

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

A. 510(k) Number:

K041951

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:

Remel Inc.

F. Proprietary and Established Names:

Xpect™ *Clostridium difficile* 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):
Xpect™ *Clostridium difficile* Toxins A/B is a rapid *in vitro* immunochromatographic test for the direct, qualitative detection of *Clostridium difficile* Toxin A and/or B in human fecal specimens from patients suspected of having *Clostridium difficile*-associated disease (CDAD). The test is intended for use as an aid in the diagnosis of CDAD. The test can also be used for confirmation of toxigenic *Clostridium difficile* from Brain Heart Infusion (BHI) broth culture.

Indication(s) for use:

Remel's Xpect™ *Clostridium difficile* Toxins A/B is a rapid *in-vitro* immunochromatographic test for the direct, qualitative detection of *Clostridium difficile* Toxin A and/or B in human fecal specimens from patients suspected of having *Clostridium difficile*-associated disease (CDAD). The test is intended for use as an aid in the diagnosis of CDAD. The test can also be used for confirmation of toxigenic *Clostridium difficile* from Brain Heart Infusion (BHI) broth culture.

2. Special condition for use statement(s):

For Prescription Use Only

3. Special instrument Requirements:

Not applicable

I. Device Description:

The device consists of a chromatography strip membrane housed in a plastic frame that is coated with capture reagents. The membrane carries immobilized antibodies to toxins A and B coupled to colored microparticles. There is immobilized streptavidin as a test line and goat anti-immunoglobulin antibody as a control line. The enzyme conjugate reagent one consists of a blend of microparticles coated with mouse anti-Toxin A and rabbit anti-Toxin B. Conjugate reagent two consists of biotinylated goat anti-Toxin A and rabbit anti-Toxin B. If Toxin A and/or B is present in the patient's specimen, immunocomplexes of Toxin and conjugated antibodies form a visible band as they flow across the test line. Excess colored particle conjugates form a visible band at the control line to assure that the test is functioning properly.

J. Substantial Equivalence Information:1. Predicate device name(s):

BD ColorPac™ Toxin A

Predicate K number(s):

K980185

2. Comparison with predicate(s):

Similarities		
Item	Device	Predicate
Intended use	Used for confirmation of suspect colonies of toxigenic <i>C. difficile</i> from agar plates or BHI broth.	same
Specimen type	Fecal specimens, colonies, BHI broth	same
Level of skill	Moderately complex	same

Differences		
Item	Device	Predicate
Intended Use	A rapid <i>in vitro</i> immunochromatographic test for the direct, qualitative detection of <i>C. difficile</i> Toxin A and/or B in human fecal specimens.	A rapid <i>in vitro</i> chromatographic assay for the qualitative detection of <i>C. difficile</i> Toxin A in human stool specimens.
technology	Immunochromatographic membrane assay	Chromatographic membrane assay
Limit of detection	Toxin A ≥ 6.25 ng/ml (0.12 ng/test) Toxin B ≥ 40.0 ng/ml (0.76 ng/test)	Liquid stool 1.4- 5.2 Semi-solid stool 1.6-18.71 Solid stool 3.2-22.6
Clinical sensitivity	86.3% CI (79.8-91.3%)	81% CI (72.4-87.3%)
Clinical specificity	96.2% CI (94.5-97.5%)	97% CI (95.4-98.2%)

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:

The device consists of a chromatographic membrane strip held in a plastic frame and is coated with immobilized antibodies to toxin A and B. The enzyme conjugate reagents are composed of group specific antibodies to Toxins A and B bound colored microparticles. The sample is diluted with specimen diluent to help solubilize the Toxins. A portion of the diluted sample is then mixed with a volume of Conjugate one containing antibodies to Toxin A and B coupled to colored microparticles, plus a volume of Conjugate two containing biotinylated antibodies to Toxin A and Toxin B. An aliquot of the mixture is transferred to a test device having immobilized streptavidin as a test line and goat anti-immunoglobulin antibody as a control line.

