510(k) SUBSTANTIAL EQUIVALENCE
DETERMINATION DECISION SUMMARY

A. 510(k) Number:
k123274

B. Purpose for Submission:
New device

C. Measurand:
Salmonella, *Shigella*, *Campylobacter* (*C. jejuni and C. coli* only, undifferentiated) nucleic acids target sequences and Shiga Toxin 1 (*stx1*) and Shiga Toxin 2 (*stx2*) genes.

D. Type of Test:
A multiplexed real time PCR *in vitro* diagnostic test intended 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.

E. Applicant:
Gen-Probe Prodesse, Inc.

F. Proprietary and Established Names:
Proprietary Name: ProGastro SSCS Assay

Common Name: Nucleic acid amplification assay for *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni* and *Campylobacter coli*, and Shiga Toxin 1 and 2 genes.

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
PCH	Class II	21 CFR 866.3990 Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay	Microbiology (83)
PCI	Class II	21 CFR 866.3990 Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay	Microbiology (83)

OOI	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)

H. Intended Use:

1. 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.

2. Indication for use:
   Same as Intended Use

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   The bioMerieux NucliSENS easyMAG system is used for nucleic acid isolation from stool samples.

   The Cepheid SmartCycler II Real Time instrument with Dx software version 1.7b or 3.0a/b is used to perform PCR amplification and detection of nucleic acid.

   Several other Prodesse Assays have been cleared for use with the above instrumentation systems: Prodesse ProFlu+ (k110968), Pro hMPV+ (k082688), ProGastro Cd (k090239), ProParaflu+ (k091053), ProFAST+ (k101855) and ProAdeno+ (k102952).

I. Device Description:

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 1 (*stx1*) and Shiga Toxin 2 (*stx2*) genes and an Internal Control in the STEC Mix. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2.

An overview of the procedure is as follows:

1. Collect raw stool specimens from symptomatic patients placed into Cary Blair (CB) or ParaPak C&S Transport Medium (C&S).
2. Dilute stool preserved in CB or C&S 1:10 in CB or C&S Medium. Add an Internal Control to every sample to monitor for inhibitors present in the specimens.
3. Perform isolation and purification of nucleic acids using a NucliSENS easyMAG System (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux).
4. Add purified nucleic acids to the **SSC Mix** included in the ProGastro SSCS Kit. The SSC Mix contains target-specific oligonucleotide primers and oligonucleotide probes for detection of *Salmonella, Shigella,* and *Campylobacter* (*C. jejuni* and *C. coli* only). The primers 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 Kit. The STEC Mix contains target-specific oligonucleotide primers and oligonucleotide probes for detection of the Shiga Toxins 1 and 2 genes (*stx1* and *stx2*). The primers 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).
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 the 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.

Mix	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	667 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	1,100 µL	Brown	403325	55 (2 tubes provided)
STEC Mix	• Taq DNA polymerase • oligonucleotide primers • oligonucleotide probes • Buffer containing dNTPs • $MgCl_2$ and stabilizers	1,000 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 System (Meridian Cat. No. 900612)
- Molecular Grade Water (RNase/DNase Free)
- Extraction Control *(recommended, e.g. previously characterized positive sample)*

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

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 and/or Campylobacter coli* , *Salmonella*, and *Shigella* nucleic acids **not detected**
Sample ID	Positive	NA*		**POS**	NEG	NEG	*Campylobacter jejuni* and/or *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* and/or *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* and/or *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* and/or *C. coli*, *Salmonella spp.*, and *Shigella spp.* nucleic acid **detected.** Triple infections are rare,

SSC Mix							
Sample ID[1]	**Assay Result**	**IC Result**	**Warning / Error Code**	***Campy.* Result**	***Salmonella* Result**	***Shigella* Result**	**Interpretation of Results**
							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 ≥13 is reported in the ***Campy, Salmonella, and/or Shigella*** *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.*

STEC Mix						
Sample ID[1]	**Assay Result**	**IC Result**	**Warning / Error Code**	***stx*2 Result**	***stx*1 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.*

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Luminex® xTAG™ Gastrointestinal Pathogen Panel (GPP).

2. Predicate K number(s):

   k121454

3. Comparison with predicate(s):

Similarities		
Element	**Prodesse ProGastro SSCS (k123274)**	**Luminex xTAG GPP (k121454)**
Organisms Detected	*Salmonella spp.*, *Shigella spp.*, *Campylobacter* (*C. jejuni* and *C. coli*), and STEC (*stx1* and *stx2* genes)	Same (See below for differences)
Analyte	DNA	(See below for differences)
Technological Principles	Multiplex nucleic acid	Same (See below for differences)
Specimen Types	Stool specimens	Same
User Complexity	High	Same
Sample Preparation Method	Up front sample processing is required to extract nucleic acids	Same
Controls	Internal control in each sample. External control processed with each batch of samples.	Same

Differences		
Element	**Prodesse ProGastro SSCS (k123274)**	**Luminex xTAG GPP (k121454)**
Organisms Detected	(See above for similarities)	Can also detect and distinguish *C. lari*. In addition, can detect and distinguish *Clostridium difficile toxin A/B, Cryptosporidium (C. parvum and C. hominis only), Escherichia coli (E. coli) O157,* Enterotoxigenic *E. coli* (ETEC) LT/ST, *Giardia (G. lamblia only),* Norovirus GI/GII, and Rotavirus A.
Analyte	DNA	RNA/DNA
Technological Principles	Real time multiplex PCR based on the Taqman reagent chemistry	Multiplex RT-PCR and bead hybridization followed by Fluorescence-activated sorting of labeled beads coupled to streptavidin-conjugated biotinylated products
Instrumentation	Cepheid SmartCycler II	PCR Thermocycler and Luminex 100/200 system
Time to result	Approximately 3 hours	Approximately 6 to 12 hours

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

- Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff
- Guidance for Off-the-Shelf Software Use in Medical Devices; Final
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests; Draft Guidance for Industry and FDA Reviewers
- Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff

- Draft Guidance on Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection of *Clostridium difficile*

L. Test Principle:

The real-time PCR process simultaneously amplifies and detects nucleic acid targets in two separate closed-tube reactions. 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 (the first tube), and Shiga Toxin 1 (*stx1*) and Shiga Toxin 2 (*stx2*) genes and an Internal Control in the STEC Mix (the second tube), and is based on two processes: nucleic acid isolation and Real Time polymerase chain reaction (PCR) amplification/detection. The Gastro RNA/DNA Internal Control (GIC) is added to each preserved stool specimen from symptomatic patient to monitor for inhibitors present. Nucleic acids are isolated and purified from the stool specimen. Purified nucleic acid is added to the SSC Mix and the STEC Mix. The SSC Mix and STEC Mix contain target specific oligonucleotide primers and oligonucleotide probes dual-labeled with a reporter dye attached to the 5' end and a quencher dye attached to the 3' end. Amplification proceeds during which the primers and probes anneal specifically to the templates (if present) followed by primer extension and amplification. The ProGastro SSCS Assay is based on Taqman chemistry, which utilizes the 5' – 3' exonuclease activity of the 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 the fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification product present at that time. Fluorescent intensity is monitored during each PCR cycle by the real-time instrument.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

Inter-Laboratory Reproducibility Study

An Inter-Laboratory Reproducibility study was conducted at three independent clinical laboratories: two external sites and one internal site at Gen-Probe Prodesse Inc. Panels of samples were tested by two operators at each site and each operator tested for a total of five days resulting in 10 runs per Mix per site. Panel samples were generated by dividing and spiking a ProGastro SSCS negative stool pool with combinations of five different bacterial strains detected by the ProGastro SSCS Assay including *Salmonella enterica* serotype Enteritidis (*Salmonella* Enteritidis), *Shigella sonnei, Campylobacter jejuni, Campylobacter coli*, and Shiga Toxin producing *E. coli* (STEC). The simulated positive samples were spiked at two different concentrations with each spiked sample containing the following combined targets: *Salmonella* (Sal)/*C. jejuni* (CJ) or *Shigella* (Shi)/*C. coli* (Cc)/STEC. The simulated High Negative samples

were spiked at one concentration with each spiked sample containing all five of the targets: *Salmonella/C. jejuni*/*Shigella*/*C. coli*/STEC. Each Reproducibility Panel consisted of three Medium Positives and three Low Positives for each of the two sample group (n= 2 x 6 =12), and three High Negatives (n=3) for a total of 15 samples per panel. The High Negative concentration for each organism was at 4 logs below each bacterial strain's LoD. The Low Positive concentration was at 2x Assay LoD and the Medium Positive concentration was at 1 log above the Assay LoD for all target strains except the *C. jejuni* and *C. coli* strains. *C. jejuni* Low Positives were tested at 20x LoD and Medium Positives at 2 logs above LoD and the *C. coli* Low Positives at 0.5 log above 2x LoD, and Medium Positives at 1.5 logs above LoD. The *Campylobacter* strains were tested at higher concentrations because *Campylobacter* is very sensitive to environmental stressors including freezing where it loses viability. In-house studies (QC testing of Positive Matrix Controls and Verification Panels for the Clinical Study and QC testing of spiked stool pools for Reproducibility) have demonstrated decreased amount of *Campylobacter* DNA (increasing Ct values) over time.

