Procedure: Revogene C. difficile

Procedure and revision number: SN134773 REV. 01/20

Catalog # 410300

Institution:
Address:
Department:

Prepared By:	Date Adopted	Supersedes procedure #:

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PRINCIPLE:

The Revogene® *C. difficile* assay performed on the Revogene instrument is a qualitative *in vitro* diagnostic test that utilizes automated sample processing and real-time polymerase chain reaction (PCR) to detect the toxin B (*tcdB*) gene of toxigenic *Clostridium difficile* (*C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The Revogene *C. difficle* assay is intended to aid in the diagnosis of CDI.

The Revogene automates sample homogenization, sample dilution, cell lysis, DNA amplification and detection of the amplified PCR products. User intervention is only required for discharging the patient specimen into the Sample Buffer Tube (SBT), transferring the sample from SBT into the PIE, and loading/unloading the PIEs into the Revogene carousel.

Each PIE is a completely integrated closed device in which a sample is dispensed and processed through different microfluidic chambers and channels which allow for the sample processing (i.e. sample homogenization, sample dilution, and cell lysis) and subsequent real-time PCR steps. The liquid from a single sample is transferred by centrifugation from one chamber to the next in sequence and all reagents specific for the PCR reaction are incorporated and dried within the PCR well(s) (**Figure 1**).

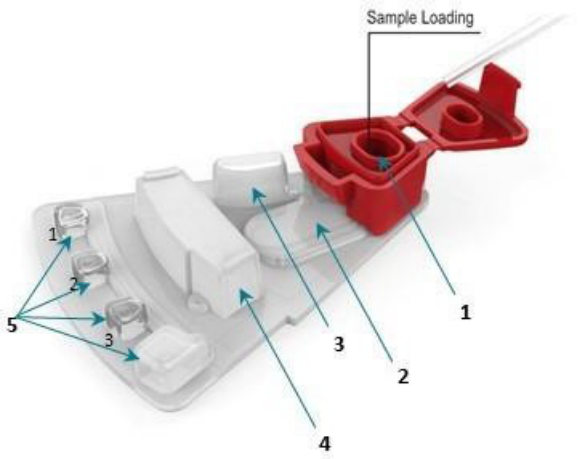

Figure 1. Top View of a PIE. 1: Sample Loading chamber, 2: Homogenization chamber, 3: Overflow chamber, 4: Dilution/Lysis chamber, 5: Three PCR wells (#1 to #3 at the left) and one waste chamber (at the right).

A Process Control (PrC) is incorporated into each PIE to verify sample processing and amplification steps. The PrC allows for the verification of potential inhibitor substances as well as microfluidic, instrument or reagent failure. The amplified products are detected in real time using target-specific TaqMan® chemistry-

based probes. No operator intervention is necessary once a PIE is loaded into the Revogene.

The Revogene can process from one up to eight samples simultaneously in the same run. The carousel must contain eight PIEs to maintain thermodynamic balance within the run. At run completion, the results are computed by the system from measured fluorescent signals and embedded calculation algorithms. Results that are displayed on the touchscreen may be printed, transferred and/or stored by the user using the USB port or the connectivity option.

SPECIMEN:

Preferred Sample Types: Unformed (liquid or soft) stool specimens obtained from patients suspected of having CDI.

Undesirable samples: Formed stools with no indication of CDAD, colonic aspirates, or specimens placed in transport media.

Collection and Storage:

1. Collect the unformed stool in a dry, clean container according to the local guidelines or procedures.
   - Transfer liquid or soft stool (but not urine) into the container. Avoid mixing toilet paper, water or soap with the specimen.
   - Label the container with the specimen or patient identification (ID) and send to the laboratory for testing.
2. Collected specimens should be stored between 2 C and 25 C during transport.
3. Stool specimens can be stored at 25 C for up to 2 days, or at 2- 8 C for up to 4 days.
4. Inoculated SBT can be stored at 25 C for up to 2 days, or at 2-8 C for up to 3 days.

