

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

A. 510(k) Number:

K090239

B. Purpose for Submission:

New device clearance

C. Measurand:

Clostridium difficile nucleic acids

D. Type of Test:

A Real Time PCR *in vitro* diagnostic test for the qualitative detection of toxigenic *Clostridium difficile* nucleic acids isolated and purified from liquid or soft stool specimens obtained from symptomatic patients.

E. Applicant:

Prodesse Incorporated

F. Proprietary and Established Names:

ProGastro™ Cd Assay

G. Regulatory Information:

1. Regulation section:

866.2660

2. Classification:

I

3. Product code:

LLH

4. Panel:

Microbiology

H. Intended Use:

The ProGastro™ Cd Assay is a Real Time PCR *in vitro* diagnostic test for the qualitative detection of toxigenic *Clostridium difficile* nucleic acids isolated and purified from liquid or soft stool specimens obtained from symptomatic patients. This test targets the *Clostridium difficile* toxin B gene (*tcdB*) and is intended for use to aid in the diagnosis of toxigenic *Clostridium difficile* infections.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Cepheid SmartCycler II Real Time Instrument

I. Device Description:

The ProGastro Cd Assay detects toxigenic *Clostridium difficile* and an Internal Control by a process of nucleic acid extraction from patient specimens followed by PCR amplification and detection. Following collection of a soft or liquid stool sample from a symptomatic patient, a portion of the sample is diluted in Stool Transport and Recovery (S.T.A.R.) Buffer and the solids separated via centrifugation (Stool Clarification). The Internal Control is added to the sample prior to extraction to monitor for PCR inhibitors that may be present. The nucleic acids from the sample are extracted and purified using the bioMérieux NucliSENS easyMAG automated extractor. Nucleic acids are added to the *C. diff* Mix for subsequent PCR amplification and detection using the Cepheid SmartCycler II.

The *C. diff* Mix contains oligonucleotide primers and probes that target the *tcdB* gene of toxigenic strains of *C. diff*. The probes are dual-labeled with a reporter dye attached to the 5'-end and a quencher dye attached to the 3'-end (see table below). During PCR amplification the primers and probes anneal to the template (if present) followed by primer extension and template amplification. The 5'-3' exonuclease activity of the Taq polymerase cleaves the probe thus separating the reporter dye from the quencher and generating an increase in fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification product present. The SmartCycler II instrument and software monitors the process, interprets the data, and presents a report upon completion.

J. Substantial Equivalence Information:

1. Predicate device name(s):
C. difficile Toxin/Antitoxin Kit
BD GeneOhm™ Cdiff Assay
2. Predicate 510(k) number(s):
K923463
K081920
3. Comparison with predicate:

Features	Prodesse ProGastro Cd Assay	TechLab C. difficile Toxin/Antitoxin Kit	BD Geneohm CDiff Assay
510(k)		K923463	K081920
Regulation	866.2660	866.2660	866.2260
Product Code	LLH	LLH	LLH
Classification Name	Regents, Clostridium difficile toxin	Regents, Clostridium difficile toxin	Regents, Clostridium difficile toxin
Device Class	Class I	Class I	Class I
Intended Use	Real Time PCR in vitro diagnostic test for the qualitative detection of toxigenic Clostridium	Use in conjunction with the tissue culture cytotoxicity assay for the confirmation of	Rapid in vitro diagnostic test for the direct, quantitative detection of C. difficile toxin B gene

Features	Prodesse ProGastro Cd Assay	TechLab C. difficile Toxin/Antitoxin Kit	BD Geneohm CDiff Assay
	difficile nucleic acids isolated and purified from stool specimens obtained from symptomatic patients. This test targets the Clostridium difficile toxin B gene (tcdB) and is intended for use to aid in the diagnosis of toxigenic Clostridium difficile infections.	Clostridium difficile toxin in patient specimens (stool).	(tcdB) in human liquid or soft stool specimens from patients suspected of having Clostridium difficile-associated disease (CDAD).
Technology/ Detection	Real Time PCR	Tissue culture cytotoxicity / cell rounding	Real Time PCR
Specimen Types	Raw stool (not formed)	Raw stool	Liquid or soft raw stool
Collection and Transport Medium	Standard stool collection and handling procedures. Specimens should be stored refrigerated (2-8°C) for up to 48 hours before processing. If Stool Clarification cannot be performed within 48 hours of collection, store specimens at ≤ - 70°C.	Standard stool collection and handling procedures. Specimens should be stored at 2-8°C for transport and storage and tested with 48 hours.	Transfer liquid or soft stool (but not urine) into a dry sterile container. Specimens should be kept at 2-25°C during transport and protected against freezing or exposure to excessive heat. Specimens can be stored at 2-8°C for up to 5 days before testing. Specimens can be kept at 15-25°C for up to 48 hours before testing. Specimens can be tested after 1 freeze-thaw cycle.
Nucleic Acid Isolation	bioMérieux NucliSENS easyMAG	N/A	N/A
Instrument /Assay Platform	Cepheid SmartCycler II System	Tissue culture/inverted microscope (visualize cell rounding)	Cepheid SmartCycler
Time to Result	2 hours	24 to 96 hours	2 hours
Assay Controls	Positive Control and Internal Control	Positive Toxin Control reagent	Control DNA (positive control) and negative control
Results	Negative Positive Unresolved Not Determined	Negative Positive Unresolved Cytotoxic activity present but is not characteristic of C. difficile toxin	Negative Positive Unresolved Not Determined