Note: 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. It appears that 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. Therefore the levels of the *C. jejuni* (ATCC 33291) and *C. coli* (ATCC BAA-371) low positives tested in this reproducibility study are acceptable.

The bacterial strains used in the Inter-Laboratory Reproducibility Study and panel member concentrations are summarized in the table below:

Bacterial Strain Information for Inter-Laboratory Reproducibility Study Panel Members		
Panel Member	**Strains**	**Panel Member Concentration**
Sal/CJ Medium Positive	*Salmonella* Enteritidis ATCC 6961	Sal = $1x10^5$ CFU/mL
	Campylobacter jejuni ATCC 33291	CJ = $1x10^5$ CFU/mL
Sal/CJ Low Positive	*Salmonella* Enteritidis ATCC 6961	Sal = $2x10^4$ CFU/mL
	Campylobacter jejuni ATCC 33291	CJ = $2x10^4$ CFU/mL
Shi/Cc/STEC Medium Positive	*Shigella sonnei* ATCC 29029	Shi = $1x10^4$ CFU/mL
	Campylobacter coli ATCC BAA-371	Cc = $1x10^{5.5}$ CFU/mL
	STEC O111:H8 (*stx1+*/*stx2+*), STEC Center Acc# TW07926, Strain 3215-99	STEC = $1x10^5$ CFU/mL
Shi/Cc/STEC Low Positive	*Shigella sonnei* ATCC 29029	Shi = $2x10^3$ CFU/mL
	Campylobacter coli ATCC BAA-371	Cc = $2x10^{4.5}$ CFU/mL
	STEC O111:H8 (*stx1+*/*stx2+*), STEC Center Acc# TW07926, Strain 3215-99	STEC = $2x10^4$ CFU/mL
ALL High Negative	*Salmonella* Enteritidis ATCC 6961	Sal = $1x10^0$ CFU/mL
	Shigella sonnei ATCC 29029	Shi = $1x10^{-1}$ CFU/mL
	Campylobacter jejuni ATCC 33291	CJ = $1x10^{-1}$ CFU/mL
	Campylobacter coli ATCC BAA-371	Cc = $1x10^0$ CFU/mL
	STEC O111:H8 (*stx1+*/*stx2+*), STEC Center Acc# TW07926, Strain 3215-9	STEC = $1x10^0$ CFU/mL

The Panels were stored at ≤–70°C and delivered frozen. One Panel was used for each of the five testing days per operator per site, generating a total of 90 data points per organism target per concentration (i.e., 3 replicates/organism/concentration/operator/day/site x 2 operators/site x 3 sites x 5 days = 90 data points/organism/concentration). The samples were extracted each day using the bioMérieux NucliSENS easyMAG and the extracted nucleic acids were tested with the ProGastro SSCS Assay on the Cepheid SmartCycler II. ProGastro SSCS Assay controls (Positive, Positive Matrix, and Negative) were also included with each panel run. Two lots of ProGastro SSCS Assay SSC and STEC Mixes were used by each of the three sites.

A summary of the Reproducibility Study data is presented in the two tables below:

		SSC Mix											
	Panel Member ID	***C. jejuni* Low Positive**	***C. jejuni* Medium Positive**	***C. jejuni* High Negative**	***C. coli* Low Positive**	***C. coli* Medium Positive**	***C. coli* High Negative**	***Salmonella* Low Positive**	***Salmonella* Medium Positive**	***Salmonella* High Negative**	***Shigella* Low Positive**	***Shigella* Medium Positive**	***Shigella* High Negative**
	Concentration	20X* LoD	100X* LoD	0.0001X LoD	6X* LoD	30X* LoD	0.0001X LoD	2X LoD	10X LoD	0.0001X 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%
	Mean Ct Value	37.6	35.2	n/a	35.9	33.5	n/a	35.9	33.2	n/a	35.8	33.2	n/a
	% CV	3.5	3.0	n/a	3.9	3.7	n/a	1.4	1.2	n/a	1.7	1.4	n/a
Site 2	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%	29/30 96.7%	30/30 100%	30/30 100%
	Mean Ct Value	37.3	34.8	n/a	35.8	33.4	n/a	36.0	33.1	n/a	35.6	33.2	n/a
	% CV	3.0	2.8	n/a	3.1	3.2	n/a	1.7	1.6	n/a	1.9	1.8	n/a
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%
	Mean Ct Value	37.0	34.4	n/a	35.2	32.9	n/a	35.7	32.7	n/a	35.0	32.8	n/a
	% CV	2.6	2.2	n/a	2.1	1.9	n/a	1.4	1.2	n/a	1.8	1.3	n/a
	Total Agreement with Expected Result	90/90 100%	90/90 100%	90/90 100%	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%
	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%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	94.0%-99.8%	95.9%-100.0%	95.9%-100.0%
	Overall Mean Ct Value	37.3	34.8	n/a	35.6	33.3	n/a	35.9	33.0	n/a	35.5	33.1	n/a
	Overall % CV	3.1	2.8	n/a	3.2	3.1	n/a	1.6	1.5	n/a	2.0	1.6	n/a

* Note: The *Campylobacter* strains were tested at higher concentrations because *Campylobacter* is very sensitive to environmental stressors including freezing where it loses viability. 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.

		SSC Mix					
	Panel Member ID	**STEC (*stx 1*) Low Positive**	**STEC (*stx 1*) Medium Positive**	**STEC (*stx 1*) High Negative**	**STEC (*stx 2*) Low Positive**	**STEC (*stx 2*) Medium Positive**	**STEC (*stx 2*) High Negative**
	Concentration	2X LoD	10X LoD	0.0001X 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%
	Mean Ct Value	36.1	33. 4	n/a	36.7	34. 5	n/a
	% CV	2.0	1.6	n/a	1.8	1.3	n/a
Site 2	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	Mean Ct Value	36.2	33.6	n/a	36.8	34.7	n/a
	% CV	2.5	1.6	n/a	1.9	1.9	n/a
Site 3	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	Mean Ct Value	35.6	33.1	n/a	36.5	34.2	n/a
	% CV	1.7	1.3	n/a	1.6	1.0	n/a
	Total Agreement with Expected Result	90/90 100%	90/90 100%	90/90 100%	90/90 100%	90/90 100%	90/90 100%
	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%
	Overall Mean Ct Value	35.9	33.4	n/a	36.7	34.5	n/a
	Overall % CV	2.2	1.6	n/a	1.8	1.5	n/a

Analysis of variance (ANOVA) for each source of variation was also performed and the results are presented in the tables below.

ProGastro SSCS Assay Inter-laboratory Reproducibility Analysis – SSC Mix																
			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Lot		Between-Site		Total	
Panel	**N**	**Mean Ct**	**SD**	**CV (%)**	**SD**	**CV(%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV(%)**
Salmonella Low Positive	90	35.86	0.483	1.35	0.189	0.53	0.180	0.50	0.063	0.18	0.110	0.31	0.047	0.13	0.566	1.58
Salmonella Medium Positive	90	32.98	0.341	1.03	0.000	0.00	0.269	0.81	0.184	0.56	0.000	0.00	0.177	0.54	0.504	1.53
Shigella Low Positive*	89	35.47	0.549	1.55	0.000	0.00	0.277	0.78	0.272	0.77	0.202	0.57	0.303	0.85	0.764	2.16
Shigella Medium Positive	90	33.07	0.394	1.19	0.069	0.21	0.272	0.82	0.201	0.61	0.127	0.38	0.155	0.47	0.561	1.70
C. coli Low Positive	90	35.64	0.486	1.36	0.714	2.00	0.000	0.00	0.829	2.33	0.000	0.00	0.000	0.00	1.198	3.36
C. coli Medium Positive	90	33.28	0.462	1.39	0.577	1.73	0.178	0.53	0.770	2.31	0.000	0.00	0.000	0.00	1.082	3.25
C. jejuni Low Positive	90	37.30	0.549	1.47	0.734	1.97	0.152	0.41	0.752	2.02	0.000	0.00	0.000	0.00	1.195	3.20
C. jejuni Medium Positive	90	34.81	0.381	1.09	0.634	1.82	0.091	0.26	0.694	1.99	0.000	0.00	0.000	0.00	1.018	2.92
High Negative (GIC)	90	33.23	0.622	1.87	0.000	0.00	0.054	0.16	0.124	0.37	0.000	0.00	0.318	0.96	0.711	2.14

Note: 1.Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD=0 and CV=0%.
**One sample False Negative for Shigella, data not included in analysis.