This facility's procedure for specimen collection is: _____
_____

This facility's procedure for transporting specimens is: _____
_____

This facility's procedure for rejected specimens is: _____
_____

MATERIALS AND EQUIPMENT:

MATERIALS PROVIDED:

The CDiff kit contains sufficient reagents and materials to process 24 specimens. The kit contains the following:

1. 24 **Disposable Transfer Loop (DTL)** on the side compartment: DTL consists of a single-use 5 microliters (µL) loop for transferring the unformed (liquid or soft) stool specimen to the SBT.
2. 24 individual pouches and each pouch contains the following materials:
   - 1 **Disposable Transfer Tool** (DTT): DTT consists of a single-use transfer pipette for transferring the sample from the SBT to the PIE.
   - 1 **Sample Buffer Tube** (SBT): Barcode-labeled tube containing TE 1X buffered solution (Tris-HCl pH 8.0/EDTA.Na_2) as a dilution and preservation buffer for sample.
   - 1 ***C. difficile*** **PIE**: Barcode-labeled integrated device, composed of dried reagents allowing sample process and real-time PCR steps for PrC DNA and *C. difficile* toxin B gene DNA simultaneous amplification/detection. Each PIE contains PrC, PrC- specific primers and Taqman® chemistry-based probe, *C. difficile tcdB* gene-specific primers and Taqman® chemistry-based probe, dNTPs, buffer and DNA polymerase.

MATERIALS NOT PROVIDED

1. Revogene® (cat# 610210)
2. Disposable gloves; powderless
3. Vortex mixer with a maximal speed of at least 3,200 rpm
4. Sample rack (cat# 132539; optional)
5. MOCK PIE (cat# 610208 ; optional)

PERFORMANCE CONSIDERATIONS:

1. This product can only be used on the Revogene.
2. Do not use the kit if the label that seals the outer box is broken upon arrival.
3. Do not use *C. difficile* PIEs if the protective pouches are open or broken upon arrival.
4. Do not interchange DTT, SBT, and PIE between kit lots.
5. Each single-use DTL, DTT and *C. difficile* PIE are used to process one sample. Do not reuse DTL, DTT or PIE.
6. Always handle specimens as if they are infectious and in accordance with Good Laboratory Practices such as those described in Biosafety in Microbiological and Biomedical Laboratories and in CLSI Document M29-A4.
7. Wear disposable powderless gloves while handling specimens and thoroughly wash hands afterwards.
8. The *C. difficile* PIE contains dried reagents. The protective pouch should not be opened until ready to perform the test.
9. Dispose of unused materials and reagents and waste, including used PIEs, in accordance with country, federal, provincial, state and local regulations.
10. Do not open or break apart the PIE after use to avoid contamination with amplification products and/or infectious particles.

11. Do not use a PIE that has been dropped, shaken or inverted after the sample has been loaded as this may cause invalid results.
12. The *C. difficile* assay does not provide susceptibility results. Additional time is required to culture and perform susceptibility testing.
13. Do not use a kit that has passed its stated expiration date.
14. Do not refrigerate the loaded PIE.
15. An amount of stool exceeding the recommended amount may inhibit the *C. difficile* assay.
16. Each run must be performed with eight PIEs in the Revogene carousel to maintain thermodynamic and mechanical balance within the run.

SHELF LIFE AND STORAGE:

1. Store the *C. difficile* kit at 2-25 C. The expiration date is indicated on the box kit's label.
2. Do not open a pouch until ready to perform testing. Use the PIE within 1 hour after opening the pouch.

At this facility, kits are stored:_____

CALIBRATION:

Not applicable to this assay.

QUALITY CONTROL:

Quality control procedures monitor the accuracy and precision of the analytical process. Each laboratory must establish the number, type and frequency of testing control materials per applicable regulations or accrediting agencies. The procedure described below may be employed, if appropriate, based on local policies and procedures.

NOTE: Separate DTL, DTT, SBT and PIE must be used for each External Control preparation.

1. Each *C. difficile* PIE contains a Process Control (PrC) that verifies for sample homogenization, sample dilution, cell lysis, inhibition of DNA amplification and assay reagents failure.
2. Good laboratory practice recommends the use of control materials. User should follow the appropriate guidelines concerning the running of External Controls. It is recommended that one Positive External Control and one Negative External Control should be run at least on a daily basis until adequate process validation is achieved with the *C. difficile* assay on the Revogene in each laboratory setting.
3. External Control materials are not provided by Meridian Bioscience, Inc. External Controls are not used by the revogene software for the purpose of sample test result interpretation. External Controls are treated as if they are specimens.

