

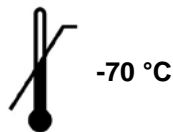
Prodesse® ProGastro™ Cd Assay

Instructions for Use

For the detection of toxigenic Clostridium difficile



100



REF

E38VK00 / E38VK55

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Intended Use

The Prodesse® 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.

Summary and Explanation

Clostridium difficile (*C. diff*) is a gram-positive, spore-forming, anaerobic bacillus.¹ There are two major groups of *C. diff* including toxigenic and nontoxigenic strains, with the toxigenic strains responsible for *Clostridium difficile* Infection (CDI). The clinical presentation of CDI varies from antibiotic associated diarrhea (AAD) to pseudomembranous colitis and toxic megacolon.¹⁻³ The mode of infection is the oral-fecal route. CDI occurs in patients after an alteration in the normal microflora of the colon often due to antibiotic treatment and after exposure and ingestion of spores and vegetative cells.³ Symptoms of CDI usually begin within two to three days after initiation of antibiotic treatment, however instances have occurred where symptoms were not evident until two to ten weeks after completion of antibiotic therapy.^{1,4}

CDI is a toxin-mediated disease and the majority of *C. diff* strains produce two toxins including Toxin A, an enterotoxin, and Toxin B, a cytotoxin; however, strains have been isolated that are Toxin A-/B+.⁵ Toxin A+/B- strains have been reported but the incidence of these variants appears to be extremely low with only a few cases reported and only one case confirmed.⁶ Although both Toxin A+/B+ and Toxin A-/B+ strains circulate in the population, strains that are Toxin A+/B+ are currently dominant.⁵

Sporadic epidemics of CDI occur and are due to the nosocomial nature of the infection including the difficulty in decontamination of the bacterial spores after introduction into the hospital environment.³ The populations at risk for infection are primarily institutionalized patients (e.g., hospitals and nursing homes), who have received antibiotic treatment. The highest prevalence of CDI is among the elderly and immunocompromised with 80% of cases occurring in individuals over 65, however CDI can occur in all age groups.¹ While CDI is generally considered a healthcare-associated infection, there are recent reports of community-associated *Clostridium difficile* infection.⁷⁻¹⁰

Principles of the Procedure

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 NucliSENS easyMAG System (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux). Nucleic acids are added to the *C. diff* Mix for subsequent PCR amplification and detection using the Cepheid SmartCycler II instrument.

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.

Analyte	Gene Targeted	Probe Fluorophore	Absorbance Peak	Emission Peak	Instrument Channel
<i>Clostridium difficile</i>	<i>tcdB</i> , Toxin B	FAM	495 nm	520 nm	FAM
Internal Control	NA	Quasar 670	647 nm	667 nm	Cy5

Materials Provided

ProGastro Cd Assay Kit (Cat. # E38VK00)

Reagents	Description	Quantity/Tube	Cap Color	Cat. #	Reactions/Tube
<i>C. diff</i> Mix	<ul style="list-style-type: none"> ↻ Taq DNA polymerase ↻ 2 oligonucleotide primer pairs ↻ 2 oligonucleotide probes ↻ Buffer containing dNTPs ↻ MgCl₂ and stabilizers 	1 mL x 2 tubes	Brown	ESM38	50
Internal Control (IC) – Internal DNA Control II	↻ Non-infectious double-stranded DNA	30 µL	Clear	GCT02	100
Positive Control (PC) – <i>C. diff</i> DNA Control	↻ Non-infectious DNA plasmid containing a portion of the targeted gene (<i>tcdB</i>)	30 µL	Blue	ECT64	15

S.T.A.R. Buffer Kit (Cat. # E38VK55)

Reagents	Description	Quantity/Bottle	Cat. #	Reactions/Bottle
S.T.A.R. Buffer	↻ Stool Transport and Recovery Buffer	100 mL	GLS36	200

Materials Required But Not Provided

Plasticware and Consumables

- RNase/DNase-free 1.5 mL polypropylene microcentrifuge tubes
- Sterile RNase/DNase-free filter or positive displacement micropipettor tips (wide bore 200 µL tips are useful for pipetting stool samples, otherwise standard 200 µL tips may be cut off for pipetting stool)
- Disposable plastic spatulas for stool samples that are not pipettable.
- bioMérieux NucliSENS easyMAG Disposables (Biohit pipette tips, sample strips)
- Greiner Break Four uncoated plates (*bioMérieux Cat. # 278303*)
- Cepheid PCR reaction tubes, 25 µL