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

N/A

L. Test Principle:

The ProGastro Cd Assay detects toxigenic *Clostridium difficile* and an Internal Control by a process of nucleic acid extraction from patient specimens followed by PCR amplification and detection. Following collection of a soft or liquid stool sample from a symptomatic patient, a portion of the sample is diluted in Stool Transport and Recovery (S.T.A.R.) Buffer and the solids separated via centrifugation (Stool Clarification). The Internal Control is added to the sample prior to extraction to monitor for PCR inhibitors that may be present. The nucleic acids from the sample are extracted and purified using the bioMérieux NucliSENS easyMAG automated extractor. Nucleic acids are added to the *C. diff* Mix for subsequent PCR amplification and detection using the Cepheid SmartCycler II.

The *C. diff* Mix contains oligonucleotide primers and probes that target the *tcdB* gene of toxigenic strains of *C. diff*. The probes are dual-labeled with a reporter dye attached to the 5'-end and a quencher dye attached to the 3'-end (see table below). During PCR amplification the primers and probes anneal to the template (if present) followed by primer extension and template amplification. The 5'-3' exonuclease activity of the Taq polymerase cleaves the probe thus separating the reporter dye from the quencher and generating an increase in fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification product present. The SmartCycler II instrument and software monitors the process, interprets the data, and presents a report upon completion.

M. Performance Characteristics (if/when applicable):

Expected Values

The prevalence of *C. difficile* Infection can vary between institutions. Variables that affect the frequency of the disease are patient population, type of institution and epidemiology. *C. difficile* is responsible for approximately 20% of all cases of antibiotic associated diarrhea and the overall incidence of disease is approximately 0.1 - 2% among all hospitalized patients¹. In the ProGastro Cd multicenter prospective study, the prevalence among symptomatic patients varied from 10.4% to 15.8% by site and averaged 13.4% overall. The number and percentage of *C. diff* positive cases determined by the ProGastro Cd Assay during this study, stratified by patient age group, are presented in the following table:

Age Group	Total (N)	Total # <i>C. diff</i> Positive by the ProGastro Cd Assay	Observed Prevalence
2 – 5 years	60	8	13.3%
6 – 10 years	43	5	11.6%
11 – 15 years	67	11	16.4%
16 – 21 years	53	9	17.0%
22 – 59 years	292	37	12.7%
≥ 60 years	256	33	12.9%
Total	771	103	13.4%

Clinical Performance

Performance characteristics of the ProGastro Cd Assay were established during a prospective study at three U.S. clinical laboratories from July through October 2008. Samples used for this study were leftover raw stool specimens that were collected for routine *Clostridium difficile* testing from patients over two years of age by each site. The reference method was tissue culture cytotoxin assay (CTA). Demographic details for this patient population are summarized in the following table:

Age	Number of Subjects (Percentage of Total)
2 – 5 years	60 (7.8 %)
6 – 21 years	163 (21.1%)
22 – 59 years	292 (37.9%)
≥ 60 years	256 (33.2%)

A total of 771 raw stool samples were tested with the ProGastro Cd Assay and by CTA. None of the 771 samples were inhibited when tested with the ProGastro Cd Assay.

		<i>CTA</i>		Total	Comments
		Positive	Negative		
<i>ProGastro Cd Assay</i>	Positive	66	37 ^a	103	Sensitivity 91.7% (83.0% - 96.1%) 95% CI
	Negative	6 ^b	662	668	Specificity 94.7% (92.8% - 96.1%) 95% CI
Total		72	699	771	

Discrepant analysis for samples where ProGastro Cd Assay and CTA results were in disagreement was performed using a predetermined algorithm including a molecular (PCR) test (which targeted a different region of the *tcdB* gene than that of the ProGastro Cd Assay) followed by bidirectional genetic sequencing, enzyme immunoassay (EIA), and culture followed by PCR and bidirectional sequencing.