Immunocomplexes of toxin and conjugated antibodies form a visible band as they flow across the test line. If A and/or B toxins are present, the development of a blue color in the test line region indicates a positive test. Excess colored particle conjugates form a visible band at the control line to confirm that the test is functioning properly.

M. Performance Characteristics (if/when applicable):1. Analytical performance:a. *Precision/Reproducibility:*

Reproducibility testing was conducted at three sites, including one in-house site, on four separate days with six blinded samples. The samples consisted of known positive and negative stool specimens.

The samples produced the expected result with the Xpect™ *C. difficile* Toxin A/B test 98.6% (71/72) of the time.

- b. *Linearity/assay reportable range:*
- c. *Not applicable*
- d. *Traceability (controls, calibrators, or method):*
Not applicable
- e. *Detection limit:*
The Xpect™ *C. difficile* Toxin A/B test kit detects Toxin A at ≥ 6.25 ng/ml (0.12 ng/test) and Toxin B at levels of ≥ 40.0 ng/ml (0.76 ng/test).
- f. *Analytical specificity:*
Fifty-four microorganisms were evaluated with the Xpect™ *C. difficile* Toxin A/B test. No cross-reactivity was observed. Bacteria and yeast isolates were tested at 10^8 colony-forming units per ml concentration. Viral isolates were tested at concentrations of 10^4 to 10^5 TCID₅₀ (tissue culture infectious dose) per ml concentration. The following organisms were tested in the Xpect™ *C. difficile* Toxin A/B test.

<i>Aeromonas hydrophila</i>	<i>Proteus mirabilis</i>
<i>Bacillus cereus</i>	<i>Proteus vulgaris</i>
<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>
<i>Bacteroides fragilis</i>	<i>Salmonella Typhimurium</i>
<i>Campylobacter coli</i>	<i>Serratia liquefaciens</i>
<i>Campylobacter fetus subsp. fetus</i>	<i>Shigella boydii</i>
<i>Campylobacter jejuni subsp. jejuni</i>	<i>Shigella dysenteriae</i>
<i>Campylobacter lari</i>	<i>Shigella flexneri</i>
<i>Candida albicans</i>	<i>Shigella sonnei</i>
<i>Clostridium botulinum</i> (toxin 20 µg/ml)	<i>Staphylococcus aureus</i>
<i>Clostridium beijerinickii</i>	(Cowan)
<i>Clostridium difficile</i> (non-toxigenic)	<i>Staphylococcus epidermidis</i>
<i>Clostridium haemolyticum</i>	<i>Vibrio cholerae</i>
<i>Clostridium histolyticum</i>	<i>Vibrio parahaemolyticus</i>
<i>Clostridium innocuum</i>	<i>Yersinia enterocolitica</i>
<i>Clostridium novyi</i>	<i>Giardia intestinalis</i>
<i>Clostridium perfringens</i>	<i>Entamoeba histolytica</i>
<i>Clostridium septicum</i>	<i>Adenovirus type 2</i>
<i>Clostridium sordellii</i>	<i>Adenovirus type 40</i>
<i>Clostridium sporogenes</i>	<i>Adenovirus type 41</i>
<i>Clostridium subterminale</i>	<i>Coxsackievirus B4</i>
<i>Clostridium tetani</i>	<i>Cytomegalovirus</i>
<i>Enterobacter aerogenes</i>	<i>Echovirus (type 22)</i>
<i>Enterobacter cloacae</i>	<i>Enterovirus (type 69)</i>
<i>Enterococcus faecalis</i>	<i>Rotavirus</i>
<i>Enterococcus faecium</i>	
<i>Escherichia coli</i>	
<i>Klebsiella pneumoniae</i>	
<i>Peptostreptococcus anaerobius</i>	