Reagents

- bioMérieux NucliSENS easyMAG reagents (*Buffer 1 Cat. # 280130, Buffer 2 Cat. # 280131, Buffer 3 Cat. # 280132, Magnetic Silica Cat. # 280133, and Lysis Buffer Cat. # 280134*)
- Molecular Grade Water (RNase/DNase Free)
- Extraction Control - recommended (*e.g. previously characterized positive sample or simulated sample*)

Equipment

- ≤ -70°C Freezer
- Vortexer
- Benchtop microcentrifuge capable of accommodating 1.5 mL polypropylene microcentrifuge tubes and 13,000 x g
- bioMérieux NucliSENS easyMAG System with software version 1.0.1 or 2.0
- Biohit multi-channel pipettor
- Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b or 3.0a / 3.0b
- Micropipettors (range between 1-10 µL, 10-200 µL and 100-1000 µL)
- Mini-centrifuge with adapter for Cepheid Reaction Tubes
- Cepheid cooling block
- Waterbath or Incubator
- Ice/Ice Bucket or -20°C Cold Block
- Biosafety Cabinet

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Limit use of this product to personnel who are trained in the techniques of Real-Time PCR.
- This assay is for use with liquid or soft raw stool specimens.
- Performance characteristics were not established for patients < 2 years of age.
- Handle specimens as if infectious using safe laboratory procedures such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* and in the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*. Thoroughly clean and disinfect all surfaces with 10% bleach. Autoclave any biohazardous waste generated from contact with clinical specimens before discarding.
- Use micropipettes with aerosol barrier or positive displacement tips for all procedures.
- Always pre-plan, organize and segregate workflow. Proceed with laboratory workflow in a unidirectional manner, beginning in the Pre-Amplification Area and moving to the Amplification/Detection Area.
 - Begin pre-amplification activities with reagent preparation and proceed to specimen preparation.
 - Dedicate supplies and equipment to separate areas for reagent preparation and specimen preparation; no cross-movement allowed between areas.
 - Do not use equipment and supplies used for reagent preparation for specimen preparation activities or for pipetting or processing other sources of target nucleic acid.
 - Keep all amplification supplies and equipment in the Amplification/Detection Area at all times.
 - Always wear disposable gloves in each area and change them before entering a different area.
 - Do not open sample tubes following PCR.
- Take care to preserve the purity of kit reagents. Avoid contamination from the Positive Control, Internal Control, and specimens by following good laboratory practices.
- Do not use reagents after their expiration date.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- Material safety data sheets (MSDS) are available on the manufacturer's website at www.gen-probe.com.

Reagent Storage, Handling and Stability

- Store all reagents (opened and unopened), except S.T.A.R. Buffer, at ≤ -70°C. Once thawed, store the S.T.A.R. Buffer at room temperature.
- **Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.**
- Always check the expiration date on the reagent tubes. **For Intermediate stocks of the Positive Control and Internal Control, use the expiration date of the originating stock control tube.** Do not expose controls to more than one (1) freeze-thaw cycle.
- ProGastro Cd reagents are shipped frozen and should arrive frozen. The *C. diff* Mix and Controls should be stored frozen after receipt. If they are not frozen upon receipt or if the tubes have been compromised during shipment, contact Customer Service for assistance. The S.T.A.R. Buffer should be stored at room temperature after receipt.
- An internal study demonstrated that performance of the *C. diff* Mix is not affected for up to 5 freeze-thaw cycles.
- Visually examine reagents for adequate reagent volume before beginning any test procedures.
- Protect the *C. diff* Mix from light.

**Recommendation**

Aliquoting of kit components to maintain less than 5 freeze/thaw cycles is recommended for labs with smaller numbers of samples.

**Specimen Collection, Handling and Storage****Collecting the Specimen**

Standard stool collection and handling procedures are appropriate to obtain raw stool. Place raw stool in a sterile screw-cap container that can be adequately sealed.

Transporting Specimens

Ensure that when transporting human stool specimens, all applicable regulations for the transport of etiologic agents are met.

Storing Specimens

Store specimens 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.

Storing Purified Nucleic Acid

Store purified nucleic acids at ≤ -70°C.

**Note**

Inadequate or inappropriate specimen collection, storage and transport are likely to yield false negative results.

**Reagent and Control Preparation****Reagents****Note**

Prepare reagents for the bioMérieux NucliSENS easyMAG System following the manufacturer's instructions.

Controls**Recommendation**

- ❖ *For Intermediate stocks of the Positive and Internal Controls, use the expiration date of the originating tube.*
- ❖ *Controls and aliquots of controls must be thawed and kept on ice/cold block at all times during preparation and use. It is recommended to prepare controls in a sample prep area, such as a Biological Safety Cabinet.*

Positive Control (PC)**Note**

The positive control must be included in each run.

