

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

A. 510(k) Number:

K102242

B. Purpose for Submission:

To determine substantial equivalence for the OSOM *Clostridium difficile* (*C. difficile*) Toxin A/B Test

C. Measurand:

C. difficile toxins A and/ or B

D. Type of Test:

Sandwich immunoassay employing immunochromatographic dipstick technology

E. Applicant:

Genzyme Diagnostics

F. Proprietary and Established Names:

OSOM *C. difficile* Toxin A/B Test

G. Regulatory Information:

1. Regulation section:

21 CFR 866.2660 – Microorganism differentiation and identification device.

2. Classification:

Class I

3. Product code:

LLH – Reagents, *C. difficile* toxin

4. Panel:

H. Intended Use:

1. Intended use:

The OSOM *C. difficile* Toxin A/B Test is an immunochromatographic assay intended for the qualitative detection of *Clostridium difficile* toxins A and/or B in human stool samples. This test is intended as an aid in the diagnosis of *C. difficile*-associated disease (CDAD) in patients with symptoms of CDAD.

2. Indications for use:

The OSOM *C. difficile* Toxin A/B Test is an immunochromatographic assay intended for the qualitative detection of *Clostridium difficile* toxins A and/or B in human stool samples. This test is intended as an aid in the diagnosis of *C. difficile*-associated disease (CDAD) in patients with symptoms of CDAD.

3. Special conditions for use statement:

For Prescription Use

4. Special instrument requirements:

None

I. Device Description:

The OSOM *C. difficile* Toxin A/B test is a rapid test which can detect the presence of *Clostridium difficile* toxins A and B in human stool samples. The test kit contains 25 OSOM test stick devices and 25 disposable pipettes, 10 applicator sticks, 35 test tubes, 2 bottles sample diluent: 20 mL each (buffered solution with protein, surfactant, 0.09% sodium azide and 0.05% Proclin 300), 2 diluent dropper tops, 1 bottle reagent: 8 mL (antibody conjugate, buffered solution with protein, 0.09% sodium azide and 0.05% ProClin 300), 1 positive control: 1mL *C. difficile* Toxoid A and Toxoid B (contains 0.09% sodium azide), 1 negative control: 1mL (contains 0.09% sodium azide), 1 directional insert, 1 result interpretation guide and 1 workstation.

J. Substantial Equivalence Information:

1. Predicate device name:

Remel Xpect *Clostridium difficile* toxin A/B

2. Predicate K number(s):

K041951

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Rapid immunochromatographic test for the qualitative detection of <i>C. difficile</i> toxins A and/or B in human fecal specimens	Same
Matrix	Human fecal specimen	Same
Assay time	20 minutes	Same

Differences		
Item	Device	Predicate
Sample volume	Solid stool: pea sized portion	Solid stool: 0.2g
	Liquid stool: 0.05mL	Liquid stool: 0.2mL
Antibodies	Capture: goat polyclonal anti-toxin A and rabbit polyclonal anti-toxin B Detection: goat polyclonal anti-toxin A and mouse monoclonal anti-toxin B	Capture: mouse anti-toxin A and rabbit anti-toxin B. Detection: Biotinylated goat anti-toxin A and rabbit anti-toxin B

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

Not referenced

L. Test Principle:

The OSOM *C. difficile* toxin A/B test is a qualitative assay using immunochromatographic dipstick technology. It is a sandwich immunoassay with a single test zone on the nitrocellulose dipstick to detect toxin A and/or toxin B and a single control line zone to indicate proper sample flow. If the toxins are present in the sample, they will bind to blue colored latex particles conjugated to a monoclonal antibody against toxin B or a polyclonal antibody against toxin A and will form a partial immune complex. The test stick, when placed in the sample mixture, initiates sample migration along the nitrocellulose membrane. If toxin A or toxin B is present, a blue/gray line will appear in the test line region indicating a positive result. A red control line must appear for the results to be valid. If *C. difficile* toxins are not

present, only the red control line will appear. An invalid test occurs when no control line appears.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The reproducibility study was conducted by 2 lab personnel per day at 3 sites—one in-house site and two health centers (a small and large hospital clinical laboratory). A coded panel of 12 samples was prepared in a stool matrix and included *C. difficile* toxin A and toxin B. The panel was comprised of 3 negative, 3 high negative, 3 low positive and 3 moderate positive samples. Each set was run twice per day for a period of 5 days at the three sites. Positive and negative assay controls were included. The two runs on any given day at each site were performed by a different operator. The OSOM *C. difficile* Toxin A/B test gave the expected result 99.2% (357/360) of the time.