4. Various types of External Controls are recommended to allow the user to select the most appropriate for its laboratory quality control program. Process and test External Control preparations according to the **Sample Preparation and Handling** section.

External Positive Control:

- A freshly prepared cell suspension of a toxigenic *C. difficile* strain, bearing the *tcdB* gene, from commercially available control material (e.g., ATCC® 43255™) prepared at 0,5 ± 0,05 McFarland and diluted 1/2 in saline (e.g., BD BBL™ Prepared Saline Solution, cat# 221819) is recommended for use as a Positive External Control.
- Alternatively, a previously characterized stool specimen positive for toxigenic *C. difficile* could also be used as a Positive External Control.

External Negative Control:

- A freshly prepared cell suspension of a non-toxigenic *C. difficile* strain from commercially available control material (e.g., ATCC® 43593™) prepared at 0,5 ± 0,05 McFarland in saline (e.g., BD BBL™ Prepared Saline Solution, cat# 221819) is recommended for use as a Negative External Control.
- Alternatively, a previously characterized stool specimen negative for toxigenic *C. difficile* could also be used as a Negative External Control.

QC Testing Frequency and Documentation:

For this facility, External QC is run: _____

Results of External QC and action(s) taken when control results are unacceptable are documented: _____

PROCEDURE:

NOTE: Start the test within 1 hour after opening the pouch containing the PIE.
NOTE: The content of one pouch is required for each specimen to be tested.

SBT PREPARATION

1. For each specimen to be tested, unseal the right side of the pouch (when facing label) containing DTT and SBT and remove the SBT from the pouch.
2. Identify (or label) the SBT with the appropriate specimen identification without obscuring or writing over the barcodes. Place the SBT on the Sample rack, if used.
3. Vortex the specimen at maximal speed for 15 seconds. Dip the provided DTL into the stool. Remove any excess of soft stool present on the outside of the loop in order to take approximately 5 µL.
4. Remove the cap from the SBT, shake the DTL into the SBT for 2-3 seconds and/or swirl to dislodge sample from the loop. Make sure that only one SBT is open at once.
5. Replace the cap on the SBT, tightly close the SBT cap, and place it on the Sample

rack, if used.

6. Prepare any additional specimens for testing by repeating steps 1 to 5 then proceed to step 7.
7. When all samples are prepared, proceed to *C. difficile* PIE preparation (next section).

PIE PREPARATION

NOTE: Processing one sample at a time:

8. Vortex the SBT for 15 seconds at maximal speed.
9. Unseal the left side of the pouch (when facing label) containing the PIE, removing it from the pouch.
10. Place the *C. difficile* PIE on the Sample rack, if used.
11. Using the DTT, aspirate the Sample Buffer by squeezing the entire bulb. The liquid level into the DTT must be anywhere between the two marks (**Figure 2**). If the liquid level is not between the two marks, discharge the SB volume completely in the SBT by squeezing the entire bulb and repeat step 11.
12. Discharge completely the SB into the Sample Loading chamber of the PIE (**Figure 1**).
13. Close the cap of the PIE tightly, making sure the cap lock is well in place. Do not refrigerate the loaded PIE. Make sure that only one PIE is open at once.
14. Repeat steps 8 to 13 for any additional samples then proceed to step 15.
15. The stool specimens and the inoculated SBT can be stored at 2-8 C or at 25 C within the timeframe defined in the **Storage and Stability** section.

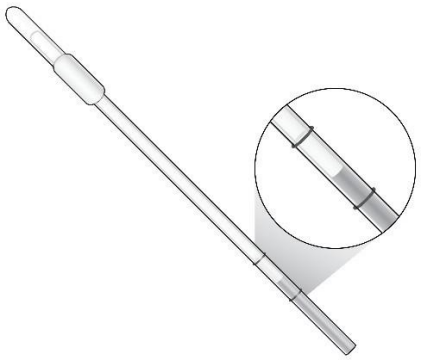

Figure 2. Representation of an Appropriate Sample Buffer Level Using the Disposable Transfer Tool (DTT).