1. Thaw the Positive Control (blue cap tube) on ice.
2. Create Intermediate stock tubes of the Positive Control using the following dilution scheme: (Mix Well)

$$25 \mu\text{L Positive Control} + 225 \mu\text{L Molecular Grade Water} = 250 \mu\text{L total volume}$$

3. Make 15 aliquots of 15 µL each, label and store at ≤ -70°C. Ensure aliquots do not undergo more than one (1) freeze-thaw cycle.
4. Dilute the Intermediate stock of the Positive Control just prior to setup of the PCR reaction (see [Step 3 \(a\)](#) of the [Assay Procedure](#)).

**Note**

Do not spike Positive Control with the Internal Control. Do not take Positive Control through the nucleic acid isolation procedure.

Internal Control (IC)

1. Thaw Internal Control (clear cap tube) on ice.
2. Create Intermediate stock tubes of the Internal Control using the following dilution scheme: (Mix Well)

$$28 \mu\text{L Internal Control} + 252 \mu\text{L S.T.A.R. Buffer} = 280 \mu\text{L total volume}$$

3. Make 8 aliquots of 35 µL each, label and store at ≤ -70°C (when diluted to its Working concentration, one aliquot will yield enough volume to add to 30 samples). Make aliquots of larger or smaller volumes based on the number of samples expected to be processed in a single extraction run. Ensure aliquots do not undergo more than one (1) freeze-thaw cycle.
4. Dilute the Intermediate stock of the Internal Control to its Working concentration just prior to spiking samples. Add the appropriate volume of the Working concentration of the Internal Control to each sample prior to nucleic acid isolation (see [Step 1 \(b\)](#) of the [Assay Procedure](#)).

Negative Control (NC)

1. Use S.T.A.R. Buffer as the Negative Control.
2. Add the appropriate volume of the Working concentration of the Internal Control to the Negative Control prior to nucleic acid isolation (see [Step 1 \(c\)](#) of the [Assay Procedure](#)).

Extraction Control (recommended)

Good laboratory practice recommends including a positive Extraction Control in each nucleic acid isolation run. The Extraction Control may consist of a clinical sample previously determined as positive for toxigenic *C. diff* by the ProGastro Cd Assay or a simulated sample. Treat the Extraction Control as a sample.

To create a simulated sample:

1. Obtain a commercially available toxigenic strain of *C. diff*.
2. Spike the *C. diff* into negative stool at a concentration that results in a Ct value of approximately 20 – 40.
3. Clarify the spiked stool (see [Step 1 \(a\)](#) of the [Assay Procedure](#)).
4. Make 30 µL aliquots of the Clarified Stool, label, and store at ≤ -70°C.

 Assay Procedure

Assay Overview

Get Ready: Create the Research Assay Protocol for the Cepheid SmartCycler instrument using the Dx Software (first time only).

1. **Prepare the Samples and Negative Control.**
2. **Isolate the Nucleic Acid (Pre-Amplification Area I) - bioMérieux NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents**
3. **Set up the RT-PCR Reaction (Pre-Amplification Area II).**
4. **Run the ProGastro Cd Assay.**
5. **Print Report.**



Note

❖ Instructions provided for the Cepheid SmartCycler II instrument with Dx Software versions 1.7b and 3.0a / 3.0b.

❖ Do NOT deviate from the protocol settings defined in this section.

Get Ready: Create the Research Assay Protocol for the Cepheid SmartCycler instrument using the Dx Software (first time only)



Recommendation

❖ The protocol is only created for first-time use; it does not need to be recreated with each sample run.

❖ Refer to SmartCycler Dx Software Operator Manual for assistance in defining assay protocols.

❖ To **Define** and **Edit** Research Assay protocols, administrative access rights are required, otherwise, the fields are grayed out.



Note

The Cepheid Dx Software interprets the data and reports the run as either VALID or INVALID based on the results of the Positive and Negative Controls.

Create the ProGastro Cd Assay protocol:

- a. Launch the Cepheid Dx software application.
- b. Click the **Define Assays** box at the top of the screen.
- c. Click the **New Assay** box at the bottom left corner of the screen (Dx 3.0a / 3.0b: Click the **New** box at the bottom left corner of the screen).
- d. Enter **ProGastro Cd Assay** for the assay protocol in the window that opens.
- e. Click **OK**.
- f. In the middle of the **Define Assays** screen, select **FTTC25** for the **Dye Set** (Dx 3.0a / 3.0b: Click the **Analysis Settings** tab and in the middle of the screen, select **FTTC25** for the **Dye Set**).
- g. Select **NC fails if: Any target criterion is positive** (Dx 3.0a / 3.0b: this is the default setting in the **Control Settings** tab).
- h. Check the box to **Require Lot Number** (Dx 3.0a / 3.0b: Click the **Advanced Settings** tab and check **Require Lot Number** box).
- i. Deselect the box to **Use Patient IDs** (Dx 3.0a / 3.0b: Under the **Advanced Settings** tab, verify that the Use Patient IDs box is **deselected** by default).