ProGastro SSCS Assay Inter-laboratory Reproducibility Analysis – STEC Mix																
			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Lot		Between-Site		Total	
Panel	**N**	**Mean Ct**	**SD**	**CV (%)**	**SD**	**CV(%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV(%)**
*Stx*1 Low Positive	90	35.94	0.699	1.95	0.273	0.76	0.000	0.00	0.000	0.00	0.000	0.00	0.279	0.78	0.801	2.23
*Stx*1 Medium Positive	90	33.38	0.433	1.30	0.258	0.77	0.040	0.12	0.000	0.00	0.000	0.00	0.261	0.78	0.568	1.70
*Stx*2 Low Positive	90	36.67	0.638	1.74	0.000	0.00	0.084	0.23	0.000	0.00	0.000	0.00	0.145	0.40	0.659	1.80
*Stx*2 Medium Positive	90	34.46	0.429	1.24	0.268	0.78	0.000	0.00	0.000	0.00	0.000	0.00	0.212	0.62	0.548	1.59
High Negative (GIC)	90	32.90	0.423	1.29	0.000	0.00	0.000	0.00	0.000	0.00	0.057	0.17	0.295	0.90	0.519	1.58

Note: 1.Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small.
When this occurs, SD=0 and CV=0%.

Intra-Laboratory Reproducibility Study

An Intra-Laboratory Precision study was conducted internally at Gen-Probe Prodesse Inc. Panels of samples were tested by two operators and each operator tested for a total of 12 days resulting in 24 runs per PCR Mix. Panel samples were generated as described previously in the Inter-Laboratory Reproducibility Study. One Panel was used for each of the 12 testing days per operator. The samples were extracted each day using the bioMérieux NucliSENS easyMAG and the extracted nucleic acids were tested with the ProGastro SSCS Assay on the Cepheid SmartCycler II. ProGastro SSCS Assay controls (Positive, Positive Matrix, and Negative) were also included with each panel run. Two lots of ProGastro SSCS Assay SSC and STEC Mixes were used by each of the operators.

A summary of the Intra-Laboratory Precision Study data is presented in the tables below:

Panel Member ID	SSC Mix											
	***C. jejuni* Low Positive**	***C. jejuni* Medium Positive**	***C. jejuni* High Negative**	***C. coli* Low Positive**	***C. coli* Medium Positive**	***C. coli* High Negative**	***Salmonella* Low Positive**	***Salmonella* Medium Positive**	***Salmonella* High Negative**	***Shigella* Low Positive**	***Shigella* Medium Positive**	***Shigella* High Negative**
Concentration	20X* LoD	100X* LoD	0.0001X LoD	6X* LoD	30X* LoD	0.0001X LoD	2X LoD	10X LoD	0.0001X LoD	2X LoD	10X LoD	0.0001X LoD
Total Agreement with Expected Result	72/72 100%	72/72 100%	71/72 98.6%	72/72 100%	72/72 100%	71/72 98.6%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%	72/72 100%
95% CI	94.9%-100.0%	94.9%-100.0%	92.5%-99.8%	94.9%-100.0%	94.9%-100.0%	92.5%-99.8%	94.9%-100.0%	94.9%-100.0%	94.9%-100.0%	94.9%-100.0%	94.9%-100.0%	94.9%-100.0%
Overall Mean Ct Value	36.7	34.2	n/a	35.0	32.7	n/a	35.6	32.7	n/a	35.1	32.7	n/a
Overall % CV	2.6	2.4	n/a	2.1	2.3	n/a	1.5	1.1	n/a	1.6	1.3	n/a

* Note: The *Campylobacter* strains were tested at higher concentrations because *Campylobacter* is very sensitive to environmental stressors including freezing where it loses viability. 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.

Panel Member ID	SSC Mix					
	STEC (*stx 1*) Low Positive	**STEC (*stx 1*) Medium Positive**	**STEC (*stx 1*) High Negative**	**STEC (*stx 2*) Low Positive**	**STEC (*stx 2*) Medium Positive**	**STEC (*stx 2*) High Negative**
Concentration	2X LoD	10X LoD	0.0001X 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%
95% CI	94.9%-100.0%	94.9%-100.0%	94.9%-100.0%	94.9%-100.0%	94.9%-100.0%	94.9%-100.0%
Overall Mean Ct Value	35.6	33. 0	n/a	36.5	34. 2	n/a
Overall % CV	1.8	1.3	n/a	1.8	1.2	n/a

b. Linearity/assay reportable range:

Not applicable, qualitative assay

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

Assay Controls

The following controls are provided in the ProGastro SSCS Assay kit:

Positive Controls (PCs): The ProGastro SSCS Assay kit contains an SSCS Positive DNA Control and a *C. Coli* Positive DNA Control. Both PCs are DNA plasmids containing portions of the Assay target genes of interest. The SSCS Control (PC1) plasmid contains approximately 200 bp of target sequence for each of the following organisms or genes: *Campylobacter jejuni*, *Salmonella*, *Shigella*, *stx1* and *stx2*. The SSCS Control is used with both the SSC Mix and the STEC Mix. The *C. coli* Control (PC2) plasmid contains a 403 bp target sequence specific for *Campylobacter coli*. The *C. Coli* Control is used with the SSC Mix only. The PCs do not go through nucleic acid isolation and purification, but are included during set-up of the PCR reaction. The PCs in conjunction with the IC are used to verify reagent and system performance. The SSCS Control (PC1, SAP# 403324) and the *C. coli* Control (PC2, SAP# 403328) are sold at $1x10^4$ c/µL and should be used at $1x10^3$ copies/µL.

Because the PC is meant to assess global failure of the assay (reagent, technical, instrument failure, etc.) and the customer variance components were to be determined during the clinical trial, a broad Ct range was desired. The acceptable Ct range was set to 20.0 to 45.0 in the Clinical Trial Protocol for all targets (except *Shigella*: 20.0 to 37.0).

Of a total of 77 PC1 tested by the ProGastro SSCS Assay during the clinical trial, 100% (77/77) of these controls gave correct results. The average Ct of the PC1s tested was 31.5 (Min 29.1- Max 34.4), with 1.1 Standard Deviation (STDEV) and 3.6% CV; 27.6 (Min 25.3- Max 30.8), with 1.1 STDEV and 4.0%; 29.2 (Min 27.2- Max 31.2), with 1.1 STDEV and 3.6 % CV; 30.7 (Min 29.6- Max 32.5), with 0.6 STDEV and 1.8 % CV; and 28.5 (Min 27.5- Max 30.3), with 0.6 STDEV and 2.2%, for the *Campylobacter jejuni, Salmonella, Shigella, stx1, and stx2*, respectively. The prospective clinical study data validated the pre-determined PC1 Ct acceptance range of 20.0 to 45.0 for *Campylobacter jejuni, Salmonella, stx1, and stx2,* and 20.0 to 37.0 for *Shigella*.

Of a total of 77 PC2 tested by the ProGastro SSCS Assay during the clinical trial, 100% (77/77) of these controls gave correct results. The average Ct of the PC2s tested was 28.9 (Min 26.8- Max 34.2), with 1.6 Standard Deviation (STDEV) and 5.6% CV for *Campylobacter coli*. The prospective clinical study data validated the pre-determined PC2 Ct acceptance range of 20.0 to 45.0 for *Campylobacter coli.*

Internal Control (IC): A Gastro RNA/DNA Internal Control (GIC) consisting of a pooled non-infectious DNA plasmid and RNA transcript is incorporated into every sample and is carried through all steps of the procedure from nucleic acid isolation and purification through amplification to monitor for inhibitors present in the specimen or reaction tube. The GIC contains a DNA and an RNA portion to allow for one nucleic acid extract to be used with multiple RT-PCR/PCR assays whether the assay detects a DNA or an RNA target. The GIC also serves as a general process control ensuring that each step of the procedure was performed correctly, assay and instrument parameters were set correctly, and that general reagents were working. The GIC should be used at $5x10^5$ c/µL for the RNA and $1x10^3$ c/µL DNA portion, and sold at $5x10^7$ c/µL for the RNA and $1x10^5$ c/µL for the DNA portion.