REVOGENE OPERATION

NOTE: A maximum of eight samples can be processed simultaneously in a single run using the Revogene (including External Controls).

NOTE: Each run must be performed with eight PIEs in the Revogene. When less than eight samples are processed, the empty places must be filled with MOCK PIEs*.

NOTE: Refer to the Revogene Operator's Manual for further information regarding Revogene set-up and operation.

1. Power on the Revogene (if not already done). The software will launch automatically.
2. Log in by entering the **<user name>** and **<password>** and tap **<Login>**. The main menu will appear automatically.
3. Tap **<Setup Run>**.
4. Enter the sample identification using either the barcode scanner or manual entry. Manual entry can be done by tapping the **pencil** icon of the **<Scan or Enter Sample ID>** line.
5. Enter the SBT and *C. difficile* PIE barcodes using the Revogene barcode scanner. Gently positioning the PIE vertically in front of the scanner. Alternatively, SBT and PIE barcodes may be entered manually (tap the **pencil** icon of their respective lines). Handle the PIE carefully without dropping, shaking or inverting it.
6. (Optional) Tap the **pencil** icon of the **<Add Comments>** line and type a comment.
7. Insert the *C. difficile* PIE into the Revogene, at any position of the carousel. The software will automatically associate sample and SBT to the correct *C. difficile* PIE.
8. Confirm that the PIE is inserted into instrument by tapping **<OK>** on the **<insert PIE into instrument>** line and repeat steps 4 to 8 for all samples.
9. When all *C. difficile* PIEs are inserted into instrument, and MOCK PIEs when necessary, tap **<Next>**.
10. Scan the retention ring and place it on the carousel. Close the instrument lid.
11. Initiate the test run by tapping **<Start>**.

*If MOCK PIEs are not available, use unused assay PIEs filled with un-inoculated SB (BLANK) or with External Controls.

VIEWING AND EXPORTING RESULTS

NOTE: Refer to the Revogene Operator's Manual for further information regarding the acquisition of test results.

1. Once the run is completed, the lid opens automatically.
2. Tap the **home** icon.
3. If the Revogene has logged-out, re-enter **<user name>** and **<password>** and tap **<Login>**. The main menu will appear automatically.
4. Tap **Results** icon to access test results.
5. Tap **<Last Run>** to see the latest test results.
6. From the **<Last Run>,** select samples for which results report(s) has (have) to be exported. All samples can be selected in one time by clicking the first box to the left of the "Sample ID" column.
7. Tap **<Export>** and save where appropriate (e.g. USB key).
8. Remove the retention ring and *C. difficile* PIEs from the Revogene. Used CDiff PIEs should be discarded in appropriate waste containers according to the institution's standard practices.

REPEAT TESTING PROCEDURE

- **UNRESOLVED OR INDETERMINATE RESULT FOR A SPECIMEN**

  When an Unresolved (UNR) or an Indeterminate (IND) result is obtained for a specimen, a repeat test from the corresponding inoculated SBT must be performed within the specified timeframe described in the **Storage and Stability** section. Only one repeat testing from the SBT is allowed.

  Vortex the SBT for a minimum of 15 seconds at maximal speed using a vortex mixer. Using a new pouch, follow steps 9 to 13 of the **Sample Preparation and Handling / PIE Preparation** section, then follow the **Revogene Operation** section.

- **UNRESOLVED, INDETERMINATE, FALSE NEGATIVE OR FALSE POSITIVE RESULT FOR AN EXTERNAL CONTROL**

  When an unresolved, an indeterminate, a false negative or a false positive result is obtained for an External Control, the run is invalid. Specimens included in the run should be repeated using the corresponding inoculated SBT, along with freshly prepared External Controls, within the specified timeframe described in the **Storage and Stability** section. Refer to the next **Quality Control** section for preparation of fresh External Controls.

  For the repeat testing using the corresponding inoculated SBT, vortex the SBT for a minimum of 15 seconds at maximal speed using a vortex mixer. Using a new pouch, follow steps 9 to 13 of the **Sample Preparation and Handling / PIE Preparation** section, then follow the **Revogene Operation** section.