- j. Enter **Thermocycler Parameters** in the Protocol section (bottom half of **Define Assay** screen).

Stage 1			Stage 2			Stage 3		
Hold			Repeat 5 times			Repeat 40 times		
2- Temperature Cycle			2- Temperature Cycle			2- Temperature Cycle		
Temp	Secs	Optics	Temp	Secs	Optics	Temp	Secs	Optics
95	600	OFF	95	30	OFF	95	10	OFF
			55	60	ON	55	60	ON

Stages 4 – 10 remain UNUSED

- k. Enter information in **Analysis Settings** tab as follows:

- Gray boxes are default settings.

Analysis Settings

Channel	Dye Name	Target	Usage	Curve Analysis	Thresh Setting	Manual Thresh	Auto Thresh	Auto Min. Cycle	Auto Max. Cycle	Valid Min. Cycle	Valid Max. Cycle	Bkgnd Sub	Bkgnd Min. Cycle	Bkgnd Max. Cycle	Boxcar Avg	EndPt Thresh	NC IC %	IC Delta
1	FAM*	C. diff	Assay**	Primary Curve	Manual Threshold	30	NA	5	10	13.0	45.0	On	5	40	0	30	10.0/NA †	NA
2	TET*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	TxR*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Cy5*	Internal Control	Internal Control	Primary Curve	Manual Threshold	32	NA	5	10	13.0	45.0	On	5	40	0	32	10.0/NA †	NA

Channel 1 = C. diff; Channel 2 = not used; Channel 3 = not used; Channel 4 = Internal Control

*If the Dye Names are incorrect, check that FTTC25 Dye Set is being used.

** Dx 3.0a / 3.0b = Target

† Dx 1.7b = 10; Dx 3.0a / 3.0b = NA

- l. Enter information in the **Control Settings** tab.



Note

- ❖ Use PC1 (Pos Cntrl 1) for the Positive Control.
- ❖ Enter 0 Replicates to inactivate Positive Controls PC2 and PC3.
- ❖ Use NC1 (Neg Cntrl 1) for the Negative Control.
- ❖ Enter 0 Replicates to inactivate the Negative Controls NC2 and NC3.
- ❖ Gray boxes are default settings.
- ❖ Enter the Extraction Control (if used) as a sample within the PCR run. Do not define it in the Control Settings tab.

Control Settings

Control ID	Control Name	Replicate	C. diff Valid Min Cycle	C. diff Valid Max Cycle	C. diff EndPt Thresh	IC +/-	IC Valid Min Cycle	IC Valid Max Cycle	IC EndPt Thresh
PC1	Pos Cntrl 1	1	26	45	30	-	15	45	32
PC2	Pos Cntrl 2	0	13	45	10	+	13	45	10
PC3	Pos Cntrl 3	0	13	45	10	+	13	45	10
NC1	Neg Cntrl 1	1	15	45	30	+	28	43	32
NC2	Neg Cntrl 2	0	13	45	10	+	13	45	10
NC3	Neg Cntrl 2	0	13	45	10	+	13	45	10

m. Confirm **Probe Check Settings** tab.



Note

The probe check is not used for the ProGastro Cd Assay Protocol.

Probe Check Settings

Channel	Dye Name	Prb1 Min	Prb1 Max	Prb2 Min	Prb2 Max	Prb3 Min	Prb3 Max	Delta 12 Min	Delta 12 Max	Delta 23 Min	Delta 23 Max	Delta 13 Min	Delta 13 Max
1	FAM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	TET	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	TxR	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Cy5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

n. Select **Save Assay**.

1. Prepare the Samples and Negative Control (Pre-Amplification Area)

a. Prepare Clarified Stool

i. KEEP RAW STOOL ON ICE AT ALL TIMES.

ii. Verify that the S.T.A.R. Buffer is clear and colorless. White precipitates may form when the buffer is stored below room temperature. If precipitates have formed, warm the solution to 30-40°C in a waterbath or incubator until precipitates have dissolved.

iii. Mix stool samples well before sampling, either by vortexing or mixing with a pipet tip. Use a new pipet tip for each sample.

