HOLOGIC®

Prodesse ProGastro™ SSCS Assay

Instructions for Use
Rx Only

For the detection and differentiation of Salmonella, Shigella, Campylobacter (C. jejuni and C. coli only, undifferentiated) nucleic acids, and Shiga Toxin Producing E. coli (STEC) Shiga Toxin 1 (stx1) and Shiga Toxin 2 (stx2) genes.

Rx only

100

REF

303278

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Intended Use

The Prodesse ProGastro™ SSCS Assay is a multiplex real time PCR *in vitro* diagnostic test for the qualitative detection and differentiation of *Salmonella*, *Shigella*, and *Campylobacter* (*C. jejuni and C. coli* only, undifferentiated) nucleic acids and Shiga Toxin 1 (*stx1*) and Shiga Toxin 2 (*stx2*) genes. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2. Nucleic acids are isolated and purified from preserved stool specimens obtained from symptomatic patients exhibiting signs and symptoms of gastroenteritis. This test is intended for use, in conjunction with clinical presentation and epidemiological risk factors, as an aid in the differential diagnosis of *Salmonella, Shigella, Campylobacter jejuni/Campylobacter coli*, and STEC infections in humans.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative ProGastro SSCS Assay results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

Summary and Explanation

Bacterial gastroenteritis is inflammation of the stomach and intestines that results in acute diarrhea (three or more episodes per day) lasting less than 14 days and may also include symptoms such as nausea, vomiting, and abdominal cramping.[1] In the United States it is estimated that there are >200 million cases of diarrheal illness per year resulting in 73 million physician consultations, 1.8 million hospitalizations, and up to 6000 deaths.[1,2] According to the Centers for Disease Control Food Net data (data compilation from 10 state health departments), in 2010 the number of reported infections and incidence per 100,000 population were as follows: *Salmonella* (8256; 17.6), *Campylobacter* (6365; 13.6), *Shigella* (1780; 3.8), STEC O157 (442; 0.9), and STEC non-O157 (451; 1.0).[3] These four bacteria are the most common cause of bacterial gastroenteritis. The populations most at risk due to bacterial gastroenteritis infection are children (≤ 5), the elderly, and immunocompromised. However, infection can occur in all age groups. The mode of infection is via the fecal-oral route typically from ingesting contaminated food or water or as a result of poor hygiene (hand-washing).

Salmonella are gram-negative, aerobic, rod-shaped bacilli. There are two species of *Salmonella* including *enterica* and *bongori*. *Salmonella enterica* is further divided into 6 subspecies with only a fraction of *Salmonella enterica* subspecies I being responsible for human illness.[4] *Salmonella* serotypes Typhimurium, Enteritidis, and Newport account for about half of the culture-confirmed *Salmonella* isolates in the U.S.[5,6] *Salmonella* serotype Typhi, the strain that causes typhoid fever, is uncommon in the U.S. while *Salmonella* serotypes Mississippi and Javiana are increasingly identified as a source of illness.[7]

Campylobacter are curved, motile, microaerophilic, gram-negative rods. They exhibit rapid, darting motility in a corkscrew fashion using 1 or 2 flagella and also have a lipopolysaccharide endotoxin.[8] Two species of *Campylobacter, C. jejuni* and *C. coli,* are responsible for the vast majority of human infections.[9-11]

Shigella are gram-negative, aerobic, rod-shaped bacteria that are closely related to *E. coli*.[12,13] There are four species of *Shigella,* all of which can cause disease in humans and include *S. sonnei* (subgroup D)*, S.flexneri* (subgroup B)*, S. boydii* (subgroup B)*,* and *S. dysenteriae* (subgroup A).[14] According to the 2006 *Shigella* annual summary published by the CDC, *S. sonnei* is the most prevalent cause of infections at 76%, followed by *S. flexneri* (14%), *S boydii* (1.1%), and *S. dysenteriae* (0.5%).[15]

Shiga Toxin-producing *Escherichia coli* (STEC, also referred to as enterohemorrhagic *E. coli* – EHEC, and verocytotoxic *E. coli* – VTEC) are gram-negative, rod-shaped, aerobic bacteria. The majority of *E. coli* strains are harmless while some can cause illness such as diarrhea, urinary tract infections, respiratory illness, and pneumonia.[16] STEC cause disease by producing Shiga Toxin(s), either Shiga Toxin 1 and/or Shiga Toxin 2. The most commonly identified serogroup is O157:H7; in addition, serogroups O26, O45, O103, O111, O118, O121, and O145 also cause illness in the U.S.[16]

Principles of the Procedure

The ProGastro SSCS Assay enables detection and differentiation of *Salmonella, Shigella, Campylobacter* (*C. jejuni* and *C. coli* only, undifferentiated) and an Internal Control in the SSC Mix and Shiga Toxin Producing *E. coli* (STEC, *stx1* and *stx2* differentiated) and an Internal Control in the STEC Mix.

An overview of the procedure is as follows:

1. Collect raw stool specimens from symptomatic patients and place into Cary Blair Transport Medium or ParaPak C&S (C&S) Transport Medium (refer to **Materials Required but not Provided**).
2. Add the Gastro RNA/DNA Internal Control (GIC) to every sample to monitor for inhibitors present in the specimens.
3. Perform isolation and purification of nucleic acids using a NucliSENS easyMAG System and the Automated Magnetic Extraction Reagents (bioMérieux).
4. Add purified nucleic acids to the **SSC Mix** included in the ProGastro SSCS Assay Kit. The SSC Mix contains target-specific oligonucleotide primers and probes for detection of *Salmonella*, *Shigella*, and *Campylobacter* (*C. jejuni* and *C. coli* only). The primers and probes are complementary to highly conserved regions of genetic sequences for these organisms. The probes are dual-labeled with a reporter dye and a quencher (see table below).
5. Add purified nucleic acids to the **STEC Mix** included in the ProGastro SSCS Assay Kit. The STEC Mix contains target-specific oligonucleotide primers and probes for detection of Shiga Toxin 1 and 2 genes (*stx1* and *stx2*). The primers and probes are complementary to highly conserved regions of these genes. The probes are dual-labeled with a reporter dye and a quencher (see table below).
6. Perform amplification of DNA in a Cepheid SmartCycler II instrument. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The ProGastro SSCS Assay is based on Taqman reagent chemistry, which utilizes the 5' – 3' exonuclease activity of Taq polymerase to cleave the probe thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle, additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time. Fluorescent intensity is monitored during each PCR cycle by the real-time instrument.

Supermix	Analyte	Gene Targeted		Probe Fluorophore	Absorbance Peak	Emission Peak	Instrument Channel
SSC Mix	*Campylobacter* (*C. jejuni* and *C. coli* only)	*C. jejuni*	*C. coli*	FAM	495 nm	520 nm	FAM
		glyA	*cadF*				
SSC Mix	*Salmonella spp.*	*orgC*		CAL Fluor Orange 560	538 nm	559 nm	TET
SSC Mix	*Shigella spp.*	*ipaH*		CAL Fluor Red 610	590 nm	610 nm	Texas Red
STEC Mix	Shiga Toxin 1	*stx1*		CAL Fluor Orange 560	538 nm	559 nm	TET
STEC Mix	Shiga Toxin 2	*stx2*		FAM	495 nm	520 nm	FAM
SSC Mix and STEC Mix	Internal Control	NA		Quasar 670	647 nm	670 nm	Cy5

Materials Provided

ProGastro SSCS Assay Kit (100 Reactions, Cat. # 303278)

Reagents	Description	Quantity/ Tube	Cap Color	Cat. #	Reactions/ Tube
SSC Mix	➲ Taq DNA polymerase ➲ oligonucleotide primers ➲ oligonucleotide probes ➲ Buffer containing dNTPs ➲ $MgCl_2$ and stabilizers	1100 µL	Brown	403325	55 (2 tubes provided)
STEC Mix	➲ Taq DNA polymerase ➲ oligonucleotide primers ➲ oligonucleotide probes ➲ Buffer containing dNTPs ➲ $MgCl_2$ and stabilizers	1000 µL	Blue	403323	50 (2 tubes provided)
RNase Inhibitor IV	➲ 40U/µL	50 µL	Lilac	403326	100
SSCS Control	➲ Non-infectious DNA plasmid containing a portion of the targeted genes (*Salmonella, Shigella, C. jejuni, stx1,* and *stx2*)	400 µL	Red	403324	20
C. coli Control	➲ Non-infectious DNA plasmid containing a portion of the targeted gene for *C. coli* only	400 µL	Yellow	403328	20
Gastro RNA/DNA Internal Control (GIC)	➲ Non-infectious DNA plasmid ➲ Non-infectious *in vitro* transcribed RNA	30 µL	Green	403327	>100

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 sterile RNase/DNase-free filter 200 µL micropipettor tips
- ☐ easyMAG System Disposables (Sample Strips and Aspiration Tips)
- ☐ Biohit Pipette Tips for use with easyMAG System
- ☐ Greiner Break Four uncoated plates for use with easyMAG System
- ☐ Cepheid SmartCycler PCR reaction tubes

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)*
- ☐ Cary Blair Transport Medium *(Remel, Inc. Cat. No. R21610, R21617 or R21925),* or ParaPak C&S Transport Medium (Meridian Cat. No. 900612)
- ☐ Molecular Grade Water (RNase/DNase Free)
- ☐ Extraction Control *(recommended, e.g. previously characterized positive sample or negative sample spiked with a well characterized ProGastro SSCS Assay target strain)*

Equipment

- ☐ ≤ -70°C Freezer
- ☐ bioMérieux NucliSENS easyMAG System with Software version 1.0.1 or 2.0
- ☐ Biohit multi-channel pipettor for use with easyMAG System
- ☐ Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b, 3.0a, or 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
- ☐ 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.
- ➲ Use a trained health care professional to carefully interpret the results from the ProGastro SSCS Assay in conjunction with patient's clinical presentation, epidemiological risk factors, and results of other diagnostic tests.
- ➲ National, state and local public health authorities have published guidelines for notification of reportable diseases in their jurisdictions including *Salmonella*, *Shigella*, and Shiga-like Toxin producing *E. coli* (STEC) *stx*1/*stx*2 to determine necessary measures for verification of results to identify and trace outbreaks. Refer to the CDC's Nationally Notifiable Disease Surveillance System (NNDSS) (http://wwwn.cdc.gov/nndss/) for additional information and resources. Laboratories are responsible for following their state or local regulations for submission of clinical material or isolates on positive specimens to their state public health laboratories.

- *Campylobacter* is sensitive to environmental stressors including freezing where it loses viability. Avoid storing preserved stool samples at freezing temperatures for prolonged periods prior to testing.
- Perform the ProGastro SSCS Assay on the Cepheid SmartCycler II instrument only.
- Once the SSC Mix and STEC Mix are thawed, start the run within two hours.
- Do not update the SmartCycler Dx Software beyond version 3.0b until Hologic communicates that the updated software version is validated for use with the ProGastro SSCS Assay.
- This assay is for use with stool specimens placed into Cary Blair Transport Medium or ParaPak C&S Transport Medium according to the manufacturer's instructions only.
- Handle all 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 equipment or materials that have contacted clinical specimens before discarding.
- Real-Time PCR testing in general requires meticulous effort by the operator in reducing the chance of cross-contamination of samples during all steps of the procedure including: extraction, transfer of purified nucleic acids, and preparation of the PCR reactions.
- Use personal protective equipment such as (but not limited to) gloves and lab coats when handling kit reagents and other materials including samples, pipettes, and other equipment.
- 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.
    - Always dedicate supplies and equipment to a specified area; no cross-movement allowed between areas.
    - Do not use equipment and supplies used for reagent preparation for specimen preparation activities or for pipeting 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 Positive Controls and specimens by following good laboratory practices.
- Perform the procedure given in this Instructions for Use as described, any deviation from the outlined protocols may result in assay failure or generate erroneous results.
- Do not use kit after its 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.hologic.com.