^a 34 samples positive by discrepant analysis. Of these 33 were positive by sequencing, and one (1) was positive by culture followed by sequencing.

^b Four (4) samples positive by discrepant analysis. Of these, one (1) was positive by sequencing, one (1) was positive by EIA, and two (2) were positive by culture followed by sequencing.

Reproducibility

The reproducibility of the ProGastro Cd Assay was evaluated at three laboratory sites. Reproducibility was assessed using a panel of six simulated samples that included medium positive, low positive (near the assay limit of detection, $\geq 95\%$ positive) and “high negative” ($< 5\%$ positive) samples. Panels and controls were tested at each site by two operators for five days (6 samples and 4 controls and 2 operators for 5 days at 3 sites = 300). The overall percent agreement with the expected result for the ProGastro Cd Assay was 99.0%.

Panel Member ID	Site 1			Site 2			Site 3			Total Agreement with expected result (%)	95% Confidence Interval	Overall Average C _T Value	Overall %CV
	Agreement with expected result	AVE C _T	%CV	Agreement with expected result	AVE C _T	%CV	Agreement with expected result	AVE C _T	%CV				
High Negatives ¹	19/20	35.2	1.52	20/20	35.3	1.05	20/20	35.5	1.30	59/60 (98.3%)	91.1% - 99.7%	35.3	1.35
Low Positives	19/20	36.2	1.04	20/20	36.2	1.30	20/20	36.3	0.75	59/60 (98.3%)	91.1% - 99.7%	36.2	1.05
Medium Positives	19/20	34.1	0.99	20/20	33.8	0.89	20/20	33.9	1.07	59/60 (98.3%)	91.1% - 99.7%	33.9	1.04
Positive Control	10/10	36.7	3.45	10/10	34.6	1.03	10/10	34.1	1.22	30/30 (100%)	88.7% - 100%	35.1	3.88
Positive Matrix Control	10/10	26.8	1.26	10/10	26.5	0.43	10/10	26.4	0.76	30/30 (100%)	88.7% - 100%	26.6	1.05
Negative Control ¹	10/10	35.0	0.98	10/10	35.0	1.44	10/10	35.4	1.46	30/30 (100%)	88.7% - 100%	35.1	1.38
Negative Matrix Control ¹	10/10	35.1	1.06	10/10	35.2	1.03	10/10	35.6	2.62	30/30 (100%)	88.7% - 100%	35.3	1.80
Total Agreement All	97/100 (97%)			100/100 (100%)			100/100 (100%)			297/300 (99.0%)	97.1% 99.7%		

¹ Average Ct value is calculated for the Internal Control (IC).

An additional reproducibility study was performed to assess samples that were at an intermediate concentration, below the assay's LoD but above the "high negatives" tested during the original reproducibility study. The percent positive for the intermediate member across all sites was 42.2%. This result was expected as the intermediate concentration should be positive in the range of 5 - 95% and the samples were lower in concentration than the LoD concentration ($\geq 95\%$ positive) and higher than the "high negative" concentration ($< 5\%$ positive).

Panel Member ID	Site 1			Site 2			Site 3			Total Agreement with expected result (%)	95% Confidence Interval	Overall Average C _T Value	Overall %CV
	Agreement with expected result	AVE C _T	%CV	Agreement with expected result	AVE C _T	%CV	Agreement with expected result	AVE C _T	%CV				
Intermediate	13/30*	40.4	2.10	12/30*	40.5	3.50	13/30*	40.5	2.07	38/90* (42.2%)	32.5% - 52.5%	40.5	2.55
Positive Control	10/10	35.2	0.88	10/10	34.4	0.37	10/10	35.1	2.64	30/30 (100%)	88.7% - 100%	34.9	1.88
Positive Matrix Control	10/10	26.5	0.78	10/10	26.5	0.86	10/10	26.3	1.12	30/30 (100%)	88.7% - 100%	26.4	1.00
Negative Control ¹	10/10	34.9	1.30	10/10	35.1	1.33	10/10	35.0	1.26	30/30 (100%)	88.7% - 100%	35.0	1.28
Negative Matrix Control ¹	10/10	35.4	1.29	10/10	35.1	1.72	10/10	35.6	1.43	30/30 (100%)	88.7% - 100%	35.4	1.55

* Number positive

¹ Average Ct value is calculated for the Internal Control (IC).