The IC is meant to assess global failure (reagent or process) and monitor for PCR inhibition. Because the IC is assessing global failure and the customer variance components were to be determined during the clinical trial, a broad Ct range was desired. The acceptable range for the Internal Control (IC) was set to 13 - 45 Ct in the Clinical Trial Protocol. For the SSC Mix, the average Ct of all eligible prospective ProGastro SSCS Assay negative clinical specimens was 33.8 (Min 31.2- Max 44.2), with 1.60 Standard Deviation (STDEV) and 4.70 % CV. The average Ct of all eligible ProGastro SSCS Assay Negative Control was 34.4 (Min 33.0- Max 36.2), with 0.6 Standard Deviation (STDEV) and 1.90% CV. For the STEC Mix, the average Ct of all eligible prospective ProGastro SSCS Assay negative clinical specimens was 33.6 (Min 31.3- Max 42.6), with 1.60 Standard Deviation (STDEV) and 4.80 % CV. The average Ct of all eligible ProGastro SSCS Assay Negative Control was 34.1 (Min 33.1- Max 36.2), with 0.5 Standard Deviation (STDEV) and 1.50% CV. The prospective clinical study data validated the pre-determined IC Ct acceptance range of 13.0 to 45.0.

Kit Control Final Release Specifications

Final release specifications were established during Test Method Characterization (TMC) and then validated against the appropriate analytical performance characteristics of specificity and total imprecision.

Final release specifications were developed to ensure First Pass Acceptance (FPA) rates of ≥ 99% at lot release and customer rejection rate of ≤ 1% for a lot released at Acceptable Quality Level (AQL) (i.e. 95% probability of QC lot acceptance at lot release). For customer specifications, the maximum allowable customer lot rejection rate at the Rejectable Quality Level (RQL) Limit (i.e. 10% probability of QC acceptance at lot release) is 1%. A false valid risk of ≤ 5% was used to determine the validity specifications.

QC release specification ranges were set using Operator Characteristics (OC) curve analysis. Lot, technician, instrument, run, and replicate variance components were obtained during test method characterization. Observed Ct means were adjusted for stability effects and specifications determined to meet the validity and specification requirements above.

Positive Control and Internal Control Specifications – SSC Mix

Control	Customer Ct Specification	Kit Control Final Release Specifications
SSCS PC (*Campylobacter*)	20 - 45	29.2 - 35.8
SSCS PC (*Salmonella*)	20 - 45	25.0 - 31.6
SSCS PC (*Shigella*)	20 - 37	26.8 - 33.4
C. coli PC (*Campylobacter*)	20 - 45	26.3 - 32.9
Internal Control	13 - 45	26.9 - 39.9

Positive Control and Internal Control Specifications – STEC Mix

Control	Customer Ct Specification	Kit Control Final Release Specifications
SSCS PC (*stx1*)	20 - 45	24.9 - 31.5
SSCS PC (*stx2*)	20 - 45	27.2 - 33.8
Internal Control	13 - 45	26.1 - 39.9

Negative Control: A Negative Control is not provided with the kit, but is required and described in the ProGastro SSCS Assay package insert. Stool preservation and transport media (SPTM, Para-Pak C&S or Cary-Blair) spiked with the GIC is to be used as the negative control and processed starting from nucleic acid isolation. The negative control serves to monitor for contamination.

A Positive Matrix Control was included in the ProGastro SSCS Clinical Study but is not provided in the ProGastro SSCS Assay kit:

Positive Matrix Control (PMC): The Positive Matrix Control used in the clinical study is a pool of the ProGastro SSCS Assay target organisms (see the table below) diluted in SPTM and spiked into negative stool matrix. The PMC was included with each nucleic acid isolation run performed for the Clinical and Reproducibility/Precision studies. The PMC served to monitor for lysis and during nucleic acid isolation.

Bacterial strains used for the Positive Matrix Control

Strain information	Final concentration in PMC
Campylobacter jejuni ATCC# 33291	1 x 10^4 CFU/mL(082211) 1 x 10^5 CFU/mL (022812)
Campylobacter coli ATCC# BAA-371	1 x 10^5 CFU/mL(082211) 1 x 10^6 CFU/mL (022812)
Salmonella enterica serotype Typhimurium ATCC# BAA-191	1 x 10^6 CFU/mL
Shigella flexneri ATCC# 12022	1 x 10^6 CFU/mL
STEC Strain 97-3250 O26:H11 (*stx1*+/*stx*2+)	1 x 10^6 CFU/mL

A total of 136 runs were attempted in the clinical trial and there were no run failures due to PMC failure.

The sponsor is also recommending the following in the product package insert: "*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. The extraction control should be treated like a sample during assay performance and analysis.*"

Specimen and Nucleic Acid Stability Study (Fresh vs. Frozen Study)

An analytical study was conducted to determine the effect that storing (refrigerated) and freezing stool preserved in Stool Preservation and Transport Media (SPTM, ParaPak C&S) as well as the effects of freezing the resultant purified nucleic acids have on the performance of the ProGastro SSCS Assay

In this study, contrived samples were generated using separate aliquots from a ProGastro SSCS negative stool pool spiked with one strain of *Salmonella, Shigella, Campylobacter jejuni*, and Shiga Toxin producing *E. coli* (STEC) representing the four bacterial targets of the ProGastro SSCS Assay. Due to the fact that natural fresh positive samples were not available for this study, samples were made at varying concentrations in negative stool matrix preserved in SPTM (ParaPak C&S).
The contrived sample panel was prepared so that approximately 60% of the samples had analyte levels close to their LoD. Of the samples near LoD, approximately 50% were tested at LoD and the other half were Moderate Positive (3 x LoD) samples. The remaining positive samples were tested at 1, 2, and 3 logs above the LoD for each bacterial strain. Therefore, for each of the four strains, ten samples were generated (n=40) in the following manner: one sample each at 3 logs, 2 logs, and 1 log above LoD, four samples of 3x LoD, and three samples at LoD. See the table below:

Bacterial Strains and Concentrations					
Strain	**Concentration (CFU/mL)**				
	1x LoD (3 Replicates)	**3 x LoD (4 Replicates)**	**1 Replicate each**		
			1 log above LoD	**2 logs above LoD**	**3 logs above LoD**
Salmonella Enteritidis ATCC 6961, 1.06×10^9 CFU/mL	1×10^4 CFU/mL	3×10^4 CFU/mL	1×10^5 CFU/mL	1×10^6 CFU/mL	1×10^7 CFU/mL
Shigella flexneri ATCC 12025, 3.30×10^9 CFU/mL	1×10^4 CFU/mL	3×10^4 CFU/mL	1×10^5 CFU/mL	1×10^6 CFU/mL	1×10^7 CFU/mL
Campylobacter jejuni ATCC 33291, 7.80×10^7 CFU/mL	1×10^3 CFU/mL	3×10^3 CFU/mL	1×10^4 CFU/mL	1×10^5 CFU/mL	1×10^6 CFU/mL
STEC O157:HN (*stx1+*/*stx2+*), STEC Center Acc# TW07700, 1.55×10^9 CFU/mL	1×10^4 CFU/mL	3×10^4 CFU/mL	1×10^5 CFU/mL	1×10^6 CFU/mL	1×10^7 CFU/mL

After sample generation, a portion of each fresh prepared sample was extracted on the bioMérieux NucliSENS easyMAG. The remaining portions of the samples were stored refrigerated (2-8°C) for five days (120 hours) and then extracted again as described above. After refrigeration for five days, the remaining portion of each sample were frozen solid at ≤-70ºC for a minimum of 24 hours and then thawed and extracted as described above. At each time point, the nucleic acids were run "fresh" (i.e., immediately subsequent to extraction) in a single replicate using one lot of ProGastro SSCS reagents. The remaining nucleic acids (NA) were then frozen at <-70°C for a minimum of 24 hours and later thawed on ice and subject to PCR as described above. The data generated was analyzed for possible negative effects of storing and freezing preserved stool samples and freezing purified nucleic acids when used in conjunction with the ProGastro SSCS Assay.