Reagent Storage, Handling and Stability

- Store all reagents (opened and unopened) at ≤ -70°C until the expiration date listed on the kit.
- Always check the expiration date on the reagent tubes. For the working stock of the Gastro RNA/DNA Internal Control, SSCS Positive DNA Control and *C. coli* Positive DNA Control, use the expiration date of the originating stock control tube. Do not expose controls to more than one (1) freeze-thaw cycle.
- ProGastro SSCS Assay Kits are shipped frozen, should arrive frozen and should be stored frozen after receipt. If the kit contents are not frozen, contact Customer Service for assistance.
- An internal study demonstrated that performance of the SSC Mix and STEC Mix are not affected for up to 10 freeze-thaw cycles.
- Visually examine reagents for adequate reagent volume before beginning any test procedures.
- Protect the SSC Mix and STEC Mix from light.
- **Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.**

Recommendation

Aliquoting of kit components to maintain less than 10 freeze/thaw cycles is recommended for labs with smaller batch sizes.

Specimen Collection, Handling and Storage

Collecting the Specimen

Standard stool collection and handling procedures are appropriate to obtain liquid or soft raw stool. Specimens must be collected into a sterile screw-cap container that can be adequately sealed.

a. Place raw stool in Cary Blair or C&S Transport Medium within 2 hours of collection.
b. Remove cap and place approximately 1 gram of the raw stool into the transport medium or a sufficient amount to bring the liquid level up to the “fill to here” line.
c. Replace cap and tighten.
d. Agitate the vial to permit adequate mixing of the specimen with the transport medium. When mixing is completed the solutions should appear homogenous.
e. Submit the specimen to the laboratory for processing.

Transporting Specimens

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

Storing Specimens

Store specimens in Cary Blair or C&S Transport Medium refrigerated (2-8°C) for up to 5 days (120 hours) before processing. Store any leftover specimens at ≤ -70°C. If retesting a frozen specimen, thaw the specimen quickly (1 to 2 minutes) in a 37°C water bath and immediately place on ice or thaw specimen on ice. Test frozen samples after no more than one (1) freeze-thaw cycle. *Campylobacter* is sensitive to environmental stressors including freezing where it loses viability. Avoid storing preserved stool samples at freezing temperatures for prolonged periods prior to testing.

Storing Purified Nucleic Acid

Store purified nucleic acids at ≤ -70°C. Test after no more than one (1) freeze-thaw cycle.

Note

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

Training in specimen collection is highly recommended because of the importance of specimen quality.

Recommendation

Reagent and Control Preparation

Reagents

Note

Prepare reagents from the bioMérieux easyMAG Automated Magnetic Extraction Reagents following the manufacturer's instructions.

Controls

Note

- *For aliquots of the Positive Controls and Gastro RNA/DNA Internal Control, use the expiration date of the originating stock control 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 preparation area, such as a Biological Safety Cabinet.*

Positive Controls (PC)

1. Thaw Positive Controls on ice.
2. For each Positive Control, make 20 aliquots of 20 µL, label and store at ≤ -70°C. **Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle.**
3. Dilute the Positive Controls just prior to setup of the PCR reaction (see ***Step 3a*** of the ***Assay Procedure***).

Note

Do not spike Positive Controls with the Gastro RNA/DNA Internal Control. Do not take the Positive Controls through the nucleic acid isolation procedure.

Gastro RNA/DNA Internal Control (GIC)

1. Thaw Gastro RNA/DNA Internal Control and RNase Inhibitor on ice.
2. Create working stock tubes of the Gastro RNA/DNA Internal Control (GIC) using the following dilution scheme:

20 µL Gastro Internal Control	+	*50 µL RNase Inhibitor*	+	*1930 µL molecular grade water*	=	*2000 µL total volume*

3. Make aliquots of 250 µL, label, and store at ≤ -70ºC (this is enough volume to add to 24 samples at 10 µL per sample). Make aliquots of larger or smaller volumes based on the number of samples expected to be processed in a single run. **Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle**.
4. Add the appropriate volume of working stock of the Gastro RNA/DNA Internal Control to each sample prior to nucleic acid isolation (see ***Step 1a*** of the ***Assay Procedure***).

Negative Control (NC)

1. Use Cary Blair or ParaPak C&S Transport Medium as the Negative Control.
2. Add the appropriate volume of working stock of the Gastro RNA/DNA Internal Control to the Negative Control prior to nucleic acid isolation (see ***Step 1b*** of the ***Assay Procedure***).

Extraction Control

Good laboratory practice recommends including a positive extraction control (e.g. previously characterized positive sample or negative sample spiked with a well characterized ProGastro SSCS Assay target strain) in each nucleic acid isolation run. Treat the extraction control like a sample during assay performance and analysis.

Assay Procedure

Assay Overview

Get Ready: Create the Assay Protocols for the Cepheid SmartCycler instrument using the Dx Software *(performed first time only).*

1. ***Prepare the Samples, Extraction Control (if included), and Negative Control.***
2. ***Isolate the Nucleic Acid –*** *NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents.*
3. ***Set up the SSC Mix and STEC Mix PCR Reactions.***
4. ***Run the ProGastro SSCS Assay on the SmartCycler II instrument.***
5. ***Print Reports.***

Note

- *Instructions provided for the Cepheid SmartCycler II Real Time Instrument with Dx Software version 3.0a / 3.0b (Instructions for version 1.7b noted).*
- *Do NOT deviate from the protocol settings defined in this section.*

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

Note

- *Each protocol is created prior to first time use; it does not need to be recreated with each run on the instrument.*
- *Refer to SmartCycler Dx Software Operator Manual for assistance in defining assay protocols.*
- *To **Define** and **Edit** Assay protocols, you must have administrative access rights. Otherwise, the fields are not accessible for data entry and editing (they are grayed out).*
- *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.*
- *Interpret the control results and determine if the run is VALID or INVALID. You must meet Positive and Negative Control criteria for the run to be VALID (see **Interpretation of Control Results**).*
- *Gray boxes in all tabs are the default settings.*

1. Create the **ProGastro SSC Mix Assay protocol**:

- ***a.*** Launch the Cepheid Dx software application.
- ***b.*** Click the **Define Assay** box at the top of the screen.
- ***c.*** Click the **New Assay** box at the bottom of the screen.
- ***d.*** Enter **ProGastro SSC Mix** for the assay protocol in the window that opens.
- ***e.*** Click **OK**.
- ***f.*** Enter **Thermocycler Parameter** in the Protocol section (bottom half of ***Define Assay*** screen).

Stage 1			**Stage 2** Repeat **5** times			**Stage 3** Repeat **40** times		
Hold			**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

g. Enter information in **BOLD** in the Analysis Settings tab as follows:

i. Select **FTTC25** for the **Dye Set**
ii. **Analysis Type: Qualitative** (default)
iii. **Customize Result Text: Target-based Result Text** (default)

Channel	Dye Name	Channel Name*	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	Bknd Max. Cycle	Boxcar Avg	EndPt Thresh	NC IC %	IC Delta
1	FAM	**Campy**	**Target****	Primary	Manual	**60.0**	NA	5	10	13.0	**45.0**	ON	5	40	0	**60**	**NA†**	**NA**
2	TET	***Salmonella***	**Target****	Primary	Manual	**35.0**	NA	5	10	13.0	**45.0**	ON	5	40	0	**35**	**NA†**	**NA**
3	TxR	***Shigella***	**Target****	Primary	Manual	**25.0**	NA	5	10	13.0	**37.0**	ON	5	40	0	**25**	**NA†**	**NA**
4	Cy5	**Internal Control**	**Internal Control**	Primary	Manual	**35.0**	NA	5	10	13.0	**45.0**	ON	5	40	0	**35**	**NA†**	**NA**

** Dx 1.7b = Target*
*** Dx 1.7b = Assay*
† Dx1.7b = 10

h. Enter information in **BOLD** in the **Control Settings** tab**.**

i. Select **NC Fails if any target criterion is positive**.
ii. *Use **two** of the three Positive Controls available in the Control Settings tab as described below. For the SSC Mix, PC1 corresponds to the SSCS Control and PC2 corresponds to the C. coli Control. Enter **0** Replicates to inactivate the Positive Control (PC3).*
iii. *Use only **one** Negative Control (NC1). Enter **0** Replicates to inactivate the Negative Controls NC2 and NC3.*

Control ID	Control Name	Replicate	IC +/-	Campy Valid Min Cycle	Campy Valid Max Cycle	Campy EndPt Thresh	*Salmonella* Valid Min Cycle	*Salmonella* Valid Max Cycle	*Salmonella* EndPt Thresh	*Shigella* Valid Min Cycle	*Shigella* Valid Max Cycle	*Shigella* EndPt Thresh	IC Valid Min Cycle	IC Valid Max Cycle	IC EndPt Thresh
PC1	Pos Cntrl 1	**1**	**NA**	**20**	**45**	**60**	**20**	**45**	**35**	**20**	**37**	**25**	NA	NA	NA
PC2	Pos Cntrl 2	**1**	**NA**	**20**	**45**	**60**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	NA	NA	NA
PC3	Pos Cntrl 3	0	+	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10
NC1	Neg Cntrl 1	**1**	**+**	**13**	**45**	**60**	**13**	**45**	**35**	**13**	**37**	**25**	**13**	**45**	**35**
NC2	Neg Cntrl 2	0	+	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10
NC3	Neg Cntrl 3	0	+	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10

i. Click the **Advanced Tab** and select **Require Lot Number**.
j. **Probe Check Settings** tab, **Advance to New Stage** tab, and **Standards** tab are not used for the ProGastro SSC Mix protocol.
k. Select **Save Assay.**

2. Create the **ProGastro STEC Assay protocol**:
   - ***a.*** Launch the Cepheid Dx software application.
   - ***b.*** Click the **Define Assay** box at the top of the screen.
   - ***c.*** Click the **New Assay** box at the bottom of the screen.
   - ***d.*** Enter **ProGastro STEC Mix** for the assay protocol in the window that opens.
   - ***e.*** Click **OK**.
   - ***f.*** Enter **Thermocycler Parameter** in the Protocol section (bottom half of ***Define Assay*** screen).