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the ProGastro Cd Assay was determined using 10-fold serial dilutions of two *C. diff* strains that were spiked into raw stool and processed according to the Instructions for Use. The LoD concentration of each bacterial strain was confirmed by extracting and testing 20 replicates. Portions of the same serial dilutions were cultured and quantified in units of CFU/mL and used to determine CFU/reaction. Analytical sensitivity (LoD) was defined as the lowest concentration at which $\geq 95\%$ of all replicates tested positive.

Bacterial Strain	LoD
NAP1 , Ribotype 027, Toxinotype III (Tox A+/B+), clinical isolate	9 CFU/reaction
ATCC 43598 , Strain 1470, Toxinotype VIII, (Tox A-/B+)	29 CFU/reaction

In addition, the analytical sensitivity was determined using quantified genomic DNA that was serially diluted and run in PCR according to the Instructions for Use. Analytical sensitivity of the ProGastro Cd Assay is 5 genomic copies/reaction.

Reactivity

In addition to the strains used in the Analytical Sensitivity (LoD) Study, an additional 35 well characterized isolates representing at least 25 different strains, five different toxinotypes, nine different restriction enzyme analysis (REA) types, five different serogroups, and three different ribotypes were used to evaluate the reactivity of the ProGastro Cd Assay. The strains were tested in triplicate at a concentration of 3 times each strain's LoD and all strains were reactive with the ProGastro Cd Assay.

Analytical Specificity

The analytical specificity of the ProGastro Cd Assay was evaluated by testing a panel of 50 cultures consisting of 41 bacteria, 8 viruses, and one yeast strain representing common gastrointestinal pathogens or microorganisms. Bacteria and yeast were tested at concentrations of 1.35×10^8 organisms/mL. Viruses were tested at concentrations of $10^{3.5}$ to $10^{6.5}$ TCID₅₀/mL. The cultures were spiked with Internal Control and were extracted using the bioMérieux NucliSENS easyMAG and tested in triplicate. The ProGastro Cd Assay did not cross react with any of the organisms tested except the toxigenic Loyola 02 NAP1 strain.

Strains	Concentration†	ProGastro Cd Result
<i>Aeromonas hydrophila</i>	0.45 McFarland	-
<i>Bacillus cereus</i>	0.45 McFarland	-
<i>Bacillus subtilis</i>	0.45 McFarland	-
<i>Bacteroides fragilis</i>	0.45 McFarland	-
<i>Campylobacter coli</i>	0.45 McFarland	-
<i>Campylobacter fetus</i>	0.45 McFarland	-
<i>Campylobacter jejuni</i>	0.45 McFarland	-
<i>Clostridium beijerinckii</i>	0.45 McFarland	-
<i>Clostridium bifermentans</i>	0.45 McFarland	-
<i>Clostridium difficile</i> (non-toxigenic ATCC 43593)	0.45 McFarland	-
<i>Clostridium difficile</i> (non-toxigenic ATCC 43601)	0.45 McFarland	-
<i>Clostridium difficile</i> (non-toxigenic ATCC 43603)	0.45 McFarland	-
<i>Clostridium difficile</i> (non-toxigenic ATCC 700057)	0.45 McFarland	-
<i>Clostridium difficile</i> (non-toxigenic Strain 3232)	0.45 McFarland	-
<i>Clostridium difficile</i> (toxigenic Loyola 02 NAP1 strain)	3.3×10^{-4} McFarland (1×10^5 orgs/mL)	+
<i>Clostridium haemolyticum</i>	0.45 McFarland	-
<i>Clostridium histolyticum</i>	0.45 McFarland	-
<i>Clostridium novyi</i>	0.45 McFarland	-
<i>Clostridium perfringens</i>	0.45 McFarland	-
<i>Clostridium septicum</i>	0.45 McFarland	-
<i>Clostridium sordellii</i>	0.05 McFarland*	-
<i>Clostridium sporogenes</i>	0.45 McFarland	-
<i>Clostridium tetani</i>	0.45 McFarland	-
<i>Enterobacter cloacae</i>	0.45 McFarland	-
<i>Enterococcus faecalis</i>	0.45 McFarland	-
<i>Escherichia coli</i>	0.45 McFarland	-
<i>Escherichia coli</i> 0157:H7	0.45 McFarland	-
<i>Klebsiella pneumoniae</i>	0.45 McFarland	-
<i>Peptostreptococcus anaerobius</i>	0.45 McFarland	-
<i>Porphyromonas asaccharolytica</i>	0.45 McFarland	-
<i>Proteus vulgaris</i>	0.45 McFarland	-
<i>Pseudomonas aeruginosa</i>	0.45 McFarland	-