CALCULATIONS:

There are no calculations associated with this procedure.

INTERPRETATION OF RESULTS:

The results are computed by the Revogene from measured fluorescent signals and embedded calculation algorithms and are available on the "Results" window. Possible results are:

Patient Specimen		Positive	Toxigenic *C. difficile* target DNA detected.
		Negative	Toxigenic *C. difficile* target DNA not detected.
		Unresolved	Amplification/detection failure of the Process Control as well as for the toxigenic *C. difficile* target DNA. Could be caused by inhibitory specimens, microfluidic or reagent failure. Repeat testing must be performed (refer to the **Repeat Testing Procedure** section for further guidance).
		Indeterminate	No reportable result due to possible Revogene detection error during the assay processing, the data analysis, or if the run is interrupted by the user. Repeat testing must be performed (refer to the **Repeat Testing Procedure** section for further guidance).
Positive External Control		Positive	Valid Positive External Control result.
		Negative	An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. **The run is invalid.** Review the specimen handling/preparation technique.
		Unresolved	Incorrect Positive External Control result. **The run is invalid.** Repeat testing must be performed (refer to the **Repeat Testing Procedure** section for further guidance).
		Indeterminate	Incorrect Positive External Control result. **The run is invalid.** Repeat testing must be performed (refer to the **Repeat Testing Procedure** section for further guidance).
Negative External Control		Positive	An External Negative Control that yields a positive test result is indicative of a specimen handling and/or contamination event. **The run is invalid.** Review the specimen handling technique.
		Negative	Valid Negative External Control result.
		Unresolved	Incorrect Negative External Control result. **The run is invalid.** Repeat testing must be performed (refer to the **Repeat Testing Procedure** section for further guidance).
		Indeterminate	Incorrect Negative External Control result. **The run is invalid.** Repeat testing must be performed (refer to the **Repeat Testing Procedure** section for further guidance).

REPORTING OF RESULTS:

Positive Test: Toxigenic *C. difficile* target DNA detected.

Negative Test: No toxigenic *C. difficile* detected or is below the limit of detection.

EXPECTED VALUES:

The prevalence of *C. difficile* infection (CDI) depends upon a variety of factors including predisposition for infection due to prior therapy with broad-spectrum

antibiotics, the presence of symptoms and the standard of care test. In a combined prospective and retrospective study, specimens were collected at 8 geographically diverse clinical sites from 2,581 subjects in the range of age between 0 –2 years old to more than 60 years old. Of the 2,461 specimens that met all inclusion criteria without meeting any of the exclusion criteria, 333 were positive based on the combined results of direct and enriched toxigenic culture for an observed prevalence of 13.5% [333/2461; 95%CI: 12.2 – 15%]. The percentage of positive results observed with the Revogene *C. difficile* assay in the study population was 11.5% [283/2461; 95%CI: 10.3 – 12.8%].

ASSAY REACTIVITY:

CROSS-REACTIVITY

The cross-reactivity of the *C. difficile* assay was assessed with high loads of organisms that are not targeted by the test, or phylogenetically related to *C. difficile*, or non-toxigenic *C. difficile* strains, or present in the normal intestinal flora. The study included 50 bacteria, 1 yeast, 7 viruses and human DNA (**Table 8**). Bacteria and yeast were tested at a load of ≥10^6 CFU/mL of SB. Nucleic acids from 6 viruses and human DNA were tested at a load of ≥10^5 DNA or RNA copies/mL of SB. These organisms were tested using quantitated cell cultures or nucleic acid solutions spiked in a *C. difficile*-negative liquid stool matrix. Each organism was tested in PCR triplicates.

Under the conditions of the study, *Clostridium sordellii* was detected by the *C. difficile* assay at a load of approximately 10^6 CFU/mL of SB for one replicate out of 3, but was found non- reactive at a load of approximately 10^5 CFU/mL of SB. *Clostridium novyi* and *Clostridium scindens* strains produced false positive reactions in one replicate out of six tested at approximately 10^6 CFU/mL of SB. No reactivity was observed for three replicates tested at 10^5 CFU/mL of SB. *Enterococcus faecalis* strain produced false positive reactions in one replicate out of three tested at approximately 10^7 CFU/mL of SB. No reactivity was observed for three replicates tested at 10^6 CFU/mL of SB. The other organisms and nucleic acids tested were found non-reactive with the *C. difficile* assay.

