicity Assay

Testing was conducted at three laboratories in North America. For all specimens evaluated, the overall sensitivity of the ProSpecT® Clostridium difficile Toxin A/B Microplate Assay when compared to Tissue Culture Cytotoxicity Assay (CTA) was 90.3% (149/165) and the overall specificity when compared to CTA was 96.2% (576/599).

		CTA Results	
OVERALL		+	-
ProSpecT® EIA Results	+	149	23
	-	16	576
Total		165	599

90.3% Sensitivity (149/165); 95% CI = 84.7% - 94.4%
96.2% Specificity (576/599); 95% CI = 94.3% - 97.5%

Visual Interpretation of Test

Visual read data was collected at two of the three laboratories for a total of 586 specimens. The overall sensitivity when compared to CTA was 85.0% and the overall specificity was 95.5%. The visual read results were in 99.0% (580/586) agreement with the spectrophotometric results obtained for each specimen.

		CTA Results	
		+	-
ProSpecT® EIA Visual Results	+	85	22
	-	15	464
Total		100	486

85.0% Sensitivity: (85/100); 95% CI = 76.5% - 91.4%
95.5% Specificity: (464/486); 95% CI = 93.2% - 97.1%

The ProSpecT® Clostridium difficile Toxin A/B Microplate Assay was also compared to two commercially available Enzyme Immunoassays (predicate devices). The performance of the ProSpecT® Clostridium difficile Toxin A/B Microplate Assay and the predicate devices when compared to a CTA (using the same specimens) are as follows:

EIA	**Performance versus CTA**			
	Sensitivity		**Specificity**	
	#	**%**	**#**	**%**
ProSpecT®	33/40	82.5	263/268	98.1
Predicate 1	33/40	82.5	260/268	97.0
ProSpecT®	115/124	92.7	302/320	94.3
Predicate 2	98/124	79.0	309/320	96.5

b. Clinical specificity:
Refer to (a) above

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

4. Clinical cut-off:
   See assay cut-off above
5. Expected values/Reference range:

Expected values were established from the literature.
C difficile colitis occurs at a much higher frequency in patients who are hospitalized and is the fourth most common nosocomial disease reported to the Centers for Disease Control and Prevention. C. difficile is responsible for 20-30% of antibiotic-associated diarrhea and more than 90% of pseudomembranous colitis. The incidence rate of nosocomial CDAD may vary with hospital populations and is influenced by the presence of predisposing factors, such as increased patient age, type and duration of antimicrobial therapy, severity of underlying illness(es), and length of hospital stay. C. difficile is found in 3-5% of healthy adults and up to 50% of infants and young adults asymptomatically carry both the bacteria and its toxins.

M. Conclusion:

In clinical settings, the ProsPecT® Clostridium difficile Toxin A/B Microplate assay is substantially equivalent in performance to the predicate devices for the detection of Clostridium difficile Toxins A & B from human fecal specimens.