iv. Using a wide-bore 200 µL pipet filter tip (or a 200 µL filter tip with the end cut off to generate a wide bore tip) create a 1:5 dilution of stool to S.T.A.R. Buffer by adding 100 µL of each stool sample to individual 1.5 mL microcentrifuge tubes containing 400 µL S.T.A.R. Buffer. If the sample is too thick to pipet, use a disposable spatula to sample approximately 100 µL (the size of a small pea) and add it to the 400 µL of S.T.A.R. Buffer.

v. Mix to homogeneity by vortexing.

vi. With some thick stool samples it may be necessary to add more S.T.A.R. Buffer to generate the Clarified Stool. If necessary, add extra S.T.A.R. Buffer in 100 µL increments to achieve a consistency that allows for the generation of pipettable Clarified Stool.

vii. Centrifuge the sample at 13,000 x g for 1 minute.

viii. Pipet off the supernatant avoiding any particulate matter and place into a new 1.5 mL microcentrifuge tube. This supernatant is the Clarified Stool. It is not necessary to keep the Clarified Stool on ice.

ix. The Clarified Stool may be stored at 2-8°C for up to 7 days before spiking with IC and extracting.

b. Add Internal Control to Clarified Stool samples.

i. Thaw the appropriate number of aliquots of Intermediate stock of the Internal Control (enough volume needed for each sample, the Negative Control, and the Extraction Control) on ice.

ii. Make a 1:10 dilution of the Intermediate stock of the Internal Control as follows:

$$30 \mu\text{L Intermediate stock of Internal Control} + 270 \mu\text{L S.T.A.R. Buffer} = 300 \mu\text{L Working concentration of Internal Control}$$

iii. Add 10 µL of the Working concentration of the Internal Control to each sample vessel in the easyMAG disposable.

- iv. Remove 20 µL of each Clarified Stool sample and pipet into the specific sample vessel in the easyMAG disposable in which it is to be purified. Pipet up and down a minimum of 5 times to mix. Use a new pipet tip for each sample.



Note

When mixing, be sure not to introduce air bubbles or contaminate neighboring sample vessels.

- v. Store any remaining Clarified Stool samples at 2-8°C for up to one week. Store samples for longer durations at ≤ -70°C.

c. Add Internal Control to the Negative Control.

- i. Include one (1) Negative Control in each extraction run.
- ii. Add 10 µL of the Working concentration of the Internal Control to the sample vessel in the easyMAG disposable.
- iii. Add 20 µL of S.T.A.R. Buffer into the specific sample vessel in the easyMAG disposable in which it is to be purified. Pipet up and down a minimum of 5 times to mix.
- iv. Discard remaining volume of Internal Control – DO NOT reuse.



Do not reuse


Do not reuse Internal Control.

2. Isolate the Nucleic Acid (Pre-Amplification Area I) – bioMérieux NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents

a. Start Instrument and Software.

Power on the easyMAG instrument and once the LED on the instrument turns green, power on the computer and log into the software.

b. Prepare the software for a run.

To prepare for a run, touch “Settings”  in the main toolbar, which defaults to “Application Settings”, and choose the following run settings:

Default Request: Generic 1.0.6 or 2.0.1 (for software version 1.0.1 or 2.0, respectively)

Run Name Prefix: N/A (leave as default)

Sample ID prefix: N/A (leave as default)

Sample Type: Primary (on-board lysis)



Default On-board Lysis Dispensing: Yes

Default On-board Lysis Incubation: Yes



Sample Addition Guidance: Off

Reagent Tracking: Off

c. Input buffer information.

Touch “Instrument”  to default to the “Reagent Inventory”  icon and input the buffer barcodes by first scanning the instrument position (A, B, C, or D) and then its corresponding buffer. For example, scan position A and then scan the bottle of Lysis buffer in that position and then move on to position B and its corresponding bottle.

d. Create a worklist.

- i.** Touch the “Daily Use”  icon which defaults to the “Define Extraction Request”  icon and select the following settings:

Sample ID: Manually enter the sample name.

Matrix: Stool


Request: Generic 1.0.6 or 2.0.1 (for software version 1.0.1 or 2.0, respectively)

Volume (mL): 0.030 (input volume of sample)






Eluate (µL): 110

Type: Primary

Priority: Normal

- ii.** Press **Enter** on the keyboard or touch the “New Extraction Request”  icon after each manual sample addition. The settings above remain as the default settings for each subsequent entry as long as you do not navigate to other pages.


e. Create a run and add samples from the worklist.

- i.** Touch “Organize Runs”  then “Create Run”  to display the *New Run Window*. In this window, name the run appropriately and verify that the **auto-number** box is left unchecked (not selected) and that **Yes** is selected for both the On-Board Lysis Dispensing and On-Board Lysis Incubation options.
- ii.** Touch **OK**. The *New Run Window* closes and the “Organize Runs” screen is displayed. Assign samples to the run using the positioning arrows .
- iii.** Touch “Load Run”  and select the run. Print the worklist with the “Print worklist”  icon if needed. The worklist helps keep track of the order of the samples loaded into the sample vessels.

f. Load samples, including the Negative Control. Load aspiration tips and scan the barcode on the sample strip(s).