Stage 1			**Stage 2** Repeat **5** times			**Stage 3** Repeat **40** times		
Hold			**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

   - ***g.*** Enter information in **BOLD** in the **Analysis Settings** tab as follows:
     - ***i.*** Select **FTTC25** for the **Dye Set**
     - ***ii.*** **Analysis Type: Qualitative** (default)
     - ***iii.*** **Customize Result Text: Target-based Result Text** (default)

Channel	Dye Name	Channel Name*	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	Bknd Max. Cycle	Boxcar Avg	EndPt Thresh	NC IC %	IC Delta
1	FAM	***stx2***	**Target****	Primary	Manual	**70**	NA	5	10	13.0	**45**	ON	5	40	0	**70**	NA†	NA
2	TET	***stx1***	**Target****	Primary	Manual	**40**	NA	5	10	13.0	**45**	ON	5	40	0	**40**	NA†	NA
3	TxR	*Leave Blank*	**Unused**	Primary	Manual	25	NA	5	10	13.0	45	ON	5	40	0	25	NA†	NA
4	Cy5	**Internal Control**	**Internal Control**	Primary	Manual	**35**	NA	5	10	13.0	**45**	ON	5	40	0	**35**	NA†	NA

**Dx 1.7b = Target*
***Dx 1.7b = Assay*
† Dx 1.7b = 10

   - ***h.*** Enter information in **BOLD** in the **Control Settings** tab**.**
     - ***i.*** Select **NC Fails if any target criterion is positive**.
     - ***ii.*** Use only **one** Positive Control as described below. For the STEC Mix, PC1 corresponds to the SSCS Control. Enter **0** Replicates to inactivate the Positive Controls PC2 and PC3.
     - ***iii.*** Use only **one** Negative Control (NC1). Enter **0** Replicates to inactivate the Negative Controls NC2 and NC3.

Control ID	Control Name	Replicate	IC +/-	*stx2* Valid Min Cycle	*stx2* Valid Max Cycle	*stx2* EndPt Thresh	*stx1* Valid Min Cycle	*stx1* Valid Max Cycle	*stx1* EndPt Thresh	IC Valid Min Cycle	IC Valid Max Cycle	IC EndPt Thresh
PC1	Pos Cntrl 1	**1**	**NA**	**20**	**45**	**70**	**20**	**45**	**40**	NA	NA	NA
PC2	Pos Cntrl 2	0	+	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10
PC3	Pos Cntrl 3	0	+	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10
NC1	Neg Cntrl 1	**1**	**+**	**13**	**45**	**70**	**13**	**45**	**40**	**13**	**45**	**35**
NC2	Neg Cntrl 2	0	+	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10
NC3	Neg Cntrl 3	0	+	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10

i. Click the **Advanced Tab** and select **Require Lot Number**.
j. **Probe Check Settings** tab, **Advance to New Stage** tab and **Standards** tab are not used for the ProGastro STEC Mix protocol.
k. Select **Save Assay.**

1. Prepare the Samples, Extraction Control (if included), and Negative Control (Pre-Amplification Area)

a. **Add Gastro RNA/DNA Internal Control (GIC) to all samples and Extraction Control (if included).**

i. Thaw the appropriate number of aliquots of working stock GIC (enough volume needed for each sample, the Extraction Control - if included, and the Negative Control) on ice.

ii. Add 450 µL of Cary-Blair or C&S medium to the appropriate number of microcentrifuge tubes. (Note: Aliquots of 450 µL of Cary-Blair/C&S medium may be made in advance and stored at room temperature).

iii. Mix the stool sample thoroughly either via inversion or vortexing. Using a wide-bore 200 µL pipet tip, remove 50 µL of stool in Cary-Blair/C&S medium from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube containing 450 µL Cary Blair/C&S medium. Mix thoroughly by vortexing for up to 5 seconds.

iv. Mix the Extraction Control (if included) thoroughly either by inversion or vortexing. Remove 50 µL of Extraction Control from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube containing 450 µL Cary Blair/C&S medium. Mix thoroughly by vortexing for up to 5 seconds.

v. Add 10 µL of working stock of the GIC to each easyMAG sample vessel well to be used (enough for the samples, Extraction Control - if included, and Negative Control).

vi. Using a regular (i.e. not wide-bore) 200 µL pipet tip, add 100 µL of the diluted stool specimen and Extraction Control (if included) to its specific easyMAG sample vessel well. **Pipet up and down a minimum of 5 times to mix.**

vii. Store any remaining sample at ≤ -70°C. Discard remaining volume of the diluted Extraction Control. DO NOT reuse.

Note

It is important to use a regular 200 µL tip to add 100 µL of the diluted specimen directly to the easyMAG vessel to avoid having particulate matter present that could potentially clog the aspiration tips on the easyMAG system during the purification process.

b. **Add Gastro RNA/DNA Internal Control to the Negative Control.**

i. Include one (1) Negative Control in each extraction run.

ii. Add 100 µL of Cary Blair/C&S medium to the specific easyMAG sample vessel well that contains 10 µL of the working stock of the GIC. **Pipet up and down a minimum of 5 times to mix.**

iii. Discard remaining volume of Gastro RNA/DNA Internal Control – DO NOT reuse.

Do not reuse Internal Control.

2. Isolate the Nucleic Acid (Pre-Amplification Area I) - 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, turn on the computer and log into the software.

b. Prepare the software for a run.

To prepare for a run, touch the "**Settings**"  icon in the main toolbar which defaults to the "Application Settings" icon 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 the "**Instrument**"  icon 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:

Request: Specific A 1.0.2
Matrix: Feces
Sample ID: Manually enter the sample name.
Volume (mL): 0.110 (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.

Note

*Be sure to select the **Specific A 1.0.2** protocol in the request section as the default setting for the easyMAG system is the Generic protocol*

iii. Enter samples requests by clicking on  or  to enter multiple samples.

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 screen, 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 (arrow)  .

iii. Touch "**Load Run"**  and select the run. Touch "**Print Worklist"**  to print the list. The worklist helps keep track of the order of the samples.

f. **Load the Sample vessel(s) and aspiration tips and barcode the sample vessel(s).**

Insert the aspiration tips into the instrument correctly. Insert the sample vessel(s) in the correct order as noted in the worklist and scan the sample vessel(s) position on the instrument and then the sample vessel itself. For example, scan position A and then the sample vessel in that position, then B and then C, if necessary. After scanning the sample vessel(s), 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 2 mL of 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. This procedure will need to be performed 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 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 transfers 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 the remaining mixture back into the tube containing the mix to reset the pipette.

iv. Press the **start** button 8 separate times to dispense the remaining mix in each of 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 multichannel pipettor (or however many samples are present in the specific sample vessel). Make sure the filter tips are very well connected with the multichannel 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 is 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 mix cycles with 1000 µL. 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 using new tips for each magnetic silica addition.

i. **Start the run.**

Touch "**Start**"  to begin the run. The instrument performs a series of washes and a heated elution. 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). **Use immediately or store at ≤ -70°C.**

3. Set up the SSC Mix and STEC Mix PCR Reactions (Pre-Amplification II)

Note

Start the SmartCycler SSC Assay and STEC Assay run within 1 hour of adding the nucleic acids to the SSC and STEC Mixes.

a. Dilute the Positive Controls.

i. Include both the SSCS Control (PC1) and *C. coli* Control (PC2) with each SSCS Assay run.Thaw one (1) aliquot of each Positive Control on ice.

ii. Add 45 µL of molecular grade water to two individual 1.5 mL microcentrifuge tubes, one for each Positive Control.

iii. Transfer 5 µL from the SSCS Control aliquot to the appropriate tube. **Pipet up and down a minimum of 5 times to mix (or vortex briefly) using a new pipet tip for each control tube.**

iv. Transfer 5 µL from the *C. coli* Control aliquot to the appropriate tube. **Pipet up and down a minimum of 5 times to mix (or vortex briefly) using a new pipet tip for each control tube.**

v. Keep tubes on ice.

vi. Discard remaining volume of Positive Controls – DO NOT reuse.

Do not reuse Positive Controls.

b. Add the SSC Mix and STEC Mix to the SmartCycler tubes.

Note

It is recommended to set up SSC Mix and STEC Mix reactions at the same time to eliminate multiple tube openings/closings for template addition to the two Mixes

i. Thaw and keep the SSC Mix and the STEC Mix on ice and protected from light before adding to SmartCycler tubes.

ii. Load the required number of tubes into the Cepheid Cooling Block.

Note

- *For the SSC Mix, 4 tubes are needed for Controls (SSCS Control, C coli Control, Extraction Control (if included), and Negative Control) plus tubes for the number of samples to test.*
- *For the STEC Mix, a total of 3 tubes are needed for Controls (SSCS Control, Extraction Control (if included), and Negative Control) plus tubes for the number of samples to test.*

iii. Pipet 20 µL of the SSC Mix into the upper part of the SmartCycler tubes.

iv. Pipet 20 µL of the STEC Mix into the upper part of the SmartCycler tubes.

v. Store any remaining SSC Mix or STEC Mix at ≤ -70°C.

c. Add 5 µL of each sample's nucleic acid to individual SmartCycler tubes containing the SSC Mix and then to the tubes containing the STEC Mix.

i. Add 5 µL nucleic acid to the specific tube containing the SSC Mix and pipet up and down 2 to 3 times in the upper part of the tube. Close the tube and discard the pipette tip.

ii. Add 5 µL of the same nucleic acid to the specific tube containing the STEC Mix and pipet up and down 2 to 3 times in the upper part of the tube. Close the tube and discard the pipette tip.

d. Add 5 µL of the diluted SSCS Control (PC1) to a separate SmartCycler tube containing the SSC Mix and then to the tube containing the STEC Mix.

i. Add 5 µL of the diluted SSCS Control (PC1) to the specific tube containing the SSC Mix and pipet up and down 2 to 3 times in the upper part of the tube. Close the tube and discard the pipette tip.

ii. Add 5 µL of the diluted SSCS Control (PC1) to the specific tube containing the STEC Mix and pipet up and down 2 to 3 times in the upper part of the tube. Close the tube and discard the pipette tip.

Do not reuse Positive Controls.

e. **Add 5 µL of the diluted *C. coli* Control (PC2) to a separate SmartCycler tube containing the SSC Mix ONLY.**

i. After adding 5 µL of diluted *C. coli* Control (PC2) to the SmartCycler tube containing the SSC Mix, pipet up and down 2 to 3 times in the upper part of the tube.

ii. Close the tube.

iii. Discard remaining volume of diluted Positive Control – DO NOT reuse.

Do not reuse diluted Positive Control.

f. **Add 5 µL of the Extraction Control (if included) nucleic acid to the SmartCycler tube containing the SSC Mix and then to the tube containing the STEC Mix.**

i. Add 5 µL of the Extraction Control nucleic acid to the specific tube containing the SSC Mix and pipet up and down 2 to 3 times in the upper part of the tube. Close the tube and discard the pipette tip.

ii. Add 5 µL of the Extraction Control nucleic acid to the specific tube containing the STEC Mix and pipet up and down 2 to 3 times in the upper part of the tube. Close the tube and discard the pipette tip.

g. **Add 5 µL of the Negative Control nucleic acid to the SmartCycler tube containing the SSC Mix and then to the tube containing the STEC Mix.**

i. Add 5 µL of the Negative Control nucleic acid to the specific tube containing the SSC Mix and pipet up and down 2 to 3 times in the upper part of the tube. Close the tube and discard the pipette tip.

ii. Add 5 µL of the Negative Control nucleic acid to the specific tube containing the STEC Mix and pipet up and down 2 to 3 times in the upper part of the tube. Close the tube and discard the pipette tip.

h. **Centrifuge all tubes**

i. Appropriately label the SmartCycler tubes on the caps.

ii. Centrifuge all tubes for 5 to 10 seconds using the Cepheid microcentrifuge specially adapted to fit the SmartCycler tubes.

iii. Return tubes to the cooling block.

Note

Keep the tubes on the cooling block before loading them into the SmartCycler instrument.