b. *Linearity/assay reportable range:*

Not applicable

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

Shelf life for the OSOM *C. difficile* toxin A/B test was initially estimated by accelerated stability testing with 2 lots at 2-8° C, 22° C, 37 and 45° C for 35 days of testing. Testing included 6 replicates on Day 0 and 3 replicates on all other days using in-house *C. difficile* Toxin A and Toxin B QC controls (Toxoid A and Toxoid B standards purified from *C. difficile* strain (ATCC: VPI 10643) with immunoreactivity confirmed by commercially available ELISA. Observed results predicted >24 months stability for both the OSOM test kit and the kit controls. Real time stability was examined for 2 kit lots stored at 2-8 ° C for up to 2.2 years. Stability for external controls was also examined when stored at 2-8°C. Observed results showed all positive control results remained positive and all negative control results remained negative for all lots and all time points. The test kit and controls have a stability in excess of 24 months.

Stool sample storage conditions were also established in a study using 2 reagent lots with 3 toxin-positive and 3 toxin-negative stool samples. Results from the study showed that stool samples for the test can be stored up to 4 hours at room temperature, up to 72 hours when refrigerated at (2-8°C), or up to 2 months if stored frozen (-20°C).

d. *Detection limit:*

The OSOM *C. difficile* toxin A/B test detected 15 ng/mL for toxin A and 40 ng/mL for toxin B. These studies were conducted with 3 representative lots using a serial dilution series prepared from purified *C. difficile* toxin A and toxin B in a buffer matrix.

The limit of detection was also examined in a stool matrix dilution series. An antigen dilution series was prepared by spiking the stool matrix with *C. difficile* toxin A & B standard diluted to a concentration of 480ng/mL. Two-fold dilutions of the toxin-stool-matrix were prepared to generate the series (480, 240, 120, 60, 30, 15, 8 ng/mL). Stool matrix by composition is a non-homogenous mixture which can introduce variability in a dilution series. The limit of detection results in the stool matrix were consistent with those determined in the buffer matrix.

e. *Analytical specificity:*

Cross-Reactivity

The OSOM *C. difficile* Toxin A/B Test was evaluated with bacterial and viral isolates. All testing was performed in a diarrhea matrix except where noted. Cross-reactivity testing was performed with materials obtained from ATCC. Bacterial isolates were tested at a concentration of 10^8 cfu/mL except where noted. All viruses were cultured to ensure viability and tested at the specified concentrations. All bacteria listed gave negative responses. All viruses listed produced negative responses.

Table 1: Organisms tested at 10⁸ cfu/mL except where noted

<i>Aeromonas hydrophila</i>	<i>Escherichia coli</i>
<i>Bacillus cereus</i>	<i>Escherichia coli sero:0157</i>
<i>Bacillus subtilis</i>	<i>Escherichia coli type 0124:NM (ETEC)</i>
<i>Bacteroides fragilis</i>	<i>Escherichia coli type o78:k80:h12 (EIEC)</i>
<i>Campylobacter coli</i>	<i>Giardia lamblia</i> ²
<i>Campylobacter fetus</i>	<i>Helicobacter pylori</i>
<i>Campylobacter jejuni</i>	<i>Klebsiella pneumoniae</i>
<i>Candida albicans</i>	<i>Peptostreptococcus anaerobius</i>
<i>Clostridium difficile</i> (non-toxicogenic)	<i>Porphyromonas asaccharolytica</i>
<i>Clostridium beijerinckii</i>	<i>Proteus vulgaris</i>
<i>Clostridium haemolyticum</i>	<i>Pseudomonas aeruginosa</i>
<i>Clostridium histolyticum</i>	<i>Salmonella typhimurium</i>
<i>Clostridium novyi</i>	<i>Serratia liquefaciens</i>
<i>Clostridium perfringens</i> ³	<i>Shigella dysenteriae</i> ²
<i>Clostridium septicum</i>	<i>Shigella flexneri</i>
<i>Clostridium sordellii</i>	<i>Shigella sonnei</i>
<i>Clostridium sporogenes</i>	<i>Staphylococcus aureus</i> (Cowan's serotype 1)
<i>Clostridium tetani</i>	<i>Staphylococcus aureus</i>
<i>Cryptosporidium parvum</i> ¹	<i>Staphylococcus epidermidis</i>
<i>Enterobacter aerogenes</i>	<i>Vibrio cholerae</i> ³
<i>Enterobacter cloacae</i>	<i>Vibrio parahaemolyticus</i>
<i>Enterococcus faecalis</i>	<i>Yersinia enterocolitica</i>

¹ Tested at 0.91x10⁶ cfu/mL

² Tested at 1x10⁶ cfu /mL

³ Tested 1x10⁸ cfu /mL in a buffer matrix

Table 2: Viruses tested at specified concentrations

	TCID ₅₀ /mL
Human adenovirus 40 (strain Dugan)	5.25x10 ⁴
Human coxsackievirus B4 (strain J.V.B)	2.34x10 ⁴
Human cytomegalovirus (strain Towne)	1.86x10 ²
Human echovirus 22 (strain Harris)	4.79x10 ⁶

Human enterovirus 69 (strain Toluca – 1)	9.55x10 ⁴
Human rotavirus (strain HRV-408)	1.62x10 ²

Interference Substances

The following potential interferents were tested and were found to have no effect on the performance of the OSOM *C. difficile* Toxin A/B Test.