Porphyromonas asaccharolytica

The following substances were tested with the Xpect™ C. difficile Toxin A/B test and no interference was observed in the assay for any substance tested at the indicated levels: blood, mucous, fecal fat, Pepto-Bismol® (10% v/v), Imodium® AD (10% v/v), Kaopectate® (10% v/v), Castoria® (10% v/v), vancomycin (12.5 mg/ml), metronidazole (12.5 mg/ml), and barium sulfate (12.5 mg/ml).

g. Assay cut-off

The Xpect™ C. difficile Toxin A/B test kit detects Toxin A at ≥ 6.25 ng/ml (0.12 ng/test) and Toxin B at levels of ≥ 40.0 ng/ml (0.76 ng/test).

2. Comparison studies:

a. Method comparison with gold standard:

The performance of the Xpect™ Clostridium difficile Toxin A/B was evaluated at four geographically diverse regions of the United States. A total of eight hundred fifteen specimens were tested with the Xpect™ Clostridium difficile Toxin A/B test and compared to results obtained from the cytotoxin assay (CTA).

Overall		CTA Results	
		+	-
Xpect™ Results	+	132	25
	-	21	637
TOTAL		153	662

Sensitivity: 86.3% (95% CI = 79.8-91.3%)

Specificity: 96.2% (95% CI = 94.5-97.5%)

Positive Predictive Value: 84.1% (95% CI = 77.4-89.4%)

Negative Predictive Value: 96.8% (95% CI = 95.2-98.0%)

% Correlation: 94.4% (95% CI = 92.5-95.8%)

Discordant results were further evaluated by toxigenic culture and microwell enzyme immunoassay that detects both Toxin A and B. Four of 25 specimens that were cytotoxin negative and Xpect™ Clostridium difficile Toxin A/B positive on initial testing were positive on retest by toxigenic culture and enzyme immunoassay. Ten of 21 (47.6%) specimens that were CTA positive and Xpect™ Clostridium difficile Toxin A/B negative on initial testing were negative on retest by toxigenic culture and microwell enzyme immunoassay.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical sensitivity:

The Xpect™ C. difficile Toxin A/B test was also compared to two commercially available products. Each of the four clinical trial sites tested a chromatographic membrane assay that detects Toxin A only (Predicate Device), the Xpect™ C. difficile Toxin A/B test, and cytotoxin assay (CTA) for each sample. In addition, one clinical trial site tested a microwell enzyme immunoassay for the detection of both Toxin A and B for each sample. The results presented below are calculated using CTA as the reference.

Performance of Devices Compared to CTA

	n = 815		n = 267	
	Xpect™ C. difficile Toxin A/B	Predicate Device	Xpect™ C. difficile Toxin A/B	EIA
Sensitivity	86.3%	62.7%	91.0%	80.6%
Specificity	96.2%	98.8%	98.0%	97.5%

An in-house study was conducted using twenty-one known reference strains and thirty-six suspect *Clostridium difficile* isolates from stool specimens. BHI broth cultures were tested with the Xpect™ Clostridium difficile Toxin A/B test following 72-hours incubation. Under these conditions, the BHI broth culture of *Clostridium sordellii* ATCC® 9714 produced a positive reaction. There was 94.7% (54/57) agreement with expected values.

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 Xpect™ C. difficile Toxin A/B test kit detects Toxin A at ≥ 6.25 ng/ml (0.12 ng/test) and Toxin B at levels of ≥ 40.0 ng/ml (0.76 ng/test). This limit does not vary from solid to liquid/semi-solid stool.

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

Community-acquired CDAD cases are recognized, but the incidence is low (<1 case per 10,000 antibiotic prescriptions). This may be because diagnostic testing is not performed often enough in outpatient settings to detect CDAD. *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 that asymptotically carry both the bacteria and its toxins.¹² An overall prevalence rate of 16% was observed when the Xpect™ *C. difficile* Toxin A/B was evaluated in a prospective study conducted at four independent laboratories in the United States.

N. Conclusion:

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