4. Run the ProGastro SSCS Assays on the SmartCycler II Instrument (Amplification/Detection Area)

Run the ProGastro SSC Mix Assay

a. Create a new run by clicking **Create Run** at the top of the screen. The *Create Run* screen is displayed.

b. Under **Run Name** in the left panel of the *Create Run* screen, enter a unique run identifier.

c. Click the **Assay** arrow in the left panel of the *Create Run* screen and select the **ProGastro SSC Mix** Assay protocol from the drop-down menu.

d. Under **Assay Information** in the left panel of the *Create Run* screen, enter the **Lot Number** and **Expiration Date** of the SSC Mix.

e. In the left panel of the *Create Run* screen, enter the number of specimens and Extraction Control (if included) and click **Apply**. The **Site Table** and the SmartCycler Dx Software automatically selects the **I-Core** sites for the number of specimens requested as well as the necessary controls (defined in the protocol).

f. In the **Site Table** under the **Sample ID** column, enter the Sample Identifier for the appropriate I-Core sites.

g. Insert each reaction tube into an I-Core site of the SmartCycler by pressing down firmly on all tubes. Close each lid. Verify that the SSCS Control (PC1), *C. coli* Control (PC2), and Negative Control (NC1) are loaded into the correct I-Core sites.

h. Select the **Start Run** button located at the bottom left corner of the screen. Verify that the LED is on for the appropriate I-Core sites.

Run the ProGastro STEC Mix Assay

a. Create a new run by clicking **Create Run** at the top of the screen. The *Create Run* screen is displayed.

b. Under **Run Name** in the left panel of the *Create Run* screen, enter a unique run identifier.

c. Click the **Assay** arrow in the left panel of the *Create Run* screen and select the **ProGastro STEC Mix** Assay protocol from the drop-down menu.

d. Under **Assay Information** in the left panel of the *Create Run* screen, enter the **Lot Number** and **Expiration Date** of the STEC Mix.

e. In the left panel of the *Create Run* screen, enter the number of specimens and Extraction Control (if included) and click **Apply**. The **Site Table** and the SmartCycler Dx Software automatically selects the **I-Core** sites for the number of specimens requested as well as the necessary controls (defined in the protocol).

f. In the **Site Table** under the **Sample ID** column, enter the Sample Identifier for the appropriate I-Core sites.

g. Insert each reaction tube into an I-Core site of the SmartCycler by pressing down firmly on all tubes. Close each lid. Verify that the PC1 (SSCS Control), and NC1 (Negative Control) are loaded into the correct I-Core sites.

h. Select the **Start Run** button located at the bottom left corner of the screen. Verify that the LED is on for the appropriate I-Core sites.

5. Print Reports

a. Click **Report** at bottom of screen to open the **Report Preview** screen.

b. Click on the **Print Icon** at the top of the screen.

Interpretation of Control Results

Validation of Run

Note

You must interpret the the Extraction Control (if included) results to determine whether the run is VALID; The SmartCycler Dx software automatically interprets the Positive and Negative Control results.

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

Sample ID[1]	Assay Result	IC Result	Warning / Error Code	Sample Type	IC Ct	SSC Mix and STEC Mix Result	Ct (positive in *all target* channels for each mix)	
							SSC Mix	**STEC Mix**
Extraction Control	Positive	NA	*	SPEC	NA**	POS	13-45 (*Shigella* = 13-37)	13-45
Neg Cntrl	Valid[2]	Pass		NC1	13-45	Valid	0	

[1] *Columns and data not used for interpretation are not included.*

[2] *(Typical) an Invalid assay will display Error Code 4098.*

* *Error Code 3079: Warning/Error Code 3079 is periodically observed with Campylobacter positives. Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for the sample are reported by the Dx software as ND (Not Determined). If this code is observed for the Extraction Control, Campy Ct values ≥ 13 are considered VALID.*

***Detection of the Internal Control in the Cy5 detection channel for the Extraction Control (if included) is not required for VALID result. The presence of multiple organisms can lead to reduced or absent Internal Control signal.*

For a VALID SSC Mix PCR run, the conditions in the table below must be met. If the run is valid, specimens should be interpreted using the next section *Interpretation of Specimen Results*.

Sample ID[1]	Assay Result	IC Result	Warning / Error Code	Sample Type	IC Ct	Campy Result	Campy Ct	*Salmonella* Result	*Salmonella* Ct	*Shigella* Result	*Shigella* Ct
SSCS Control	Valid[2]	NA	**	PC1	NA	Valid	20-45	Valid	20-45	Valid	20-37
C. coli Control	Valid[2]	NA	**	PC2	NA	Valid	20-45	Valid	0	Valid	0
Neg Control	Valid[2]	Pass		NC1	13-45	Valid	0	Valid	0	Valid	0

[1] *Columns and data not used for interpretation are not included.*

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

** *Error Code 3079: Warning/Error Code 3079 is periodically observed with FAM positives. Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). If Ct values between 20-45 (20-37 for Shigella) are reported in the all target detection channels (IC Ct is NA) for the SSCS Control and a Ct value is reported in ONLY the Campy Ct column for the C. coli Control with Ct values of 0 reported in the Salmonella and Shigella (IC Ct is NA) columns, results can be recorded as positive and the run considered VALID.*

For a VALID STEC Mix PCR run, the conditions in the table below must be met. If the run is valid, interpret specimens using the next section, *Interpretation of Specimen Results*.

Sample ID[1]	Assay Result	IC Result	Warning / Error Code	Sample Type	IC Ct	*stx2* Result	*stx2* Ct	*stx1* Result	*stx1* Ct
SSCS Control	Valid[2]	NA	**	PC1	NA	Valid	20-45	Valid	20-45
Neg Control	Valid[2]	Pass		NC1	13-45	Valid	0	Valid	0

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

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

*[**] Error Code 3079: Warning/Error Code 3079 is periodically observed with FAM positives. Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). If Ct value between 20-45 are reported in the "**stx2 Ct**" and "**stx1 Ct**" columns (IC Ct is NA) for the SSCS Control, results can be recorded as positive and the run considered VALID.*

Invalid Extraction Run

If the conditions for a Valid Extraction run are not met (i.e., the Extraction Control is not positive or the Negative Control is Invalid), repeat the entire extraction run. Start from original sample(s) using a new Extraction Control and a new Negative Control (starting at ***Step 1*** of the ***Assay Procedure***) and retest with both SSC and STEC Mixes.

Invalid PCR Run

If the Positive Control is not positive within the specified Ct ranges but the Negative Control is valid, prepare all new reactions using remaining purified nucleic acids and new Positive Controls (starting with PCR at ***Step 3*** of the ***Assay Procedure***) and retest using SSC and/or STEC Mixes as appropriate.

Interpretation of Specimen Results

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

SSC Mix							
Sample ID[1]	**Assay Result**	**IC Result**	**Warning / Error Code**	**Campy Result**	***Salmonella* Result**	***Shigella* Result**	**Interpretation of Results**
Sample ID	Negative	Pass		**NEG**	**NEG**	**NEG**	*Campylobacter jejuni* / *Campylobacter coli*, *Salmonella*, and *Shigella* nucleic acids **not detected**
Sample ID	Positive	NA*		**POS**	NEG	NEG	*Campylobacter jejuni* / *Campylobacter coli* nucleic acid **detected**
Sample ID	Positive	NA*		NEG	**POS**	NEG	*Salmonella spp.* nucleic acid **detected**.
Sample ID	Positive	NA*		NEG	NEG	**POS**	*Shigella spp.* nucleic acid **detected**
Sample ID	Positive	NA*		**POS**	**POS**	NEG	*C. jejuni* / *C. coli* and *Salmonella spp.* nucleic acid **detected.** Dual infections are not common, repeat test from the purified nucleic acid or collect and test a new sample.
Sample ID	Positive	NA*		**POS**	NEG	**POS**	*C. jejuni* / *C. coli* and *Shigella spp.* nucleic acid **detected.** Dual infections are not common, repeat test from the purified nucleic acid or collect and test a new sample.
Sample ID	Positive	NA*		NEG	**POS**	**POS**	*Salmonella spp.* and *Shigella spp.* nucleic acid **detected.** Dual infections are not common, repeat test from the purified nucleic acid or collect and test a new sample.
Sample ID	Positive	NA*		**POS**	**POS**	**POS**	*C. jejuni* / *C. coli*, *Salmonella spp.*, and *Shigella spp.* nucleic acid **detected.** Triple infections are rare, repeat test from the purified nucleic acid or collect and test a new sample.
Sample ID	Unresolved	Fail		**NEG**	**NEG**	**NEG**	Unresolved – PCR inhibition or reagent failure. Repeat test from the purified nucleic acid or collect and test a new sample.
Sample ID	ND	ND	3079[2]	ND	ND	ND	Not Determined – error code 3079
Sample ID	Invalid		4098[3]	ND	ND	ND	Not Determined – error code 4098

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

*[2] Error Code 3079: Warning/Error Code 3079 is periodically observed with Campy positives (Positive Control, Campy positive stool samples). Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). If a Ct value between 13-45 is reported in the **Campy and/or Salmonella** Ct columns, the sample results can be recorded as POS for the specific analyte(s). If a Ct value between 13-37 is reported in the **Shigella** Ct column, the sample results can be recorded as POS for the specific analyte.*

[3] 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 bacterial load can lead to reduced or absent Internal Control signal.*

STEC Mix						
Sample ID[1]	**Assay Result**	**IC Result**	**Warning / Error Code**	***stx2* Result**	***stx1* Result**	**Interpretation of Results**
Sample ID	Negative	Pass		**NEG**	**NEG**	*stx1* and *stx2* nucleic acids **not detected**
Sample ID	Positive	NA*		**POS**	NEG	*stx2* nucleic acid **detected**
Sample ID	Positive	NA*		NEG	**POS**	*stx1* nucleic acid **detected**
Sample ID	Positive	NA*		**POS**	**POS**	*stx1* and *stx2* nucleic acid **detected**.
Sample ID	Unresolved	Fail		**NEG**	**NEG**	Unresolved – PCR inhibition or reagent failure. Repeat test from the purified nucleic acid or collect and test a new sample.
Sample ID	ND	ND	3079[2]	ND	ND	Not Determined – error code 3079
Sample ID	Invalid		4098[3]	ND	ND	Not Determined – error code 4098

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

*[2] Error Code 3079: Warning/Error Code 3079 is periodically observed with stx2 positives (Positive Control, stx2 positive stool samples). Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). If a Ct value ≥ 13 is reported in the **stx2 or stx1 Ct** columns, the sample results can be recorded as POS for the specific analyte(s).*

[3] 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 bacterial load can lead to reduced or absent Internal Control signal.*

Not Determined Samples

If an assay result of **ND** (Not Determined) is reported with an instrument failure other than Warning/Error Code 3079, repeat testing from the purified nucleic acids (see ***Step 3*** of the ***Assay Procedure***). Refer to the Cepheid Dx Software Operator Manual for interpretation of Warning Codes.

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 to 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 493.1201.
- 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 all Positive Controls and the Gastro RNA/DNA 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 an Extraction Control with each nucleic acid isolation run. Treat the Extraction Control as a sample.
- Never run the Positive Controls through nucleic acid isolation.