Strains	Concentration†	ProGastro Cd Result
<i>Salmonella typhimurium</i>	0.45 McFarland	-
<i>Shigella dysenteriae</i>	0.45 McFarland	-
<i>Shigella flexneri</i>	0.45 McFarland	-
<i>Shigella sonnei</i>	0.45 McFarland	-
<i>Staphylococcus aureus</i>	0.45 McFarland	-
<i>Staphylococcus epidermis</i>	0.45 McFarland	-
<i>Vibrio cholerae</i>	0.45 McFarland	-
<i>Vibrio parahaemolyticus</i>	0.45 McFarland	-
<i>Yersinia enterocolitica</i>	0.45 McFarland	-
Adenovirus 18	1x10 ^{3.5} TCID ₅₀ /mL	-
Adenovirus 40	1x10 ^{5.5} TCID ₅₀ /mL	-
Coxsackie B4	1x10 ^{4.5} TCID ₅₀ /mL	-
Cytomegalovirus	1x10 ^{4.25} TCID ₅₀ /mL	-
Echovirus 11	1x10 ^{6.5} TCID ₅₀ /mL	-
Enterovirus 68	1x10 ^{3.5} TCID ₅₀ /mL	-
Norovirus	Clinical isolate**	-
Rotavirus	1x10 ^{3.5} TCID ₅₀ /mL	-
<i>Candida albicans</i>	0.45 McFarland	-

† Bacteria and fungus tested at 0.05 or 0.45 McFarland (1.5 x 10⁷ orgs/mL or 1.35 x 10⁸ orgs/mL). Viruses tested at the highest concentration available in TCID₅₀/mL.

* Higher concentrations of *Clostridium sordellii* exhibited inhibition of the Internal Control. No cross-reaction in the FAM channel was observed at 0.45 McFarland.

**Cultured and titered Norovirus was unavailable; nucleic acids from a positive clinical sample (Milwaukee Public Health Lab Real Time PCR assay with a C_T value = 20.5) were tested.

Interference

Blood, mucin, fecal fats, over the counter and prescription medicines (Kaopectate, Immodium AD, Pepto Bismol, Vancomycin, and Metronidazole) and a radiological contrast agent (barium sulfate) were spiked into aliquots of a raw fecal pool along with one cultured and titered strain of *C. difficile* (*C. diff*). *C. diff* was spiked in at a concentration of 2 times the assay's Limit of Detection (LoD). Clinically relevant amounts of the potential inhibiting substances were added to spiked samples. Samples were clarified and spiked with the Internal DNA Control (IC). Nucleic acid from the samples was extracted with the bioMérieux NucliSENS easyMAG. The nucleic acids were tested in triplicate reactions with ProGastro Cd on the Cepheid SmartCycler II. The following table shows the potential interfering substances used for this study.

Interfering Substance	Active Ingredient	Concentration Tested
Whole Blood	N/A	40% (volume/volume)
Mucin	Purified mucin protein	3.5% (weight/volume)
Fecal Fats (stearic and palmitic acid)	N/A	40% (weight/volume)
Kaopectate	Bismuth Subsalicylate	10% (volume/volume)
Pepto Bismol	Bismuth Subsalicylate	10% (volume/volume)
Immodium AD	Loperamide HCl	10% (volume/volume)
Metronidazole	N/A	12.5 mg/mL
Vancomycin	N/A	12.5 mg/mL
Barium Sulfate	N/A	5% (weight/volume)

None of the potentially interfering exogenous or endogenous substances interfered with detection of *C. diff* at a concentration near the LoD of the ProGastro Cd Assay.

Carry-over/Cross-contamination

To evaluate the level of carry-over/cross-contamination with the ProGastro Cd Assay, simulated *Clostridium difficile* (*C. diff*) high positive samples run in series alternating with *C. diff* high negative samples were tested over five separate extraction and PCR runs. High Positive samples represented the upper range of Ct values obtained in the ProGastro Cd clinical trials (lowest Ct = 25.4). High Negative samples consisted of negative stool spiked with a concentration of *C. diff* below but near the analytical LoD concentration such that > 95% of samples should be negative. The samples were processed and extracted in a “High Positive/High Negative” alternating fashion on the bioMérieux NucliSENS easyMAG and likewise processed and run on the Cepheid SmartCycler II instrument in an alternating fashion. Three out of fifty-five high negative samples tested showed potential *C. diff* contamination. Due to the fact that the high negative samples did include a low amount of *C. diff* that is detectable no more than 5% of the time, it cannot be ruled out that these results may actually fall in this category. Potential contamination could have occurred during sample preparation, during extraction, during transferring of the purified nucleic acid from the sample vessel into the microfuge tube, or during preparation of the PCR reactions.

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.