Table 3: Exogenous Substances

Potential Interferent	Concentration
Barium sulfate	5% w/v
Fecal fat	5% w/v
Hemorrhoidal Cooling Gel	5% v/v
Imodium [®] AD caplets	5% w/v
Kaopectate [®]	5% v/v
KY Jelly	5% v/v
Metronidazole	0.25% w/v
Mucin	3.5 % w/v
Pepto Bismol [®]	5% v/v
Vancomycin	0.25% w/v
Whole blood	40% v/v

f. Assay cut-off:

The assay was determined to detect Toxin A at 15 ng/ml and Toxin B at 40 ng/mL.

2. Comparison studies:

a. Method comparison with predicate device:

The OSOM *C. difficile* Toxin A/B Test was compared to a commercially available device (see Table below). All testing was performed at a clinical trial site. A total of 250 paired sample results (OSOM and Predicate) were included in this comparison. The performance of the Predicate and OSOM tests were compared to the results from the cytotoxicity assay.

Table 4: Comparison of OSOM *C. difficile* Toxin A/B Test and Commercially Available Device with Cytotoxicity Assay Results

Test Method	Agreement of Positive Samples	95% CI Positive Samples	Agreement of Negative Samples	95% CI Negative Samples	Accuracy	95% CI Accuracy
Commercially Available Device	70.8% (34/48)	56.8- 81.8%	97.5% (197/202)	94.3- 98.9%	92.4% (231/250)	89.7- 93.8%
OSOM	87.5% (42/48*)	75.3- 94.1%	90.1% (182/202)	85.2- 93.5%	89.6% (224/250)	85.9- 92.1%

* 1 sample gave an invalid OSOM test result with the frozen sample – no internal control line appeared even on repeat; included in the analysis as an incorrect result for purposes of comparison.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Stool samples were collected from a total of 1274 adult patients at 5 sites in the United States. The specimens were tested for *C. difficile* by cytotoxicity assay (CTA) and the OSOM *C. difficile* Toxin A/B Test. The samples were de-identified, excess, loose or watery stool specimens obtained from patients ranging from 18 yrs. to > 90 years old. Of the 1274 stool specimens in the study, 577 were from males and 697 were from females. Stool consistency was observed as 547 loose stools and 727 watery stools. The specimens were submitted to the lab for *C. difficile*-associated disease (CDAD) testing. Specimens were tested with the OSOM *C. difficile* Toxin A/B test within 72 hrs of receipt. A standard *C. difficile* toxin cytotoxicity assay was also performed. The performance of the OSOM *C. difficile* Toxin A/B Test was determined for clinical sensitivity and specificity against the results from the reference method, CTA. The results from this analysis with 95% confidence intervals are summarized in the Table below.

Table 5
COMPARISON OF OSOM *C. DIFFICILE* TOXIN A/B TEST TO (CTA)

		Cytotoxin		Total
		+	-	
OSOM [®] C. difficile Toxin A/B Test	+	90	37	127
	-	12	1135	1147
Total		102	1172	1274

Sensitivity: $90/102 = 88.2\%$ (95% CI, 80.5 - 93.1%)

Specificity: $1135/1172 = 96.8\%$ (95% CI, 95.7 - 97.7%)

Agreement: $1225/1274 = 96.2\%$ (95% CI, 95.1 - 96.9%)

b. *Clinical specificity:*

See 3(a) 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:

Clostridium difficile is the most frequently identified cause of nosocomial diarrhea, accounting for 15%-25% of cases of antibiotic-associated diarrhea and more than 95% of cases of pseudomembranous colitis.^{1,3} CDI is primarily a nosocomial disease, and therefore the rate of infection may vary from location to location. Risks for infection include length of hospitalization, patient age, antibiotic use, severity of underlying disease and gastrointestinal surgery or procedures.⁸ Test results should be used in conjunction with the patient's clinical information as asymptomatic colonization with *C. difficile* and its toxins can be seen in some healthy adults, up to 50% of cystic fibrosis patients and up to 50% of infants.⁴ In a prospective clinical study involving the OSOM *C. difficile* Toxin A/B Test at 5 independent sites, an overall prevalence rate of 8.0% (102/1274)

was observed in freshly acquired diarrhea samples submitted to the laboratory for CDI testing.

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.