- Always include one Negative Control *(containing Gastro RNA/DNA Internal Control)* and Positive Control(s) in each amplification/detection run performed.
- Failure of Controls (Positive, Negative, and/or Extraction (if included)) invalidates the run and results should not be reported.
- If a Positive Control(s) is not positive within the specified Ct range but the Negative Control is valid, repeat testing using the appropriate PCR Mix (SSC or STEC) starting from the purified nucleic acid and using a new aliquot of the Positive Control(s). If repeat results are still invalid, results should not be reported and testing should be repeated from the original sample or a new sample should be collected and tested.
- If the Extraction Control is not positive within the specified Ct range or the Negative Control is invalid, repeat testing using both the SSC and STEC Mixes should be done starting from the original sample using a new Extraction Control and a new Negative Control. If repeat results are still invalid, results should not be reported and a new sample collected and tested.

Limitations

- Have a trained health care professional interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- This test detects *ONLY C. jejuni* and *C. coli* and does not differentiate between these two species of *Campylobacter.* Additional testing is required to differentiate *C. jejuni* and *C. coli* and to detect other *Campylobacter* species that may be present in stool specimens.
- Positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness.
- Negative ProGastro SSCS Assay results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.
- Analyte targets (bacterial nucleic acid) may persist *in vivo*, independent of bacterial organism viability. Detection of analyte target(s) does not imply that the corresponding organisms are infectious or are the causative agents 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.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence of disease is low.
- There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
- *Campylobacter* is sensitive to environmental stressors including freezing where it loses viability. There is a risk of false negative *Campylobacter* values if samples have been stored at freezing temperature for prolonged period of time prior to testing.
- There is a risk of false negative values due to the presence of sequence variants in the gene targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
- False Negative results may occur due to the loss of nucleic acid. The Gastro RNA/DNA Internal Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR Amplification. The Gastro RNA/DNA Internal Control does not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens.
- The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the "Interference" section below can lead to erroneous results.

- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
- Cross-reactivity with gastrointestinal tract organisms other than those listed in the “Analytical Specificity” section below may lead to erroneous results.
- The performance of this test has not been established for monitoring treatment of *Salmonella*, *Shigella*, *C. jejuni* and *C. coli*, and STEC infections.
- The following *Salmonella* strains are not reactive with the ProGastro SSCS Assay: *Salmonella bongori, Salmonella enterica* subsp. *Salamae, Salmonella enterica* subsp. *Arizonae, Salmonella enterica* subsp. *Diarizonae, and Salmonella enterica* subsp. *houtenae*.
- Not all *Salmonella* serotypes were tested in analytical studies. Analytical reactivity with Salmonella *enterica* subsp. *enterica* serotype *Mississippi* has not been performed and reactivity of the ProGastro SSCS Assay with that strain is not known.
- Enteroinvasive *Escherichia coli* (EIEC) is genetically very similar to *Shigella*, and will be detected by the SSC Mix as positive for *Shigella.*
- *Shigella dysenteriae* strains typically contain a Shiga Toxin, but not always. A positive call of STEC *stx*1/*stx*2 by the STEC Mix may be from either Shiga Toxin Producing *E. coli* (STEC) or *Shigella dysenteriae.*
- In samples where *Campylobacter jejuni* is present in high concentrations and *Shigella* is present in low concentrations (near the assay limit of detection), competitive interference may occur hindering the detection of *Shigella* by the ProGastro SSCS Assay SSC Mix. In samples where *Salmonella* is present in high concentrations and *C. jejuni* or *C. coli* are present in low concentrations (near the assay limit of detection), competitive interference may occur hindering detection of these *Campylobacter* strains by the ProGastro SSCS Assay SSC Mix.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- The performance of the test has been evaluated for use with human specimen material only.
- The performance of this test has not been evaluated for sample types other than stool specimens.
- Stool samples from rectal swabs have not been evaluated with the ProGastro SSCS Assay.
- Raw stool samples (without Cary Blair or ParaPak C&S Transport Medium) are not compatible with the ProGastro SSCS Assay.
- The performance of this test has not been evaluated for immunocompromised individuals.
- The performance of this test has not been established for patients without symptoms of gastrointestinal infection.

Expected Values

In the prospective ProGastro SSCS Assay clinical study, a total of 1139 eligible prospective stool specimens were tested at four U.S. clinical laboratories in the United States. Samples were collected July 2011 thru November 2011 and May 2012 thru July 2012.

The number and percentage of *Campylobacter*, *Salmonella*, *Shigella*, and STEC positive cases as determined by the ProGastro SSCS Assay, calculated by age group and prospective sample collection time period, are presented in the following table:

Age Group	Total # SSC Mix/Total # STEC Mix	#Campy Positive/ Observed Prevalence	#*Salmonella* Positive/ Observed Prevalence	#*Shigella* Positive/ Observed Prevalence	#STEC Positive/ Observed Prevalence
< 2 years	240/238	5 (2.1%)	11 (4.6%)	2 (0.8%)	4 (1.7%)
2-5 years	149/148	8 (5.4%)	6 (4.0%)	11 (7.4%)	2 (1.4%)
6-11 years	128/130	2 (1.6%)	8 (6.3%)	6 (4.7%)	1 (0.8%)
12-18 years	157/158	1 (0.6%)	4 (2.5%)	2 (1.3%)	5 (3.2%)
19-64 years	306/306	15 (4.9%)	1 (0.3%)	0 (0.0%)	3 (1.0%)
≥ 65 years	158/158	2 (1.3%)	0 (0.0%)	0 (0.0%)	3 (1.9%)
Total	**1138/1138***	**33 (2.9%)**	**30 (2.6%)**	**21 (1.8%)**	**18 (1.6%)**

*one sample did not have age information and therefore not included in the expected values; however, 1139 valid samples were tested during the prospective clinical study (See Demographic details below).

Performance Characteristics

Clinical Performance

Prospective Study

The clinical performance of the ProGastro SSCS Assay was established during prospective studies at four U.S. clinical laboratories. Leftover stool samples were collected during July 2011 – November 2011 and May 2012 – July 2012 and tested during November 2011 thru August 2012. All specimens used in the study meeting the inclusion criteria represented excess remnants of stool specimens that were prospectively collected from symptomatic individuals suspected of gastrointestinal infection, and were submitted for routine care or analysis by each site, and that otherwise would have been discarded.

Demographic details for the patient population included in the prospective study are summarized in the following table.

Sex	Number of Samples SSC Mix	Number of Samples STEC Mix
Male	615/1214 (50.6%)	615/1214 (50.6%)
Female	581/1214 (47.9%)	581/1214 (47.9%)
Unknown	18/1214 (1.5%)	18/1214 (1.5%)
Age (yrs)		
≤ 5 years	378/1214 (31.1%)	378/1214 (31.1%)
6 - 18 years	296/1214 (24.4%)	296/1214 (24.4%)
19 – 64 years	357/1214 (29.4%)	357/1214 (29.4%)
≥ 65 years	164/1214 (13.5%)	164/1214 (13.5%)
Unknown	19/1214 (1.6%)	19/1214 (1.6%)

Performance of the ProGastro SSCS Assay was assessed and compared to the reference method of culture (*Campylobacter*, *Salmonella*, and *Shigella*) or broth enrichment followed by FDA cleared EIA test (Shiga Toxin producing *E. coli*). Samples positive for STEC by broth/EIA and/or the ProGastro SSCS Assay underwent PCR followed by bi-directional sequencing to confirm the presence of the *stx1* and/or *stx2* genes. Two PCR/sequencing assays were used that each targeted different regions of the *stx1* or *stx2* gene than the ProGastro SSCS Assay. "True" STEC positives were considered as any sample that tested positive for STEC by the broth/EIA method, and "True" STEC negatives were considered as any sample that tested negative for STEC by the broth/EIA method. "True" *stx1* or "true" *stx2* positives were considered as any sample that tested positive for STEC by the broth/EIA method and by PCR/sequencing. Bi-directional sequencing data was required to meet pre-defined quality acceptance criteria for both the forward and the reverse sequences that matched *stx1* or *stx2* sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values. The E-Value from NCBI BLAST Alignment indicates the statistical significance of a given pair-wise alignment and reflects the size of the database and the scoring system used. The lower the E-Value, the more significant the hit is. A sequence alignment that has an E-Value of 1e-3 means that this similarity has a 1 in 1000 chance of occurring by chance alone. (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=handbook.section.614).

Discrepant results between the ProGastro SSCS Assay and the reference methods were also evaluated using analytically validated PCR/sequencing assays and results are footnoted in the performance tables below.

A total of 1214 patients were initially enrolled in the prospective clinical trial. Prospective stool specimens were initially included in the prospective clinical trial. Sixty-one (61) patient/specimens were excluded from the performance calculations due to deviations from the clinical study protocol. Fourteen (14) specimens were excluded for the SSC Mix and 14 were excluded for the STEC Mix from the prospective clinical study data analysis because they remained "Unresolved" after repeat testing with the respective ProGastro SSCS Assay Mix. Unresolved results occur when the sample is negative for all target detections and the Internal Control, indicating potentially PCR-inhibited samples. This resulted in a total of 1139 eligible prospective specimens to be included in the prospective clinical study data analysis.

Campylobacter (C. jejuni / C. coli) Comparison Results

		Culture			
		Positive	**Negative**	**Total**	
ProGastro SSCS Assay	**Positive**	20	13[a]	**33**	**Sensitivity 100.0%** (83.9% - 100.0%) 95% CI
	Negative	0	1106	**1106**	**Specificity 98.8%** (98.0% - 99.3%) 95% CI
	Total	**20**	**1119**	**1139**	

[a] Six (6) samples were positive for *Campylobacter* (*C. coli* or *C. jejuni*) by bi-directional sequence analysis.

Salmonella Comparison Results

		Culture			
		Positive	**Negative**	**Total**	
ProGastro SSCS Assay	**Positive**	20	10[a]	**30**	**Sensitivity 95.2%** (77.3% - 99.2%) 95% CI
	Negative	1[b]	1108	**1109**	**Specificity 99.1%** (98.4% - 99.5%) 95% CI
	Total	**21**	**1118**	**1139**	

[a] Ten (10) samples were positive for *Salmonella* by bi-directional sequence analysis.
[b] One (1) sample was negative for *Salmonella* by bi-directional sequence analysis.

Shigella Comparison Results

		Culture			
		Positive	**Negative**	**Total**	
ProGastro SSCS Assay	**Positive**	15	6[a]	**21**	**Sensitivity 100.0%** (79.6% - 100.0%) 95% CI
	Negative	0	1118	**1118**	**Specificity 99.5%** (98.8% - 99.8%) 95% CI
	Total	**15**	**1124**	**1139**	

[a] Six (6) samples were positive for *Shigella* by bi-directional sequence analysis.

STEC Comparison Results

		Broth Enrichment/EIA			
		Positive	**Negative**	**Total**	
ProGastro SSCS Assay	**Positive**	9[a]	9[b]	**18**	**Sensitivity** 100.0% (70.1% - 100.0%) 95% CI
	Negative	0	1121	**1121**	**Specificity** 99.2% (98.5% - 99.6%) 95% CI
	Total	**9**	**1130**	**1139**	

[a] Six (6) samples positive for *stx1*, one (1) sample positive for *stx2*, and two (2) samples positive for *stx1* and *stx2* by bi-directional sequence analysis.
[b] Five (5) samples positive for *stx1* and three (3) samples positive for *stx2* by bi-directional sequence analysis.

stx1 Comparison Results

		Broth Enrichment/EIA and sequencing for stx1			
		Positive	**Negative**	**Total**	
ProGastro SSCS Assay	**Positive**	8	6[a]	**14**	**Positive Percent Agreement 100.0%** (67.6% - 100.0%) 95% CI
	Negative	0	4	**4**	**Negative Percent Agreement 40.0%** (16.8% - 68.7%) 95% CI
	Total	**8**	**10**	**18**	

[a] Five (5) samples were positive for *stx1* by bi-directional sequence analysis, but were negative by Broth Enrichment/EIA.

stx2 Comparison Results

		Broth Enrichment/EIA and sequencing for stx2			
		Positive	**Negative**	**Total**	
ProGastro SSCS Assay	**Positive**	3	3[a]	**6**	**Positive Percent Agreement 100.0%** (43.9% - 100.0%) 95% CI
	Negative	0	12	**12**	**Negative Percent Agreement 80.0%** (54.8% - 93.0%) 95% CI
	Total	**3**	**15**	**18**	

[a] Three (3) samples were positive for *stx2* by bi-directional sequence analysis, but were negative by Broth Enrichment/EIA.