Positive and Negative percent agreements were calculated and compared to the Fresh Stool Sample/Fresh NA (FR ST/FR NA) data. Each bacterial strain and each concentration were required to not test statistically significantly different in terms of positive/negative result (percent agreement) ($p<0.05$) for all testing points/concentrations to be deemed equivalent. A paired t-test of Ct values compared to FR ST/FR NA was also performed for positive samples at all testing points and analyzed for informational purposes.

First, the number of positive samples was identified at each concentration at each strain for the fresh samples. The remaining testing points were compared to those numbers to determine the percent agreement to the initial control result:

Sample and Nucleic Acid Stability Results — % Agreement to the Fresh ST / Fresh NA Data							
Strain	**Concentration**	**% Agreement**					
		FR ST/FR NA[1]	**FR ST/FZ NA[2]**	**REF ST/FR NA[3]**	**REF ST/FZ NA[4]**	**FZ ST/FR NA[5]**	**FZ ST/FZ NA[6]**
Salmonella	3 logs above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	2 logs above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	1 log above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	3x LoD	4	4/4 = 100%	4/4 = 100%	4/4 = 100%	4/4 = 100%	4/4 = 100%
	@ LoD	3	3/3 = 100%	3/3 = 100%	3/3 = 100%	3/3 = 100%	3/3 = 100%
	Overall	10	10/10 = 100%	10/10 = 100%	10/10 = 100%	10/10 = 100%	10/10 = 100%
Shigella	3 logs above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	2 logs above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	1 log above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	3x LoD	4	4/4 = 100%	4/4 = 100%	4/4 = 100%	4/4 = 100%	4/4 = 100%
	@ LoD	3	3/3 = 100%	3/3 = 100%	3/3 = 100%	3/3 = 100%	3/3 = 100%
	Overall	10	10/10 = 100%	10/10 = 100%	10/10 = 100%	10/10 = 100%	10/10 = 100%
C. jejuni	3 logs above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	2 logs above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	1 log above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	3x LoD	4	4/4 = 100%	4/4 = 100%	4/4 = 100%	4/4 = 100%	4/4 = 100%
	@ LoD	3	3/3 = 100%	3/3 = 100%	3/3 = 100%*	3/3 = 100%	3/3 = 100%
	Overall	10	10/10 = 100%	10/10 = 100%	10/10 = 100%	10/10 = 100%	10/10 = 100%
STEC	3 logs above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	2 logs above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	1 log above LoD	1	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%	1/1 = 100%
	3x LoD	4	4/4 = 100%	4/4 = 100%	4/4 = 100%	4/4 = 100%	4/4 = 100%
	@ LoD	3	3/3 = 100%	3/3 = 100%	3/3 = 100%	3/3 = 100%	3/3 = 100%
	Overall	10	10/10 = 100%	10/10 = 100%	10/10 = 100%	10/10 = 100%	10/10 = 100%
p-value	NA	NA	1.000	1.000	1.000	1.000	1.000

[1]**FR ST/FR NA**: Fresh contrived Stool samples/Freshly extracted nucleic acids
[2]**FR ST/FZ NA**: Fresh contrived Stool samples/Frozen nucleic acids
[3]**REF ST/FR NA**: Refrigerated contrived Stool samples/Freshly extracted nucleic acids
[4]**REF ST/FZ NA**: Refrigerated contrived Stool samples/Frozen nucleic acids
[5]**FZ ST/FR NA**: Frozen contrived Stool samples/Fresh nucleic acids
[6]**FZ ST/FZ NA**: Frozen contrived Stool samples/Frozen nucleic acids

The mean Ct for each sample(s) at a particular bacterial concentration was calculated and the difference in mean Ct for the latter five time points to the FR ST/FR NA was calculated and summarized in the tables below:

Mean Ct Values						
Sample ID*	**FR ST/FR NA Ct**	**FR ST/FZ NA Ct**	**REF ST/FR NA Ct**	**REF ST/FZ NA Ct**	**FZ ST/FR NA Ct**	**FZ ST/FZ NA Ct**
Salmonella at LoD	37.2	36.0	38.6	35.1	36.4	37.1
Salmonella 3X LoD	34.5	34.5	36.3	33.8	35.0	34.9
Salmonella 1 log above LoD	33.1	33.1	33.5	32.8	33.2	34.1
Salmonella 2 logs above LoD	29.6	29.5	30.8	28.7	29.6	30.0
Salmonella 3 logs above LoD	26.3	26.0	27.3	25.8	26.5	26.2
Shigella at LoD	32.4	32.0	33.3	32.9	32.8	32.2
Shigella 3X LoD	30.6	30.6	31.2	31.2	31.0	30.7
Shigella 1 log above LoD	29.0	28.8	29.8	28.8	29.4	29.1
Shigella 2 logs above LoD	25.3	24.4	26.0	25.7	26.1	25.3
Shigella 3 logs above LoD	21.8	21.5	22.3	22.4	22.6	22.2
C. jejuni at LoD	39.1	39.3	39.6	38.8	41.2	42.4
C. jejuni 3X LoD	37.1	37.3	37.3	37.3	39.6	40.8

Mean Ct Values						
Sample ID*	**FR ST/FR NA Ct**	**FR ST/FZ NA Ct**	**REF ST/FR NA Ct**	**REF ST/FZ NA Ct**	**FZ ST/FR NA Ct**	**FZ ST/FZ NA Ct**
C. jejuni 1 log above LoD	35.2	35.3	35.4	35.7	37.5	38.2
C. jejuni 2 logs above LoD	31.8	31.6	32.1	31.2	33.9	33.5
C. jejuni 3 logs above LoD	28.1	27.6	28.3	27.8	30.1	30.1
STEC at LoD {*stx2*}	37.6	38.4	37.8	37.4	39.0	38.1
STEC 3X LoD {*stx2*}	36.4	35.7	36.8	36.0	36.3	35.8
STEC 1 log above LoD {*stx2*}	34.1	34.0	34.7	33.3	34.7	33.9
STEC 2 logs above LoD {*stx2*}	31.4	31.3	31.3	30.9	31.2	31.0
STEC 3 logs above LoD {*stx2*}	27.7	27.6	27.8	27.6	28.0	27.9
STEC at LoD {*stx1*}	39.6	39.4	38.0	37.7	38.6	38.2
STEC 3X LoD {*stx1*}	36.9	35.5	36.2	35.3	36.3	35.5
STEC 1 log above LoD {*stx1*}	34.6	34.3	34.6	33.6	34.5	34.3
STEC 2 logs above LoD {*stx1*}	30.9	30.4	31.0	30.3	30.7	30.4
STEC 3 logs above LoD {*stx1*}	27.2	26.8	27.1	26.7	27.3	27.0

* Only one replicate tested at each of the 1 log above LoD, 2 logs above LoD, and 3 logs above LoD levels per the study plan.

Mean Ct Difference to initial FR ST/FR NA Data					
Sample ID	**FR ST/FZ NA**	**REF ST/FR NA**	**REF ST/FZ NA**	**FZ ST/FR NA**	**FZ ST/FZ NA**
Salmonella at LoD	-1.2	1.4	-2.1	-0.8	-0.1
Salmonella 3X LoD	0.0	1.8	-0.7	0.5	0.4
Salmonella 1 log above LoD	0.0	0.4	-0.3	0.1	1.0
Salmonella 2 logs above LoD	-0.1	1.2	-0.9	0.0	0.4
Salmonella 3 logs above LoD	-0.3	1.0	-0.5	0.2	-0.1
Shigella at LoD	-0.4	0.9	0.5	0.4	-0.2
Shigella 3X LoD	0.0	0.6	0.6	0.4	0.1
Shigella 1 log above LoD	-0.2	0.8	-0.2	0.4	0.1
Shigella 2 logs above LoD	-0.9	0.7	0.4	0.8	0.0
Shigella 3 logs above LoD	-0.3	0.5	0.6	0.8	0.4
C. jejuni at LoD	0.2	0.5	-0.3	2.1*	3.3*
C. jejuni 3X LoD	0.2	0.2	0.2	2.5*	3.7*
C. jejuni 1 log above LoD	0.1	0.2	0.5	2.3*	3.0*
C. jejuni 2 logs above LoD	-0.2	0.3	-0.6	2.1*	1.7*
C. jejuni 3 logs above LoD	-0.5	0.2	-0.3	2.0*	2.0*
STEC at LoD {*stx2*}	0.8	0.2	-0.2	1.4	0.5
STEC 3X LoD {*stx2*}	-0.7	0.4	-0.4	-0.1	-0.6
STEC 1 log above LoD {*stx2*}	-0.1	0.6	-0.8	0.6	-0.2
STEC 2 logs above LoD {*stx2*}	-0.1	-0.1	-0.5	-0.2	-0.4
STEC 3 logs above LoD {*stx2*}	-0.1	0.1	-0.1	0.3	0.2
STEC at LoD {*stx1*}	-0.2	-1.6	-1.9	-1.0	-1.4
STEC 3X LoD {*stx1*}	-1.4	-0.7	-1.6	-0.6	-1.4
STEC 1 log above LoD {*stx1*}	-0.3	0.0	-1.0	-0.1	-0.3
STEC 2 logs above LoD {*stx1*}	-0.5	0.1	-0.6	-0.2	-0.5
STEC 3 logs above LoD {*stx1*}	-0.4	-0.1	-0.5	0.1	-0.2

*Test points that indicated potential stability issue

A paired t-test was performed on Ct values for each of five "non-fresh" conditions compared to FR ST/FR NA on the samples that tested positive (all samples tested positive at all testing points) for each PCR Mix.