Insert the required aspiration tips to use in the extraction run. Insert the sample strip(s) in the correct order as noted in the worklist and scan the sample strip(s) position on the instrument and then the sample strip itself. For example, scan position A and then the sample strip in that position, then B and then C, if necessary. After scanning the sample strips), the indicator changes from red to green on the screen.


g. On-Board Lysis Dispensing.

Once the samples and tips are loaded and the strip(s) scanned, close the lid and touch “Dispense Lysis” . The instrument dispenses the Lysis buffer and incubates for 10 minutes.

h. Prepare the magnetic silica to add to the sample vessels.

During the 10-minute lysis incubation, use the Biohit multi-channel pipettor to prepare the magnetic silica. Perform this procedure for each sample vessel used in the run (1, 2, or 3 times).

- i.** Set the pipettor to **Program 1** and place a Biohit pipette tip on position 1. Program 1 provides the means to aspirate and dispense 550 µL of liquid. The magnetic silica is prepared in a 1:1 ratio of Molecular Biology Grade Water to Magnetic Silica.
- ii.** Using Program 1 of the pipettor, press the **start** button to aspirate and then again to dispense 550 µL of water into a microcentrifuge tube. Vortex the tube of magnetic silica briefly to mix and use Program 1 of the pipettor to aspirate and then dispense 550 µL of magnetic silica into the same microcentrifuge tube as the water. Eject the tip, cap the tube, and vortex to mix.
- iii.** Set the pipettor to **Program 2** and place a Biohit pipette tip on position 1. Program 2 will transfer 8 volumes of the previous mix to the 8 vessels of a strip on an ELISA plate (1 strip/sample vessel). Press the **start** button to aspirate the mix. Press the **start** button again to dispense once to set the pipette correctly for dispensing.
- iv.** Press the **start** button 8 separate times to dispense the remaining mix into each of the 8 vessels of an ELISA plate strip and eject the tip.

- v. After the 10 minute lysis incubation is done, set the pipettor to **Program 3** and place 8 Biohit pipette tips on the multi-channel pipettor (or however many samples are present in the specific sample strip). Make sure the filter tips are well connected with the multi-channel pipettor to prevent leakage errors. Program 3 first mixes the magnetic silica mixture in the ELISA plate and then aspirates it for delivery to the vessels of the sample strip where it will be mixed.
 - vi. Press the **start** button once and the pipette mixes the silica in the ELISA plate and then aspirates it for addition to the sample vessel. Verify that each tip has the same volume of silica mix before placing in the sample vessel. Place the pipettor over the sample vessel strip so the tips are below the liquid level of each sample and press the start button again to aspirate 800 µL out of each sample vessel and perform 3 mixing cycles with 1000 µL of sample/magnetic silica in the BioHit tip. As it is mixing, be sure to hold the pipette steady below the liquid/air interface to avoid introducing bubbles to the sample.
 - vii. Repeat for each sample strip in the run.
- i. **Start the run.**  Touch "**Start**" to begin the run. The instrument performs 5 washes and heat and elute. Transfer the purified nucleic acids to appropriate storage tubes (1.5 mL microcentrifuge) on ice within 30 minutes of extraction to avoid contamination by the magnetic silica stuck to the front wall of the sample vessel(s). Store for longer durations at ≤ -70°C.



Note

- ❖ Do not store purified nucleic acids in the sample vessel(s).
- ❖ Do not use nucleic acids from failed sample(s).

3. Set up the RT-PCR Reaction (Pre-Amplification Area II)



Note

Start the SmartCycler ProGastro Cd Assay run within 1 hour of thawing the *C. diff* Mix.

a. Dilute the Positive Control.

- i. Include the Positive Control with each run. Thaw 1 aliquot of the Intermediate stock of the Positive Control on ice.
- ii. Add 45 µL of molecular grade water to a 1.5 mL microcentrifuge tube.
- iii. Transfer 5 µL of the Intermediate stock of the Positive Control to the tube from step ii. Briefly vortex to mix.
- iv. Keep tube on ice.
- v. Discard remaining volume of diluted Intermediate Positive Control – DO NOT reuse.



Do not reuse

Do not reuse Intermediate stock of the Positive Control.

b. Thaw the *C. diff* Mix and add 20 µL to the SmartCycler tubes.