The ProGastro SSCS Assay detected one mixed infections in the prospective clinical evaluation. This represents 0.98% of the total positive specimens (1/102).The one mixed infection sample was a dual positive and was confirmed by the reference methods.

Distinct Co-infection Combinations Detected by the ProGastro SSCS Assay in the Prospective Clinical Trial

Distinct Co-infection Combinations Detected by ProGastro SSCS Assay			Total Co-infections	Number of Discrepant Co-infections[a]
Analyte 1	**Analyte 2**	**Analyte 3**		
Salmonella	*Campylobacter*	N/A	0	0
Salmonella	*Shigella*	N/A	0	0
Salmonella	STEC	N/A	0	0
Campylobacter	*Shigella*	N/A	0	0
Campylobacter	STEC	N/A	1	0
STEC	*Shigella*	N/A	0	0
Salmonella	*Campylobacter*	STEC	0	0
Total Co-infections			1	0
Total Double Infections			1	0
Total Triple Infections			0	0

[a] A discrepant co-infection was defined as one that was detected by the ProGastro SSCS Assay but not detected by the reference methods.

There were no co-infections that were detected by the reference method and not detected by the ProGastro SSCS Assay.

Retrospective Study

In addition to the prospective clinical study, two clinical sites also performed testing using retrospective samples that were collected from 2007 - 2011. A total of 105 stool samples were included in the retrospective study. These samples had been previously determined to be positive or negative by culture and/or Broth Enrichment/EIA. The ProGastro SSCS Assay was compared to the same reference method that was employed for the prospective study to determine positive and negative percent agreement.

Demographic details for this patient population are summarized in the table below.

Sex*	Number of Subjects
Female	24/55 (43.6%)
Male	31/55 (56.4%)
Age	**Number of Subjects**
≤ 5 years	12/105 (11.4%)
6 - 18 years	24/105 (22.9%)
19 – 64 years	51/105 (48.6%)
≥ 65 years	18/105 (17.1%)

*For all of the 50 specimens tested from one site the gender was unknown

Campylobacter Comparison Results

		Culture			
		Positive	Negative	Total	
ProGastro SSCS Assay	Positive	27	5	**32**	**Positive Percent Agreement** **96.4%** (82.3% - 99.4%) 95% CI
	Negative	1	72	**73**	**Negative Percent Agreement** **93.5%** (85.7% - 97.2%) 95% CI
	Total	**28**	**77**	**105**	

Salmonella Comparison Results

		Culture			
		Positive	Negative	Total	
ProGastro SSCS Assay	Positive	3	0	**3**	**Positive Percent Agreement** **100.0%** (43.9% - 100.0%) 95% CI
	Negative	0	102	**102**	**Negative Percent Agreement** **100.0%** (96.4% - 100.0%) 95% CI
	Total	**3**	**102**	**105**	

Shigella Comparison Results

		Culture			
		Positive	Negative	Total	
ProGastro SSCS Assay	Positive	4	0	**4**	**Positive Percent Agreement** **100.0%** (51.0% - 100.0%) 95% CI
	Negative	0	101	**101**	**Negative Percent Agreement** **100.0%** (96.3% - 100.0%) 95% CI
	Total	**4**	**101**	**105**	

STEC Comparison Results

		Culture or Broth Enrichment/EIA			
		Positive	Negative	Total	
ProGastro SSCS Assay	Positive	19[a]	0	**19**	**Positive Percent Agreement** **100.0%** (83.2% - 100.0%) 95% CI
	Negative	0	86	**86**	**Negative Percent Agreement** **100.0%** (95.7% - 100.0%) 95% CI
	Total	**19**	**86**	**105**	

[a] Five (5) samples positive for *stx1*, five (5) samples positive for *stx2*, and nine (9) samples positive for *stx1* and *stx2*.

stx1 Comparison Results

		Culture or Broth Enrichment/EIA and sequencing for stx1			
		Positive	**Negative**	**Total**	
ProGastro SSCS Assay	**Positive**	14	0	**14**	**Positive Percent Agreement** **100.0%** (78.5% - 100.0%) 95% CI
	Negative	0	5	**5**	**Negative Percent Agreement** **100.0%** (56.6% - 100.0%) 95% CI
	Total	**14**	**5**	**19**	

stx2 Comparison Results

		Culture or Broth Enrichment/EIA and sequencing for stx2			
		Positive	**Negative**	**Total**	
ProGastro SSCS Assay	**Positive**	14	0	**14**	**Positive Percent Agreement** **100.0%** (78.5% - 100.0%) 95% CI
	Negative	0	5	**5**	**Negative Percent Agreement** **100.0%** (56.6% - 100.0%) 95% CI
	Total	**14**	**5**	**19**	

Of the prospective and retrospective specimens run using the SSC Mix Assay, 98.0% (1233/1258) of these specimens were successful on the first attempt. The remaining 25 (25/1258 = 2.0%) gave “Unresolved” results on the first attempt. An “Unresolved” result is generated when the Gastro RNA/DNA Internal Control (GIC) fails to be detected in a clinical specimen. A failure of the GIC to be detected can occur if inhibitors are present in a sample or due to technical error (e.g., GIC not added prior to nucleic acid extraction). Of the 25 “Unresolved” specimens on the first attempt with sufficient nucleic acid for retest, 44.0% (11/25) gave a valid result on the second attempt. The remaining 14 were “Unresolved” on the second attempt.

Of the prospective and retrospective specimens run using the STEC Mix Assay, 97.9% (1232/1258) of these specimens were successful on the first attempt. The remaining 26 (26/1258 = 2.1%) gave “Unresolved” results on the first attempt. Of the 26 “Unresolved” specimens on the first attempt with sufficient nucleic acid for retest, 48.0% (12/25) gave a valid result on the second attempt. The remaining 14 were “Unresolved” on the second attempt.

Reproducibility

The reproducibility of the ProGastro SSCS Assay was evaluated at three laboratory sites. Reproducibility was assessed using a panel of 15 simulated samples that included medium positive, low positive (near the assay limit of detection, ≥ 95% positive), and high negative (below the assay limit of detection, ≤ 95% positive) samples for each of the assay targets. The reproducibility panel and controls were run with the ProGastro SSCS Assay (SSC and STEC Mixes) at three sites by each of two operators per site for five days.

Reproducibility Panel Member Results

		SSC Mix								STEC Mix				SSC Mix	STEC Mix
	Panel Member ID	***C. jejuni* Low Positive**	***C. jejuni* Medium Positive**	***C. coli* Low Positive**	***C. coli* Medium Positive**	***Salmonella* Low Positive**	***Salmonella* Medium Positive**	***Shigella* Low Positive**	***Shigella* Medium Positive**	**STEC (*stx 1*) Low Positive**	**STEC (*stx 1*) Medium Positive**	**STEC (*stx 2*) Low Positive**	**STEC (*stx 2*) Medium Positive**	**High Negative (IC Ct Value)**	**High Negative (IC Ct Value)**
	Concentration	20X* LoD	100X* LoD	6X* LoD	30X* LoD	2X LoD	10X LoD	2X LoD	10X LoD	2X LoD	10X LoD	2X LoD	10X LoD	0.0001X LoD	
Site 1	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	90/90 100%	90/90 100%
	Mean Ct Value	37.6	35.2	35.9	33.5	35.9	33.2	35.8	33.2	36.1	33. 4	36.7	34. 5	33.6	33.2
	% CV	3.5	3.0	3.9	3.7	1.4	1.2	1.7	1.4	2.0	1.6	1.8	1.3	3.0	1.7
Site 2	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	29/30 96.7%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	90/90 100%	90/90 100%
	Mean Ct Value	37.3	34.8	35.8	33.4	36.0	33.1	35.6	33.2	36.2	33.6	36.8	34.7	33.3	33.0
	% CV	3.0	2.8	3.1	3.2	1.7	1.6	1.9	1.8	2.5	1.6	1.9	1.9	0.9	0.9
Site 3	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	90/90 100%	90/90 100%
	Mean Ct Value	37.0	34.4	35.2	32.9	35.7	32.7	35.0	32.8	35.6	33.1	36.5	34.2	32.9	32.6
	% CV	2.6	2.2	2.1	1.9	1.4	1.2	1.8	1.3	1.7	1.3	1.6	1.0	0.8	1.1
	Total Agreement with Expected Result	90/90 100%	90/90 100%	90/90 100%	90/90 100%	90/90 100%	90/90 100%	89/90 89.9%	90/90 100%	90/90 100%	90/90 100%	90/90 100%	90/90 100%	270/270 100%	270/270 100%
	95% CI	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	94.0%-99.8%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	98.6%-100.0%	98.6%-100.0%
	Overall Mean Ct Value	37.3	34.8	35.6	33.3	35.9	33.0	35.5	33.1	35.9	33.4	36.7	34.5	33.2	32.9
	Overall % CV	3.1	2.8	3.2	3.1	1.6	1.5	2.0	1.6	2.2	1.6	1.8	1.5	2.1	1.5

* Note: The *Campylobacter* strains were tested at higher concentrations because *Campylobacter* is sensitive to environmental stressors including freezing where it loses viability.[17-19] However, the average Ct value for the *C. jejuni* (ATCC 33291) Low Positives was 37.3, which is very close to the average Ct value of 38.8 for the same *C. jejuni* strain tested at the estimated LoD level in the Analytical Reactivity Study. The average Ct value for the *C. coli* (ATCC BAA-371) Low Positives was 35.6, which is very close to the average Ct value of 34.4 for the same *C. coli* strain tested at the estimated LoD level in the Analytical Reactivity Study. Therefore, the effective DNA concentrations of the *C. jejuni* (ATCC 33291) and *C. coli* (ATCC BAA-371) low positive samples tested in the Reproducibility Study are very close to the estimated LoD DNA concentrations for these two strains tested in the Analytical Reactivity Study.

Precision

The precision of the ProGastro SSCS Assay was evaluated internally using a panel of 15 simulated samples that included medium positive, low positive (near the assay limit of detection, ≥ 95% positive), and high negative (below the assay limit of detection, ≤ 95% positive) samples for each of the assay targets. A panel of 15 contrived samples including the necessary controls was run with the ProGastro SSCS Assay (SSC and STEC Mixes) by each of two operators for twelve days.