Sample and Nucleic Acid Stability Results — Paired t-test to the FR ST/FR NA Testing Point										
Time point – SSC Mix										
FR ST/ FR NA	**FR ST/FZ NA**		**REF ST/FR NA***		**REF ST/FZ NA**		**FZ ST/FR NA***		**FZ ST/FZ NA***	
NA	p-value	Mean Ct Difference	p-value	Mean Ct Difference	p-value	Mean Ct Difference	p-value	Mean Ct Difference	p-value	Mean Ct Difference
	p=0.07	-0.2	p=0.000005	0.8	p=0.2	-0.2	p=0.0002	0.9	p=0.0003	1.2
Time point – STEC Mix										
FR ST/ FR NA	**FR ST/FZ NA***		**REF ST/FR NA**		**REF ST/FZ NA***		**FZ ST/FR NA**		**FZ ST/FZ NA***	
NA	p-value	Mean Ct Difference	p-value	Mean Ct Difference	p-value	Mean Ct Difference	p-value	Mean Ct Difference	p-value	Mean Ct Difference
	p=0.03	-0.4	p=0.3	-0.2	p=0.002	-0.9	p=0.9	0.0	p=0.01	-0.6

The data in the table above show that there are statistically significant differences between the testing points indicated in the boxes with an "*" and the original testing point (fresh stool and fresh nucleic acid). The "Mean Ct Difference to initial FR ST/FR NA Data" table shows that the largest mean difference is associated with the *C. jejuni* samples after they were frozen. A noticeable difference in the performance of simulated *Campylobacter* samples upon freezing has also been observed by comparing initial QC testing data for the Positive Matrix Control, and the Reproducibility samples. Even though statistically significant differences in Mean Ct do exist for each PCR Mix at different testing points via the paired t-test analysis, the ProGastro SSCS Assay is not a quantitative, but a qualitative assay. The fact that all samples tested positive for their correct target at all testing points meeting the requirement stated in the study plan (i.e., all samples must not test statistically significantly different ($p \leq 0.05$) from their fresh testing point in terms of positive/negative result) supports the claim that there are no significant negative effects due to freezing samples/nucleic acids. However, the observed negative effects of freezing samples with regard to *Campylobacter* detection are included in the "Sample Collection, Handling, and Storage" and the "Warnings and Precautions" sections of the package insert.

In conclusion, the results of this study demonstrate there is no significant negative effect on the ProGastro SSCS Assay in target organism detection due to storing samples at refrigerated temperature (2-8°C) for up to five days, samples undergoing a single freeze/thaw, or nucleic acids undergoing a single freeze/thaw. The data does show a marked, statistically significant difference in Mean Ct for the contrived *C. jejuni* samples once they are frozen and tested. This freezing effect had also been noted subsequent to performing this study. This phenomenon has also been described in literature in that *Campylobacter* is very sensitive to environmental stressors including freezing where it loses viability. In addition, the ProGastro SSCS Clinical Study data supports the use of frozen samples as the majority of Prospective Clinical Study samples were stored frozen prior to testing, and the performance of the ProGastro SSCS Assay is acceptable. This study results also support the use of frozen banked retrospective clinical samples in the evaluation of the ProGastro SSCS Assay to supplement the prospective clinical study data.

Reagent Stability and Freeze/Thaw Study

An Accelerated Stability study concluded that the ProGastro SSCS Assay Mixes (SSC Mix and STEC Mix) and controls (closed and open tubes) can be stored at ≤ -70°C for up to 18 months. Real time stability studies are currently ongoing at Gen-Probe Prodesse, Inc.

A Freeze/Thaw Study demonstrated that the SSC Mix and the STEC Mix can each undergo up to 10 freeze-thaws. The controls (Positive and Internal Controls) can undergo up to two freeze-thaws. For the controls, the ProGastro SSCS Assay Package Insert specifies that they should not undergo more than one freeze-thaw cycle, although internal studies demonstrated that up to two freeze-thaws of controls would not adversely affect performance.

d. Detection limit:

To determine and confirm the Limit of Detection (LoD) of the ProGastro SSCS Assay on the Cepheid SmartCycler II, an analytical study was carried out using fresh bacterial cultures for each detection target (*Salmonella, Shigella, Campylobacter* (*C. jejuni* and *C. coli* only), and Shiga Toxin producing *E. coli*, STEC). Analytical Sensitivity (LoD) is defined as the lowest concentration of bacteria detected ≥95% of the time.

Fresh bacterial cultures were used for both the LoD Determination and the LoD Confirmation studies, as well as plating for CFU/mL counting. The bacterial strains tested in the studies are outlined in the table below:

Limit of Detection Panel Strains	
Strain	**Strain ID**
Salmonella enterica serotype Typhi	ATCC6539
Salmonella enterica serotype Typhimurium	BAA-1603
Salmonella enterica serotype Enteritidis	BAA-1045
Shigella boydii	ATCC9207
Shigella dysenteriae	ATCC29027
Shigella flexneri	ATCC12025
Shigella sonnei	ATCC29029
Campylobacter jejuni	BAA-224
Campylobacter coli	ATCC43485
STEC O26:H/NM (*stx1-/stx2+*)	TW08569
STEC O157:H7 (*stx1+/stx2-*)	TW00975

The LoD Determination portion of this study included freshly cultured bacteria that were serially diluted, spiked into pooled negative stool matrix, and tested minimally at five concentrations: 1 log above, 0.5 log above, at, 0.5 logs below, and 1 log below the preliminary LoD level as predetermined during product development. Each bacterial strain was tested in quintuplicate extractions and real-time PCR reactions using either the SSC or STEC Mix (depending on the strain) for a total of five data points per bacterial concentration. LoD was estimated as the lowest concentration where 5/5

replicates were detected. The same bacterial dilutions were also cultured on the appropriate solid media for CFU/mL counting to enable calculation of LoDs in CFU/mL of stool and CFU/reaction. The estimated LoD for each strain was then confirmed by the generation of 20 independent samples/data points using the specific spiked stool concentration utilized during the LoD Determination portion of the study. For some of the strains, more than one concentration was included for the confirmation portion of the study, typically the two lowest concentrations that yielded 100% detection to ensure achievement of ≥95% detection for each strain. Each of the 20 replicates was subject to the entire test system from sample preparation and extraction to PCR with either the SSC or STEC Mix (depending on the strain). All samples were extracted using the bioMérieux NucliSENS easyMAG Instrument. In the event that the initial estimated LoD concentration was not confirmed (i.e., <19 replicates were positive), the LoD confirmation was repeated using the next half-log higher concentration. At least 95% (19/20) of the replicates were required to test positive to confirm the LoD for each bacterial target.

The table below outlines the results of the LoD Determination portion of the study. Concentrations in **bold** in the table below were tested during the LoD Confirmation portion of the study.