For the *Coxsackievirus* only, the cross-reactivity with primers and probes of the *C. difficile* assay was evaluated by an *in silico* analysis performed on all strain sequences of *Coxsackievirus* contained in the National Center for Biotechnology Information (NCBI) database between February 7th, 2017 and August 11th, 2017. The analysis suggested that the *Coxsackievirus* strains should not be reactive with the CDiff assay.

Table 8. List of Organisms Tested for Cross-Reactivity with the *C. difficile* Assay

Bacteria	
Abiotrophia defectiva	*Acinetobacter baumannii*
Alcaligenes faecalis subsp. *faecalis*	*Aeromonas hydrophila*
Campylobacter jejuni (Campylobacter coli)	*Bacillus cereus*

Clostridium bifermentans	*Bacteroides fragilis*
Clostridium butyricum	*Campylobacter jejuni* subsp. *jejuni*
Clostridium haemolyticum	*Citrobacter freundii*
Clostridium novyi	*Clostridium perfringens*
Flavonifractor plautii (Clostridium orbiscindens)	*Enterobacter aerogenes*
Clostridium scindens	*Enterobacter cloacae* subsp. *cloacae*
Clostridium septicum	*Enterococcus faecalis*
Clostridium sordellii	*Escherichia coli*
Clostridium difficile (non- toxigenic) – ATCC® 43593™	*Klebsiella oxytoca*
Clostridium difficile (non- toxigenic) – ATCC® 43601™	*Lactobacillus acidophilus*
Clostridium sporogenes	*Peptostreptococcus anaerobius*
Edwardsiella tarda	*Porphyromonas asaccharolytica*
Escherichia coli O157:H7	*Prevotella melaninogenica*
Helicobacter pylori	*Proteus mirabilis*
Listeria monocytogenes	*Pseudomonas aeruginosa*
Plesiomonas shigelloides	*Salmonella enterica* subsp. *enterica serovar Typhimurium*
Providencia alcalifaciens	*Serratia marcescens* subsp. *marcescens*
Salmonella enterica subsp. *arizonae*	*Staphylococcus aureus* subsp. *aureus*
Salmonella enterica subsp. *enterica serovar Choleraesuis*	*Staphylococcus epidermidis*
Serratia liquefaciens	*Streptococcus agalactiae*
Shigella boydii	*Vibrio parahaemolyticus*
Shigella dysenteriae	*Shigella sonnei*

Yeast	
Candida albicans	
Viruses	
Human Adenovirus 1 (DNA)	Rotavirus (RNA)
Enterovirus D68 (RNA)	Norovirus (RNA)
Echovirus 4 (RNA)	Human Herpes virus 5 (Cytomegalovirus) (DNA)
Coxsackievirus (*in silico*)	
Human DNA	
Human gDNA	

TEST FOR INTERFERING SUBSTANCES:

INTERFERING ORGANISMS

The potentially inhibitory effect of 30 organisms, that may be present in the normal intestinal flora and which are not targeted by the test, was assessed using organisms selected from the cross-reactivity study (**Table 8**). Each organism category (i.e., bacteria, yeast, viruses) was represented with a special attention to include the most frequent causative agents of intestinal tract infections. Groups of 2 to 6 organisms were prepared in toxigenic *C. difficile*-negative liquid stool matrix, and tested in duplicate in presence of either 3,750 CFU/mL of SB of the toxigenic *C. difficile* ATCC® 43255™ strain or 4,500 CFU/mL of SB of the toxigenic *C. difficile* ATCC® BAA-1805™ strain, to assess their potential interference on detection of toxigenic *C. difficile* or PrC. Each organism within group was diluted to reach a load of $\geq 10^6$ CFU/mL of SB for bacterium and yeast, and $\geq 10^5$ copies/mL of SB for virus. The 30 organisms included in the study are presented in **Table 9**.