- i. Keep the *C. diff* Mix on ice and protected from light before adding to the SmartCycler tubes.
- ii. Load the required number of tubes into the Cepheid Cooling Block.
- iii. Pipet 20 µL of the *C. diff* Mix into the upper part of the SmartCycler tubes.
- iv. Store any remaining *C. diff* Mix at ≤ -70°C.

c. Add 5 µL of each sample's nucleic acid to individual SmartCycler tubes containing the *C. diff* Mix.

- i. After adding the nucleic acid to the SmartCycler tube, pipet up and down 2 to 3 times to mix.
- ii. Close the tube. Use a new pipette tip for each sample.

- d. **Add 5 µL of the diluted Positive Control to a separate SmartCycler tube containing the *C. diff* Mix.**
- After adding the Positive Control to the SmartCycler tube, pipet up and down 2 to 3 times to mix.
 - Close the tube.
 - Discard remaining volume of diluted Positive Controls – DO NOT reuse.



Do not reuse

Do not reuse diluted Positive Control.

- e. **Add 5 µL of the Extraction Control nucleic acid to a separate SmartCycler tube containing the *C. diff* Mix**
- After adding the Extraction Control nucleic acid to the SmartCycler tube, pipet up and down 2 to 3 times to mix.
 - Close the tube.
- f. **Add 5 µL of the Negative Control nucleic acid to the last SmartCycler tube containing the *C. diff* Mix.**
- After adding the Negative Control nucleic acid to the SmartCycler tube, pipet up and down 2 to 3 times to mix.
 - Close the tube.
- g. **Centrifuge all tubes**
- Appropriately label the SmartCycler tubes on the caps.
 - Centrifuge all tubes for 5 to 10 seconds using the Cepheid microcentrifuge specially adapted to fit the SmartCycler tubes.
 - Return tubes to the cooling block.
- h. **Keep the tubes on the Cooling Block before loading them into the SmartCycler II instrument.**

4. Run the ProGastro Cd Assay (Amplification/Detection Area)

- Create a new run by clicking **Create Run** at the top of the screen. The *Create Run* screen is displayed.
- Under **Run Name** in the left panel of the *Create Run* screen, enter a unique run identifier.
- Click on the **Assay** arrow in the left panel of the *Create Run* screen and select **ProGastro Cd Assay** protocol from the drop-down menu.
- Under **Assay Information** in the left panel of the *Create Run* screen, enter the **Lot Number** and **Expiration Date** of the ProGastro Cd Assay kit.
- In the left panel of the *Create Run* screen, enter the number of specimens (including the Extraction Control) and click **Apply**. The **Site Table** is displayed and the SmartCycler Dx Software automatically selects the **I-Core** sites.
- In the **Site Table** under the **Sample ID** column, enter the Sample Identifier.
- Insert each reaction tube into an I-Core site of the SmartCycler by pressing down firmly on the tubes and close each lid. Verify that the Positive Control (PC1) and Negative Control (NC1) are loaded into the correct I-Core sites specified by the software.
- Select **Start Run** located at the bottom left corner of the screen. Verify the LED is on for the appropriate I-Core sites.

5. Print Report

- Click **Report** at the bottom of the screen to open the **Report Preview** screen.
- Print** the resulting report.

Interpretation of Control Results

Validation of Run



Note

The SmartCycler Dx software automatically interprets the Positive Control (PC1) and Negative Control (NC1) results to determine if the run is valid.

For a VALID run, the following control conditions must be met:

Sample ID ¹	Assay Result	IC Result	Warning / Error Code	Sample Type	IC Ct	C diff Result	C diff Ct
Pos Cntrl 1	Valid	NA		PC1	0	Valid	26 – 45
Neg Cntrl 1	Valid ²	Pass		NC1	28 – 43	Valid	0

¹ Columns and data not used for interpretation are not included.

² (Typical) An Invalid assay will display Error Code 4098.

Invalid Assay Run

If the conditions for a Valid run are not met (i.e., the Positive Control is not positive within the specified Ct range or the Negative Control is Invalid), prepare all new reactions using the remaining purified nucleic acids and new dilution of the Positive Control (starting with PCR at [Step 3](#) of the [Assay Procedure](#)) or start from original sample(s) or clarified stool using new dilution of the Positive Control (starting at [Step 1](#) of the [Assay Procedure](#)).