Precision Panel Member Results

	SSC Mix								STEC Mix				SSC Mix	STEC Mix
Panel Member ID	***C. jejuni* Low Positive**	***C. jejuni* Medium Positive**	***C. coli* Low Positive**	***C. coli* Medium Positive**	***Salmonella* Low Positive**	***Salmonella* Medium Positive**	***Shigella* Low Positive**	***Shigella* Medium Positive**	**STEC (*stx 1*) Low Positive**	**STEC (*stx 1*) Medium Positive**	**STEC (*stx 2*) Low Positive**	**STEC (*stx 2*) Medium Positive**	**High Negative (IC Ct Value)**	**High Negative (IC Ct Value)**
Concentration	20X* LoD	100X* LoD	6X* LoD	30X* LoD	2X LoD	10X LoD	2X LoD	10X LoD	2X LoD	10X LoD	2X LoD	10X LoD	0.0001X LoD	
Total Agreement with Expected Result	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	71/72 98.6%	72/72 100%
Overall Mean Ct Value	36.7	34.2	35.0	32.7	35.6	32.7	35.1	32.7	35.6	33.0	36.5	34.2	32.9	32.5
Overall % CV	2.6	2.4	2.1	2.3	1.5	1.1	1.6	1.3	1.8	1.3	1.8	1.2	0.9	1.0

* Note: The *Campylobacter* strains were tested at higher concentrations because *Campylobacter* is sensitive to environmental stressors including freezing where it loses viability.[17-19] However, the average Ct value for the *C. jejuni* (ATCC 33291) Low Positives was 37.3, which is very close to the average Ct value of 38.8 for the same *C. jejuni* strain tested at the estimated LoD level in the Analytical Reactivity Study. The average Ct value for the *C. coli* (ATCC BAA-371) Low Positives was 35.6, which is very close to the average Ct value of 34.4 for the same *C. coli* strain tested at the estimated LoD level in the Analytical Reactivity Study. Therefore, the effective DNA concentrations of the *C. jejuni* (ATCC 33291) and *C. coli* (ATCC BAA-371) low positive samples tested in the Precision Study are very close to the estimated LoD DNA concentrations for these two strains tested in the Analytical Reactivity Study.

Analytical Sensitivity

The Analytical Sensitivity (LoD) of the ProGastro SSCS Assay was determined and confirmed using fresh bacterial cultures for each detection target (*Salmonella, Shigella, Campylobacter* (*C. jejuni* and *C. coli*), and Shiga Toxin producing *E. coli*, STEC) in conjunction with extraction on the bioMérieux NucliSENS easyMAG system and Real Time PCR performed using the Cepheid SmartCycler II Real Time instrument.

Analytical sensitivity (LoD), defined as the lowest concentration at which ≥ 95% of all replicates tested positive, ranged from $2.47x10^4$ CFU/mL (11.21 CFU/reaction) to $3.11x10^3$ CFU/mL (0.17 CFU/reaction).

Strain	Calculation of LoD concentration	Calculation of LoD concentration
Salmonella enterica subsp. *enterica* ser. Typhi	$1.63x10^3$ CFU/mL	0.74 CFU/reaction
Salmonella enterica subsp. *enterica* ser. Typhimurium	$2.25x10^4$ CFU/mL	10.21 CFU/reaction
Salmonella enterica subsp. *enterica* ser. Enteritidis	$2.47x10^4$ CFU/mL	11.21 CFU/reaction
Shigella boydii	$6.60x10^2$ CFU/mL	0.30 CFU/reaction
Shigella dysenteriae	$1.03x10^3$ CFU/mL	0.47 CFU/reaction
Shigella flexneri	$3.11x10^3$ CFU/mL	1.42 CFU/reaction
Shigella sonnei	$1.46x10^3$ CFU/mL	0.66 CFU/reaction
Campylobacter jejuni	$1.36x10^3$ CFU/mL	0.62 CFU/reaction
Campylobacter coli	$1.99x10^3$ CFU/mL	0.91 CFU/reaction
STEC O26:H/NM (stx1-/stx2+)	$9.27x10^3$ CFU/mL	4.21 CFU/reaction
STEC O157:H7 (stx1+/stx2-)	$1.66x10^4$ CFU/mL	7.55 CFU/reaction

Reactivity

The reactivity of the ProGastro SSCS Assay was evaluated with multiple strains of bacteria listed in the table below. *Salmonella bongori, Salmonella enterica* subsp. *Salamae, Salmonella enterica* subsp. *Arizonae, Salmonella enterica* subsp. *Diarizonae, and Salmonella enterica* subsp. *houtenae* were not reactive with the ProGastro SSCS Assay.

Strain	Target	Concentration Tested	SSC Mix			STEC Mix	
			Campy Detection	*Salmonella* Detection	*Shigella* Detection	*stx2* Detection	*stx1* Detection
Salmonella bongori 43975	*Salmonella*	$9.25x10^8$ CFU/mL	-	-	-	-	-
Salmonella enterica subsp. *enterica* ser. Paratyphi 8759	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium 19585	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium 14028	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium BAA-189	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium BAA-191	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium BAA-215	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Enteritidis 13076	*Salmonella*	$2x10^5$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Enteritidis BAA-708	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-

Strain	Target	Concentration Tested	SSC Mix			STEC Mix	
			Campy Detection	*Salmonella* Detection	*Shigella* Detection	*stx2* Detection	*stx1* Detection
Salmonella enterica subsp. *enterica* ser. Enteritidis 4931	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Essen 6961	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Newport 6962	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Newport 27869	*Salmonella*	$2x10^3$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Heidelberg 8326	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Javiana BAA-1593	*Salmonella*	$2x10^6$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *enterica* ser. Montevideo BAA-710	*Salmonella*	$2x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Oranienburg 9239	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Saintpaul 9712	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Muenchen BAA-1674	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Braenderup BAA-664	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Infantis BAA-1675	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Thompson 8391	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Agona BAA-707	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Bareilly (clinical isolate)	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *Enterica* ser. Hadar 51956	*Salmonella*	$1x10^4$ CFU/mL	-	+	-	-	-
Salmonella enterica subsp. *indica* (genomic DNA) BAA-1578D-5	*Salmonella*	20 fg/µL	-	+	-	-	-
Salmonella enterica subsp. *salamae strain* BAA-1582	*Salmonella*	$1x10^4$ CFU/mL	-	-	-	-	-
Salmonella enterica subsp. *arizonae*	*Salmonella*	$1x10^4$ CFU/mL	-	-	-	-	-
Salmonella enterica subsp. *diarizonae*	*Salmonella*	$1x10^4$ CFU/mL	-	-	-	-	-
Salmonella enterica subsp. *houtenae*	*Salmonella*	$1x10^4$ CFU/mL	-	-	-	-	-
Shigella boydii 25930	*Shigella*	$2x10^3$ CFU/mL	-	-	+	-	-
Shigella dysenteriae 29026†	*Shigella*	$2x10^3$ CFU/mL	-	-	+	-	+
Shigella flexneri 12022	*Shigella*	$2x10^4$ CFU/mL	-	-	+	-	-
Shigella flexneri 25875	*Shigella*	$2x10^4$ CFU/mL	-	-	+	-	-
Shigella flexneri 700930	*Shigella*	$2x10^4$ CFU/mL	-	-	+	-	-
Shigella sonnei 29031	*Shigella*	$2x10^4$ CFU/mL	-	-	+	-	-
Shigella sonnei 9290	*Shigella*	$2x10^4$ CFU/mL	-	-	+	-	-
Shigella sonnei 11060	*Shigella*	$2x10^4$ CFU/mL	-	-	+	-	-
Shigella sonnei 25931	*Shigella*	$2x10^3$ CFU/mL	-	-	+	-	-
Shigella sonnei 29030	*Shigella*	$2x10^4$ CFU/mL	-	-	+	-	-
Shigella sonnei 29930	*Shigella*	$2x10^4$ CFU/mL	-	-	+	-	-
Campylobacter jejuni subsp. *jejuni* 29428	*Campylobacter*	$2x10^3$ CFU/mL	+	-	-	-	-
Campylobacter jejuni subsp.	*Campylobacter*	$2x10^3$ CFU/mL	+	-	-	-	-

Strain	Target	Concentration Tested	SSC Mix			STEC Mix	
			Campy Detection	*Salmonella* Detection	*Shigella* Detection	*stx2* Detection	*stx1* Detection
jejuni 33291							
Campylobacter jejuni subsp. *jejuni* BAA-222	*Campylobacter*	$2x10^3$ CFU/mL	+	-	-	-	-
Campylobacter jejuni subsp. *jejuni* BAA-223	*Campylobacter*	$2x10^3$ CFU/mL	+	-	-	-	-
Campylobacter jejuni subsp. *jejuni* BAA-219	*Campylobacter*	$2x10^3$ CFU/mL	+	-	-	-	-
Campylobacter jejuni subsp. *jejuni* BAA-220	*Campylobacter*	$2x10^7$ CFU/mL	+	-	-	-	-
Campylobacter jejuni subsp. *doylei* BAA-1458	*Campylobacter*	$2x10^5$ CFU/mL	+	-	-	-	-
Campylobacter coli BAA-370	*Campylobacter*	$2x10^4$ CFU/mL	+	-	-	-	-
Campylobacter coli BAA-371	*Campylobacter*	$2x10^4$ CFU/mL	+	-	-	-	-
Campylobacter coli BAA-372	*Campylobacter*	$2x10^5$ CFU/mL	+	-	-	-	-
Campylobacter coli 33559	*Campylobacter*	$2x10^5$ CFU/mL	+	-	-	-	-
*Campylobacter lari**	*Campylobacter*	1:10 dilution of Clinical Specimen*	-	-	-	-	-
E. coli DEC10B (O26:H11)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	+
E. coli 97-3250 (O26:H11)	*stx1 & stx2*	$2x10^4$ CFU/mL	-	-	-	+	+
E. coli DA-21 (O45:H/NM)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	+
E. coli MI03-19 (O45:H2)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	+
E. coli MT#80 (O103:H2)	*stx1*	$1x10^5$ CFU/mL	-	-	-	-	+
E. coli 3215-99 (O111:H8)	*stx1 & stx2*	$2x10^4$ CFU/mL	-	-	-	+	+
E. coli RD8 (O111:H2)	*stx2*	$2x10^4$ CFU/mL	-	-	-	+	-
E. coli 0201 9611 (O111:H11)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	+
E. coli DA-5 (O121:H19)	*stx2*	$2x10^4$ CFU/mL	-	-	-	+	-
E. coli DA-1 (O121:H/NM)	*stx2*	$2x10^4$ CFU/mL	-	-	-	+	-
E. coli GS G5578620 (O145:H/NM)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	+
E. coli IH 16 (O145:H/NT)	*stx2*	$2x10^4$ CFU/mL	-	-	-	+	-
E. coli 7:85 (O157:H/N)	*stx1 & stx2*	$2x10^4$ CFU/mL	-	-	-	+	+
E. coli 93-111 (O157:H7)	*stx1 & stx2*	$2x10^4$ CFU/mL	-	-	-	+	+
E. coli DA-34 (O157:H/NM)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	+
E. coli EDL933 (O157:H7)	*stx1 & stx2*	$2x10^4$ CFU/mL	-	-	-	+	+
E. coli 1:361 (O157:H7)	*stx2*	$2x10^4$ CFU/mL	-	-	-	+	-
E. coli DA-54 (O157:H/NM)	*stx2*	$2x10^4$ CFU/mL	-	-	-	+	-
E. coli O104:H4 Genomic DNA	*stx2*	0.5pg/µL (2.5pg/rxn)	-	-	-	+	-
E. coli O118:H16, EK36 TW08644	*stx1*	$1x10^4$ CFU/mL	-	-	-	-	+
E. coli O118:H-, RW2030 TW06407	*stx1*	$1x10^4$ CFU/mL	-	-	-	-	+
E. coli O118:H16, RW1191 TW07879	*stx1 & stx2*	$1x10^4$ CFU/mL	-	-	-	+	+

† *Shigella dysenteriae* strains typically contain a Shiga Toxin, therefore this strain tested positive with both the STEC and SSC Mixes.