Table 9. List of Organisms Tested for Interference with the *C. difficile* Assay

Group 1	
Aeromonas hydrophila	*Bacillus cereus*
Bacteroides fragilis	*Campylobacter jejuni* subsp. *jejuni*
Campylobacter jejuni (Campylobacter coli)	
Group 2	
Candida albicans	*Citrobacter freundii*
Clostridium difficile (non- toxigenic) – ATCC® 43593™	*Clostridium difficile* (non- toxigenic) – ATCC® 43601™
Clostridium perfringens	*Clostridium sordellii*
Group 3	
Enterobacter aerogenes	*Enterobacter cloacae* subsp. *cloacae*
Enterococcus faecalis	*Escherichia coli* O157:H7
Helicobacter pylori	
Group 4	
Lactobacillus acidophilus	*Peptostreptococcus anaerobius*
Plesiomonas shigelloides	*Proteus mirabilis*
Salmonella enterica subsp. *enterica serovar Choleraesuis*	*Salmonella enterica* subsp. *enterica serovar Typhimurium*
Group 5	
Shigella boydii	*Shigella dysenteriae*
Shigella sonnei	*Staphylococcus aureus* subsp. *aureus*
Streptococcus agalactiae	*Vibrio parahaemolyticus*
Group 6	
Rotavirus (RNA)	Norovirus (RNA)

None of the 30 organisms present at ≥10^6 CFU/mL of SB for bacteria and yeast and ≥10^5 copies/mL of SB for viruses interfered with detection of PrC and with the toxigenic *C. difficile* ATCC® BAA-1805™ strain.

Group 3 and group 5 showed a potentially inhibitory effect on detection of the toxigenic *C. difficile* strain ATCC® 43255™. Nevertheless, when each bacterium from these groups was tested individually at a load of ≥10^6 CFU/ mL of SB in presence of *C. difficile* strain ATCC® 43255™, none interfered.

INTERFERING SUBSTANCES

The potentially inhibitory effect of 16 exogenous and 5 endogenous substances that may be present in the intestinal tract was assessed using toxigenic *C. difficile* negative samples and toxigenic *C. difficile* strains ATCC® 43255™ and ATCC® BAA- 1805™ at two to three times the LoD (3,750 CFU/mL of SB and 4,500 CFU/mL of SB respectively) in presence of liquid stool matrix. Substances were tested at their potentially highest concentration that could be found in a stool specimen. The results for the 21 substances are presented in **Table 10**.

Results demonstrated no reportable interference on PrC. Calcium Carbonate (e.g. Tums®) and Aluminum Hydroxide/Magnesium Hydroxide (e.g. Stomaax®) showed a potentially inhibitory effect on the detection of toxigenic *C. difficile* when either of these substances was present in SBT at a concentration of 5 mg/mL (0.5% W/V) and 5 µL/mL (0.5% V/V) respectively. When tested at 0.5 mg/mL (0.05% W/V) or 0.5 µL/mL (0.05% V/V) respectively, these substances showed no reportable interference with the *C. difficile* assay.

Table 10. List of Exogenous and Endogenous Substances Tested with the *C. difficile* Assay.

Exogenous substances		
Substance (commercial name)	**Concentration or amount in SBT[1]**	**Results[2]**
Vaginal antifungal / anti-itch (Nystatin)	0.5% W/V	NI
Creams / ointments (Personnelle Hydrocortisone cream)	0.5% V/V	NI
Anti-hemorrhoidal creams / ointments (Preparation H®)	0.5% V/V	NI
Antacids (Tums®)	0.5% W/V	I[3]
Antacids (Stomaax®)	0.5% V/V	I[4]
Enemas (Life BRAND™ Heavy Mineral Oil USP)	0.5% V/V	NI
Enemas (Mesalazine or 5-aminosalicylic Acid)	0.5% W/V	NI
Condom with spermicidal lubricant (Trojan ® with spermicidal lubricant condom)	Square of 2 mm^2	NI
Anti-diarrheal medication (Pepto Bismol™)	0.5% V/V	NI
Anti-diarrheal medication (Imodium®)	0.5% V/V	NI
Laxatives (Senokot®)	0.5% V/V	NI
Oral and topical antibiotics (Vancomycin)	0.5% V/V	NI
Oral and topical antibiotics (Metronidazole)	0.5% W/V	NI
Non-steroidal anti- inflammatory (Aleve®)	0.5% W/V	NI
Moist towelettes (Equate™ Flushable Moist Wipes)	Square of 2 mm^2	NI
Moist towelettes (Wet Ones®)	Square of 2 mm^2	NI
Endogenous substances		
Substance	**Concentration or amount in SBT[1]**	**Results[2]**
Fecal fat, triglycerides mix (C2-C10)	0.5% V/V	NI
Fecal fat, Palmitic acid	1.0% W/V	NI
Fecal fat, Stearic acid	0.5% W/V	NI
Whole blood	0.5% V/V	NI
Mucus	0.5% V/V	NI