LoD Determination (Estimation) Results					
Bacterial Strain	**Dilution Conc.**	**Average C_T**	**Standard Deviation**	**%CV**	**PCR Replicates Detected**
Salmonella enterica serotype Typhi*	10^{-6}	38.1	0.6	1.6	4/5
	$10^{-6.5}$	39.6	0.6	1.5	3/5
	10^{-7}	NA	NA	NA	0/5
	$10^{-7.5}$	NA	NA	NA	0/5
	10^{-8}	NA	NA	NA	0/5
Salmonella enterica serotype Typhimurium*	10^{-6}	37.0	0.5	1.3	4/5
	$10^{-6.5}$	38.8	1.6	4.2	3/5
	10^{-7}	NA	NA	NA	0/5
	$10^{-7.5}$	40.6	NA	NA	1/5
	10^{-8}	NA	NA	NA	0/5
Salmonella enterica serotype Enteritidis	10^{-5}	33.8	0.3	0.8	5/5
	$10^{-5.5}$	**35.7**	**0.3**	**0.7**	**5/5**
	10^{-6}	**37.2**	**1.2**	**3.1**	**5/5**
	$10^{-6.5}$	38.2	0.8	2.1	4/5
	10^{-7}	39.1	0.5	1.4	4/5
Shigella boydii	$10^{-4.5}$	30.3	0.2	0.7	5/5
	10^{-5}	31.6	0.2	0.7	5/5
	$10^{-5.5}$	33.6	0.4	1.1	5/5
	10^{-6}	**34.8**	**0.5**	**1.5**	**5/5**
	$10^{-6.5}$	**36.3**	**0.4**	**1.1**	**5/5**
	10^{-7}	NA	NA	NA	0/5
Shigella dysenteriae	**10^{-6}**	**35.1**	**0.3**	**0.7**	**5/5**
	$10^{-6.5}$	36.9	NA	NA	1/5
	10^{-7}	NA	NA	NA	0/5
	$10^{-7.5}$	NA	NA	NA	0/5
	10^{-8}	NA	NA	NA	0/5
Shigella flexneri	10^{-6}	32.5	0.2	0.6	5/5
	$10^{-6.5}$	**34.7**	**0.4**	**1.3**	**5/5**
	10^{-7}	**34.8**	**0.3**	**1.0**	**5/5**
	$10^{-7.5}$	36.6	0.3	0.8	2/5

LoD Determination (Estimation) Results					
Bacterial Strain	**Dilution Conc.**	**Average C_T**	**Standard Deviation**	**%CV**	**PCR Replicates Detected**
	10^{-8}	NA	NA	NA	0/5
Shigella sonnei	10^{-5}	32.3	0.2	0.6	5/5
	$10^{-5.5}$	33.4	0.2	0.7	5/5
	10^{-6}	**35.8**	**0.7**	**2.1**	**5/5**
	$10^{-6.5}$	36.0	0.3	0.8	2/5
	10^{-7}	NA	NA	NA	0/5
Campylobacter jejuni	10^{-6}	36.4	0.4	1.1	5/5
	$10^{-6.5}$	**39.2**	**0.3**	**0.7**	**5/5**
	10^{-7}	40.1	0.7	1.8	4/5
	$10^{-7.5}$	42.0	1.2	2.9	3/5
	10^{-8}	43.6	0.3	0.7	3/5
Campylobacter coli	10^{-6}	35.7	0.4	1.2	5/5
	$10^{-6.5}$	37.8	0.6	1.7	5/5
	10^{-7}	**39.1**	**1.0**	**2.7**	**5/5**
	$10^{-7.5}$	40.1	0.9	2.2	4/5
	10^{-8}	NA	NA	NA	0/5
STEC O26:H/NM (*stx1-/stx2+*)	$10^{-4.5}$	34.0	0.3	0.8	5/5
	10^{-5}	36.2	0.3	0.8	5/5
	$10^{-5.5}$	**39.0**	**0.7**	**1.9**	**5/5**
	10^{-6}	39.0	0.3	0.8	4/5
	$10^{-6.5}$	38.9	1.4	3.6	2/5
STEC O157:H7 (*stx1+/stx2-*)	$10^{-4.5}$	32.7	0.3	0.8	5/5
	10^{-5}	34.0	0.4	1.2	5/5
	$10^{-5.5}$	37.9	1.0	2.6	5/5
	10^{-6}	**38.3**	**1.0**	**2.6**	**5/5**
	$10^{-6.5}$	40.1	1.2	2.9	3/5

* *S. Typhi* and *S. Typhimurium* were confirmed at ½ log higher dilutions in the LoD Confirmation portion of the study.

The results of the LoD Confirmation portion of the study are shown in the table below. Concentrations in **bold** are the confirmed LoD per strain.

LoD Confirmation Results								
Bacterial Strain	**Dilution Conc.**	**Average C_T**	**Standard Deviation**	**%CV**	**Min CT**	**Max Ct**	**Sample Replicates Detected**	**% Detected**
Salmonella enterica serotype Typhi	**$10^{-5.5}$**	**36.4**	**0.5**	**1.5**	**35.4**	**37.6**	**20/20**	**100%**
Salmonella enterica serotype Typhimurium	**$10^{-5.5}$**	**35.4**	**0.5**	**1.4**	**34.5**	**36.5**	**20/20**	**100%**
Salmonella enterica serotype Enteritidis	10^{-6}	37.7	0.9	2.4	36.8	39.3	18/20	90%
	$10^{-5.5}$	**35.9**	**0.5**	**1.3**	**35.2**	**37.2**	**20/20**	**100%**
Shigella boydii	$10^{-6.5}$	36.3	0.4	1.2	35.5	36.6	6/20	30%
	10^{-6}	**35.2**	**0.5**	**1.5**	**34.4**	**36.7**	**19/20**	**95%**
Shigella dysenteriae	**10^{-6}**	**35.4**	**0.5**	**1.5**	**34.6**	**36.6**	**20/20**	**100%**
Shigella flexneri	10^{-7}	36.1	0.5	1.4	35.0	37.0	17/20	85%
	$10^{-6.5}$	**33.9**	**0.5**	**1.5**	**32.9**	**34.8**	**20/20**	**100%**
Shigella sonnei	**10^{-6}**	**35.8**	**0.7**	**1.8**	**34.6**	**36.8**	**20/20**	**100%**
Campylobacter jejuni	**$10^{-6.5}$**	**39.1**	**0.8**	**2.0**	**37.7**	**41.3**	**20/20**	**100%**
Campylobacter coli	**10^{-7}**	**38.9**	**0.8**	**2.1**	**37.7**	**41.1**	**20/20**	**100%**
STEC O26:H/NM (*stx1-/stx2+*)	**$10^{-5.5}$**	**37.5**	**0.6**	**1.5**	**36.7**	**38.6**	**19/20†**	**95%**
	10^{-5}	35.8	0.5	1.3	35.1	37.2	20/20	100%
STEC O157:H7 (*stx1+/stx2-*)	**$10^{-5.5}$**	**37.0**	**1.0**	**2.6**	**34.6**	**39.0**	**20/20**	**100%**

†One replicate required retesting in duplicate due to an initial result of "Unresolved" (thus generating 2 Ct values for the same sample) and a total of 20 data points.

In conclusion, the LoDs of the ProGastro SSCS Assay calculated as CFU/mL stool and CFU/reaction are summarized in table below:

LoD Summary		
Strain	**LoD concentration in CFU/mL stool**	**LoD concentration in CFU/reaction**
Salmonella enterica serotype Typhi	1.63×10^3 CFU/mL	0.74 CFU/reaction
Salmonella enterica serotype Typhimurium	2.25×10^4 CFU/mL	10.21 CFU/reaction
Salmonella enterica serotype Enteritidis	2.47×10^4 CFU/mL	11.21 CFU/reaction
Shigella boydii	6.60×10^2 CFU/mL	0.30 CFU/reaction
Shigella dysenteriae	1.03×10^3 CFU/mL	0.47 CFU/reaction
Shigella flexneri	3.11×10^3 CFU/mL	1.42 CFU/reaction
Shigella sonnei	1.46×10^3 CFU/mL	0.66 CFU/reaction
Campylobacter jejuni	1.36×10^3 CFU/mL	0.62 CFU/reaction
Campylobacter coli	1.99×10^3 CFU/mL	0.91 CFU/reaction
STEC O26:H/NM (*stx1-/stx2+*)	9.27×10^3 CFU/mL	4.21 CFU/reaction
STEC O157:H7 (*stx1+/stx2-*)	1.66×10^4 CFU/mL	7.55 CFU/reaction

e. Analytical Reactivity:

The Analytical Reactivity study was performed to determine whether the ProGastro SSCS Assay is able to detect a variety of strains (reactivity panel) that represent the genetic diversity of each of the targeted organisms. This study expanded upon the Analytical Sensitivity Study by determining whether different strains of the same organism (*Salmonella, Shigella, Campylobacter* and Shiga Toxins 1 and 2 (*stx1* and *stx2*) producing strains of *E. coli*) can be detected at similar concentrations, near the assay detection limit.