Interpretation of Specimen Results

The SmartCycler Dx software automatically determines the specimen results. The interpretation of the assay specimen results is as follows:

Sample ID ¹	Assay Result	IC Result	Warning/ Error Code	C. diff Result	Interpretation of Results
Sample ID	Negative	Pass		NEG	Toxigenic <i>C. diff</i> not detected
Sample ID	Positive	NA*		POS	Toxigenic <i>C. diff</i> nucleic acid detected
Sample ID	Unresolved	Fail		NEG	Unresolved – PCR inhibition or reagent failure. Repeat testing from the Clarified Stool or collect and test a new sample.
Sample ID	Invalid		4098 ²	ND	Not Determined – error code 4098

¹ Columns and data not used for interpretation are not included

² An Invalid assay run will display Error Code 4098

* Detection of the Internal Control in the Cy5 detection channel is not required for positive result. High titers of *C. diff* can lead to reduced or absent Internal Control signal.

Quality Control

- Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures. It is recommended you refer to CLSI document C24-A3, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions*: [Approved Guideline – Third Edition] or other published guidelines for general quality control recommendations. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1202(c).
- Quality control procedures are intended to monitor reagent and assay performance.

Control Type	Used to Monitor
Positive	Substantial reagent failure including primer and probe integrity
Negative	Reagent and/or environmental contamination
Extraction	Failure in lysis and extraction procedure
Internal	PCR inhibition in individual samples and reagent failure or process error

- Dilute and test the Positive Control and the Internal Control prior to running samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice recommends including a positive Extraction Control (clinical sample previously determined to be positive for toxigenic *C. diff* by the ProGastro Cd Assay or simulated sample) in each nucleic acid isolation run. Treat the Extraction Control as a sample.
- Never run the Positive Control through nucleic acid isolation.
- Always include one Negative Control (*containing Internal Control*) and the Positive Control in each amplification/detection run performed.
- Failure of Controls (Positive, Negative and/or Extraction) invalidates the run; do not report results. Repeat testing starting from the purified nucleic acid and using a new aliquot of the Positive Control. If results are still invalid, do not report results. Instead, repeat testing from the original sample, clarified stool, or collect and test a new sample.

Limitations

- **Once the *C. diff* Mix has been thawed, the run must be started within one hour.**
- This test detects but does not differentiate the NAP1 (Ribotype 027) strain from other toxigenic strains of *C. diff*.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Analyte targets (bacterial nucleic acid) may persist *in vivo*, independent of toxin presence. Detection of the analyte target does not imply that the corresponding organism is the causative agent for clinical symptoms.
- The detection of bacterial nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
- There is a risk of false negative values due to the presence of sequence variants in the bacterial targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
- This test targets the *tcdB* gene for Toxin B production. This test will not detect strains of *C. diff* that do not contain the *tcdB* gene.
- Variant toxigenic *C. difficile* without the *tcdB* gene or with a non-functional Toxin B protein are very rare. The ProGastro Cd Assay targets the *tcdB* gene and it is unknown whether it would detect Toxin A+/Toxin B-variant strains.

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%

	Performance Characteristics
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Clinical Performance

Performance characteristics of the ProGastro Cd Assay were established during a prospective study at 3 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.

		CTA		Total	Comments
		Positive	Negative		
ProGastro Cd Assay	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 3 laboratory sites. Reproducibility was assessed using a panel of 6 simulated samples that included medium positive, low positive (near the assay limit of detection, ≥ 95% positive) and “high negative” (< 5% positive) samples. Panels and controls were tested at each site by 2 operators for 5 days (6 samples and 4 controls X 2 operators X 5 days X 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 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% as the samples were lower concentration than the LoD concentration (≥ 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 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 ≥ 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, 5 different toxinotypes, 9 different restriction enzyme analysis (REA) types, 5 different serogroups, and 3 different ribotypes were used to evaluate the reactivity of the ProGastro Cd Assay. The strains were tested in triplicate at a concentration of 3x 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 1 yeast strain representing common gastrointestinal pathogens or microorganisms. One toxigenic strain (NAP1) was included in the specificity panel as a positive control for the study. Bacteria and yeast were tested at concentrations of 1.35 x 10⁸ orgs/mL. Viruses were tested at concentrations of 10^{3.5} to 10^{6.5} TCID₅₀/mL. The cultures were spiked with Internal Control and extracted using the bioMérieux NucliSENS easyMAG instrument and tested in triplicate. The ProGastro Cd Assay did not cross-react with any of the specificity organisms tested.

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 x 10 ⁻⁴ McFarland (1 x 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	-

Strains	Concentration†	ProGastro Cd Result
<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> O157: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	-
<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, Imodium 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 2X 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 instrument. 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)
Imodium 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 (5) 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 instrument 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.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

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Understanding the Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Definition	Symbol	Definition
REF	Reference Number or Catalog number		Contains sufficient for < n > tests
	Batch code or Lot Number		Do not reuse
	Use By Date or Expiration Date		Manufacturer
	Upper Storage Temperature Limitation		Authorized Representative
	Contents		

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