[1] W/V: Weight/Volume; V/V: Volume/Volume
[2] I: Interference with the CDiff assay; NI: No Interference with the CDiff assay
[3] No Interference at 0.05% W/V
[4] No interference at 0.05% V/V

LIMITATIONS OF THE PROCEDURE:

1. The *C. difficile* assay must only be used with the Revogene by trained personnel.
2. The *C. difficile* assay is not intended to differentiate carriers of *C. difficile* from those with *C. difficile* infection.
3. The *C. difficile* assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing.
4. Performance characteristics of the *C. difficile* assay were established with unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection. Use of the *C. difficile* assay for clinical specimen types other than those specified has not been evaluated and performance characteristics are not established.
5. Results from the *C. difficile* assay should be used as an adjunct to clinical observations and other information available to the physician.
6. Assay results may be affected by concurrent antimicrobial therapy as *C. difficile* DNA may continue to be detected.
7. A positive test result does not necessarily indicate the presence of viable organisms. It is, however, indicative for the presence of toxigenic *C. difficile* DNA.
8. Erroneous test results may occur from improper specimen collection, handling or

storage, technical error or sample mix-up. Careful compliance with the instructions of this insert, the Revogene User Manual and to established guidelines is necessary to avoid erroneous results.

9. A negative result does not rule out the possibility of *C. difficile* colonization. False negative results may occur when the *C. difficile* concentration is below the limit of detection of the assay. If the patient has signs or symptoms of infection, other laboratory tests and clinical information should be used to confirm the negative result.
10. Contamination or false negative results may occur if a PIE cap is incorrectly closed.
11. While there are no known strains/isolates of toxigenic *C. difficile* lacking the tcdB gene, the occurrence of such a strain could lead to an erroneous result using the *C. difficile* assay.
12. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of *C. difficile tcdB* gene variants, resulting in a false negative result with the *C. difficile* assay.
13. Calcium Carbonate (e.g. Tums®) or Aluminum Hydroxide/Magnesium Hydroxide (e.g. Stomaax®) may potentially inhibit the detection of toxigenic *C. difficile* when either of these substances is present in sample buffer at a concentration of > 0.5 mg/mL or > 0.5 µL/mL respectively.
14. Presence of *Clostridium sordellii* at a load of $>10^5$ CFU/mL of sample buffer may lead to detection of false positive results.
15. A combinatory effect of *Enterobacter aerogenes*, *Enterobacter cloacae* subsp. *cloacae*, *Enterococcus faecalis*, *Escherichia coli* O157:H7 and *Helicobacter pylori*, at $\geq 10^6$ CFU/mL in sample buffer, may have an inhibitory effect on the detection of toxigenic *C. difficile*.
16. A combinatory effect of *Shigella boydii*, *Shigella dysenteriae*, *Shigella sonnei*, *Staphylococcus aureus* subsp. *aureus*, *Streptococcus agalactiae* and *Vibrio parahaemolyticus*, at $\geq 10^6$ CFU/mL in sample buffer, may have an inhibitory effect on the detection of toxigenic *C. difficile*.

PERFORMANCE CHARECTERISTICS:
Refer to Directional Insert- Revogene® **C. difficile**

REFERENCES:
Refer to Directional Insert- Revogene® **C. difficile**