* Clinical specimen received and tested was stool preserved in stool preservation and transport medium and processed per the ProGastro SSCS Assay Instructions for Use. Sample was culture positive for *Campylobacter* and was confirmed as *C. lari* by 16S genetic sequencing.

Analytical Specificity

The Analytical Specificity of the ProGastro SSCS Assay was determined using a panel of cultured and titered strains of common gastrointestinal pathogens or microorganisms including 54 bacterial, viral, parasitic, and yeast strains (43 bacteria, six viruses, four parasites, and one yeast). All the target organisms (*Salmonella, Shigella, Campylobacter jejuni, Campylobacter coli,* and STEC) were tested at concentrations of 10^6 – 10^7 CFU/mL. The bacterial strains were tested at concentrations of 10^6 – $8.8x10^8$ CFU/mL, the viruses were tested at $10^{3.5}$ – $10^{7.5}$ $TCID_{50}$/mL, the parasites were unable to be quantified and were tested as received, and the one fungus was tested at 10^7 CFU/mL. Samples were extracted and tested in triplicate. The ProGastro SSCS Assay did not react with any of the non-target organisms listed other than *Escherichia coli* (enteroinvasive) – EIEC. EIEC is genetically very similar to *Shigella*, and as expected it was detected by the SSC Mix as positive for *Shigella*.

Organism	Concentration tested	*Campy* Detection	*Salmonella* Detection	*Shigella* Detection	*Stx 1* Detection	*Stx 2* Detection
Salmonella enterica subsp. *enterica* ser. Enteritidis	$1x10^6$ CFU/mL	-	+	-	-	-
Campylobacter jejuni	$1x10^6$ CFU/mL	+	-	-	-	-
Campylobacter coli	$1x10^6$ CFU/mL	+	-	-	-	-
Shigella sonnei	$1x10^6$ CFU/mL	-	-	+	-	-
STEC O157:H7 Strain 93-111	$1x10^7$ CFU/mL	-	-	-	+	+
Aeromonas hydrophila	$1.5x10^7$ CFU/mL	-	-	-	-	-
Bacillus cereus	$1.5x10^7$ CFU/mL	-	-	-	-	-
Bacteroides fragilis	$1.5x10^7$ CFU/mL	-	-	-	-	-
Campylobacter lari	at use*	-	-	-	-	-
Campylobacter upsaliensis	$6.4x10^7$ CFU/mL	-	-	-	-	-
Campylobacter hyointestinalis	$7.44x10^8$ CFU/mL	-	-	-	-	-
Campylobacter fetus	$5.4x10^7$ CFU/mL	-	-	-	-	-
Campylobacter helveticus	$7.0x10^7$ CFU/mL	-	-	-	-	-
Campylobacter gracilis	$2.4x10^7$ CFU/mL	-	-	-	-	-
Campylobacter concisus	$1.0x10^6$ CFU/mL	-	-	-	-	-
Campylobacter curvus	$4.5x10^6$ CFU/mL	-	-	-	-	-
Campylobacter sputorum	$3.55x10^7$ CFU/mL	-	-	-	-	-
Campylobacter rectus	$2.0x10^7$ CFU/mL	-	-	-	-	-
Campylobacter showae	$4.3x10^6$ CFU/mL	-	-	-	-	-
Campylobacter mucosalis	$4.2x10^6$ CFU/mL	-	-	-	-	-
Citrobacter freundii	$4.8x10^8$ CFU/mL	-	-	-	-	-
Clostridium difficile Toxigenic Layola-02 Nap1	$1.5x10^7$ CFU/mL	-	-	-	-	-
Clostridium perfringens	$1.5x10^7$ CFU/mL	-	-	-	-	-
Enterobacter cloacae	$1.5x10^7$ CFU/mL	-	-	-	-	-
Enterococcus faecalis	$1.5x10^7$ CFU/mL	-	-	-	-	-
Escherichia coli (non-STEC)	$1.5x10^7$ CFU/mL	-	-	-	-	-
Escherichia coli (enteroinvasive)	$2.2x10^8$ CFU/mL	-	-	+	-	-
Escherichia fergusonii	$2.0x10^8$ CFU/mL	-	-	-	-	-
Escherichia hermannii	$8.8x10^8$ CFU/mL	-	-	-	-	-
Helicobacter pylori	$5.6x10^7$ CFU/mL	-	-	-	-	-
Klebsiella pneumoniae	$1.5x10^7$ CFU/mL	-	-	-	-	-
Lactococcus lactis	$1.14x10^8$ CFU/mL	-	-	-	-	-
Listeria monocytogenes	$4.2x10^6$ CFU/mL	-	-	-	-	-
Peptostreptococcus anaerobius	$3.2x10^7$ CFU/mL	-	-	-	-	-
Plesiomonas shigelloides	$1.80x10^8$ CFU/mL	-	-	-	-	-
Proteus vulgaris	$1.5x10^7$ CFU/mL	-	-	-	-	-
Pseudomonas aeruginosa	$1.5x10^7$ CFU/mL	-	-	-	-	-
Pseudomonas fluorescens	$5.6x10^8$ CFU/mL	-	-	-	-	-
Serratia marcescens	$8.6x10^8$ CFU/mL	-	-	-	-	-
Staphylococcus aureus	$1.5x10^7$ CFU/mL	-	-	-	-	-
Staphylococcus epidermidis	$1.5x10^7$ CFU/mL	-	-	-	-	-
Vibrio parahaemolyticus	$1.5x10^7$ CFU/mL	-	-	-	-	-
Yersinia enterocolitica	$3.3x10^7$ CFU/mL	-	-	-	-	-
Adenovirus Type 40	$1.0x10^{5.5}$ $TCID_{50}$/mL	-	-	-	-	-

Organism	Concentration tested	*Campy* Detection	*Salmonella* Detection	*Shigella* Detection	*Stx 1* Detection	*Stx 2* Detection
Adenovirus Type 41	$5.0x10^{4.5}$ ($1.58x10^{5}$) $TCID_{50}$/mL	-	-	-	-	-
Coxsackievirus B5/10/2006	$1.0x10^{6.5}$ $TCID_{50}$/mL	-	-	-	-	-
Echovirus 11	$1.0x10^{7.5}$ $TCID_{50}$/mL	-	-	-	-	-
Rotavirus	$1.0x10^{3.5}$ $TCID_{50}$/mL	-	-	-	-	-
Norovirus**	$2.5x10^{-2}$ Dilution from Raw Stool Clinical Specimen	-	-	-	-	-
Candida albicans	$1.66x10^{7}$ CFU/mL	-	-	-	-	-
Blastocystis hominis JNS	10^{-1} Dilution of stock	-	-	-	-	-
Giardia lamblia (Intestinalis)	10^{-1} Dilution of stock	-	-	-	-	-
Cryptosporidium parvum	10^{-1} Dilution of stock	-	-	-	-	-
Entamoeba histolytica MH-1:IMSS	10^{-1} Dilution of stock	-	-	-	-	-

* Clinical specimen received and tested was stool preserved in stool preservation and transport medium and processed per the ProGastro SSCS Assay Instructions for Use. Sample was culture positive for *Campylobacter* and was confirmed as *C. lari* by 16S genetic sequencing.
** Cultured and titered Norovirus was unavailable; Norovirus PCR positive clinical sample was tested.

Interference

A panel of endogenous substances, over the counter and prescription medicines, and miscellaneous substances which could potentially be found in stool was evaluated with the ProGastro SSCS Assay. Target organisms (*Campylobacter coli*, *Campylobacter jejuni*, *Salmonella*, *Shigella* and Shiga Toxin producing *E. coli* (*stx1+*/*stx2+*)) were spiked into negative stool at 2X Limit of Detection (LoD). Clinically relevant amounts of the potentially interfering substances were added to individual samples. The ProGastro SSCS Assay was performed in triplicate reactions for each sample. The following table outlines the Interfering Substances tested.

Substance	Active Ingredient(s)	Concentration Tested
Anti-Fungal /Anti-Itch Vaginal	Nystatin	10,000 USP units/mL (100mg/mL)
Creams/Ointments/ Suppositories	Hydrocortisone	2% (v/v)
Anti-Hemorrhoid Creams/Ointments	Phenylephrine	2% (v/v)
Antacid	Calcium Carbonate	0.5 mg/mL
Antacid	Aluminum Hydroxide	30% (v/v)
Antacid	Magnesium Hydroxide	0.2mg/mL
Enema	Mineral Oil	2% (v/v)
Enema	Mesalamine	2% (w/v)
Spermicidal Lubricant	Nonoxynol-9	1.4mg/mL
Anti-Diarrheal Medication	Loperamide Hydrochloride	0.00667 mg/mL
Anti-Diarrheal Medication	Bismuth Subsalicylate	0.87 mg/mL
Laxatives	Sennosides	0.1mg/mL
Antibiotics	Metronidazole	14mg/mL
Antibiotics	Vancomycin	1.4mg/mL
Non-Steroidal Anti-Inflammatory Medications	Naproxen Sodium	14mg/mL
Moist Towelettes	Benzethonium Chloride	10% (v/v)
Fecal Fat	Palmitic/Stearic Acid	2.65 mg stearic + 1.30 mg palmitic acids per mL
Blood	Glucose, Hormones, Enzymes, Ions, Iron, etc	5% v/v
Mucus	Mucin	3.33mg/mL

The ProGastro SSCS Assay was not affected by the presence of the substances in the panel of endogenous and exogenous potential PCR inhibitors.

Microbial Interference

Potentially cross-reactive or interfering microorganisms, the same organisms used for the Analytical Specificity Study, were spiked into simulated ProGastro SSCS Assay positive stool matrix at high clinically relevant concentrations and tested in triplicate with the ProGastro SSCS Assay. The presence of high clinically relevant concentrations of potentially interfering microorganisms in combination with low concentrations of assay target organisms (2X LoD) does not affect the performance of the ProGastro SSCS Assay.

Competitive Interference

Competitive Interference was assessed by generating contrived samples containing a single target analyte (organism) present at a concentration near the Limit of Detection (LoD) with one or more different target analytes at a higher concentration in the same sample. Competitive interference was observed with low concentrations of *Campylobacter jejuni or Campylobacter coli* in the presence of *Salmonella* at high concentrations and low concentrations of *Shigella* in the presence of *C. jejuni* at high concentrations. Multiple infections are rare and only *Salmonella* and *Campylobacter jejuni* have been found in co-infections with the most frequency.[20-23]

Carry-Over/Cross-Contamination

To evaluate the degree of carry-over/cross-contamination that may occur with the use of the ProGastro SSCS Assay in association with nucleic acid extracted on the bioMérieux NucliSENS easyMAG instrument and PCR on the Cepheid SmartCycler II instrument, a carry-over study was performed. Simulated pooled *Shigella* and Shiga Toxin producing *E. coli* (STEC) high positives were run in series alternating with true negatives once per day for a total of five days. The results of this study demonstrate no evidence of *Shigella* or STEC carry-over/cross-contamination over a 5 day course of processing high positive samples alongside negative samples using the ProGastro SSCS Assay.

Disposal

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

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