In addition to the eleven strains used for the LoD Study, the analytical reactivity of the ProGastro SSCS Assay was evaluated with multiple strains of bacteria. The strains were selected to include those isolated primarily from human infections (when available) and various geographical locations in order to incorporate the genetic variation that may be encountered by ProGastro SSCS users. A Limit of Detection (LoD) was estimated for most of the strains during pre-verification studies (Note: *Salmonella bongori* is not reactive and, thus, did not have a preliminary LoD). The strains used in this study were tested at 2x the estimated LoD, or at the highest concentration possible for the *Salmonella bongori* strain. One sample was generated for each strain by spiking cultured and quantified bacteria into aliquots of a ProGastro SSCS negative stool matrix pool. The Gastro RNA/DNA Internal Control (GIC) was added to each sample just prior to extraction on the bioMérieux NucliSENS easyMAG and each resultant nucleic acid sample was tested in triplicate PCR reaction on the Cepheid SmartCycler II (Dx Software Version 3.0).

Mean Cts and standard deviations for reactive strains were calculated and presented in the following table:

ProGastro SSCS Analytical Reactivity Study Results							
Strain	**Target**	**Concentration Tested***	**SSC Mix**			**STEC Mix**	
			Campy. Mean Ct±SD	**Sal. Mean Ct±SD**	**Shi. Mean Ct±SD**	***stx2* Mean Ct±SD**	***stx1* Mean Ct±SD**
Salmonella bongori 43975	*Salmonella*	9.25×10^8 CFU/mL	-	Not Reactive	-	-	-
Salmonella enterica subsp. *enterica* ser. Paratyphi 8759	*Salmonella*	2×10^4 CFU/mL	-	36.8±0.9	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium 19585	*Salmonella*	2×10^4 CFU/mL	-	35.5±0.4	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium 14028	*Salmonella*	2×10^4 CFU/mL	-	36.7±0.6	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium BAA-189	*Salmonella*	2×10^4 CFU/mL	-	36.9±0.2	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium BAA-191	*Salmonella*	2×10^4 CFU/mL	-	35.3±0.4	-	-	-
Salmonella enterica subsp. *enterica* ser. Typhimurium BAA-215	*Salmonella*	2×10^4 CFU/mL	-	35.3±0.3	-	-	-
Salmonella enterica subsp. *enterica* ser. Enteritidis 13076	*Salmonella*	2×10^5 CFU/mL	-	32.4±0.2	-	-	-
Salmonella enterica subsp. *enterica* ser. Enteritidis BAA-708	*Salmonella*	2×10^4 CFU/mL	-	37.0±0.2	-	-	-
Salmonella enterica subsp. *enterica* ser. Enteritidis 4931	*Salmonella*	2×10^4 CFU/mL	-	35.8±0.1	-	-	-
Salmonella enterica subsp. *enterica* ser. Enteritidis 6961	*Salmonella*	2×10^4 CFU/mL	-	35.7±0.6	-	-	-
Salmonella enterica subsp. *enterica* ser. Newport 6962	*Salmonella*	2×10^4 CFU/mL	-	36.3±0.2	-	-	-
Salmonella enterica subsp. *enterica* ser. Newport 27869	*Salmonella*	2×10^3 CFU/mL	-	38.8±0.7	-	-	-
Salmonella enterica subsp. *enterica* ser. Heidelberg 8326	*Salmonella*	2×10^4 CFU/mL	-	36.7±0.6	-	-	-
Salmonella enterica subsp. *enterica* ser. Javiana BAA-1593	*Salmonella*	2×10^6 CFU/mL	-	38.3±0.4	-	-	-
Salmonella enterica subsp. *enterica* ser. Montevideo BAA-710	*Salmonella*	2×10^4 CFU/mL	-	35.9±0.7	-	-	-
Salmonella enterica subsp. *Enterica* ser. Oranienburg 9239	*Salmonella*	1×10^4 CFU/mL	-	36.9±0.3	-	-	-
Salmonella enterica subsp. *Enterica* ser. Saintpaul 9712	*Salmonella*	1×10^4 CFU/mL	-	36.1±1.2	-	-	-
Salmonella enterica subsp. *Enterica* ser. Muenchen BAA-1674	*Salmonella*	1×10^4 CFU/mL	-	36.9±1.5	-	-	-

ProGastro SSCS Analytical Reactivity Study Results							
Strain	**Target**	**Concentration Tested***	**SSC Mix**			**STEC Mix**	
			Campy. Mean Ct±SD	**Sal. Mean Ct±SD**	**Shi. Mean Ct±SD**	***stx2* Mean Ct±SD**	***stx1* Mean Ct±SD**
Salmonella enterica subsp. *Enterica* ser. Braenderup BAA-664	*Salmonella*	$1x10^4$ CFU/mL	-	36.4±0.3	-	-	-
Salmonella enterica subsp. *Enterica* ser. Infantis BAA-1675	*Salmonella*	$1x10^4$ CFU/mL	-	35.8±0.3	-	-	-
Salmonella enterica subsp. *Enterica* ser. Thompson 8391	*Salmonella*	$1x10^4$ CFU/mL	-	36.3±0.5	-	-	-
Salmonella enterica subsp. *Enterica* ser. Agona BAA-707	*Salmonella*	$1x10^4$ CFU/mL	-	36.1±0.7	-	-	-
Salmonella enterica subsp. *Enterica* ser. Bareilly (clinical isolate)	*Salmonella*	$1x10^4$ CFU/mL	-	35.6±0.4	-	-	-
Salmonella enterica subsp. *Enterica* ser. Hadar 51956	*Salmonella*	$1x10^4$ CFU/mL	-	36.0±0.4	-	-	-
Salmonella enterica subsp. *indica* (genomic DNA) BAA-1578D-5	*Salmonella*	20 fg/µL	-	37.8±0.3	-	-	-
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	-	-	35.4±0.4	-	-
Shigella dysenteriae 29026†	*Shigella*	$2x10^3$ CFU/mL	-	-	35.8±0.5	-	39.8±0.5
Shigella flexneri 12022	*Shigella*	$2x10^4$ CFU/mL	-	-	34.0±0.2	-	-
Shigella flexneri 25875	*Shigella*	$2x10^4$ CFU/mL	-	-	32.3±0.1	-	-
Shigella sonnei 29031	*Shigella*	$2x10^4$ CFU/mL	-	-	34.0±0.1	-	-
Shigella sonnei 9290	*Shigella*	$2x10^4$ CFU/mL	-	-	34.6±0.6	-	-
Shigella sonnei 11060	*Shigella*	$2x10^4$ CFU/mL	-	-	32.6±0.3	-	-
Shigella sonnei 25931	*Shigella*	$2x10^3$ CFU/mL	-	-	34.5±0.2	-	-
Shigella sonnei 29030	*Shigella*	$2x10^4$ CFU/mL	-	-	33.4±0.1	-	-

ProGastro SSCS Analytical Reactivity Study Results							
Strain	**Target**	**Concentration Tested***	**SSC Mix**			**STEC Mix**	
			Campy. Mean Ct±SD	**Sal. Mean Ct±SD**	**Shi. Mean Ct±SD**	***stx2* Mean Ct±SD**	***stx1* Mean Ct±SD**
Shigella sonnei 29930	*Shigella*	$2x10^4$ CFU/mL	-	-	33.5±0.2	-	-
Shigella flexneri 700930	*Shigella*	$2x10^4$ CFU/mL	-	-	33.7±0.5	-	-
Campylobacter jejuni subsp. *jejuni* 29428	*Campylobacter*	$2x10^3$ CFU/mL	37.7±0.3	-	-	-	-
Campylobacter jejuni subsp. *jejuni* 33291	*Campylobacter*	$2x10^3$ CFU/mL	38.8±2.7	-	-	-	-
Campylobacter jejuni subsp. *jejuni* BAA-222	*Campylobacter*	$2x10^3$ CFU/mL	38.8±0.3	-	-	-	-
Campylobacter jejuni subsp. *jejuni* BAA-223	*Campylobacter*	$2x10^3$ CFU/mL	38.6±0.5	-	-	-	-
Campylobacter jejuni subsp. *jejuni* BAA-219	*Campylobacter*	$2x10^3$ CFU/mL	38.8±0.2	-	-	-	-
Campylobacter jejuni subsp. *jejuni* BAA-220	*Campylobacter*	$2x10^7$ CFU/mL	37.8±0.7	-	-	-	-
Campylobacter jejuni subsp. *doylei* BAA-1458	*Campylobacter*	$2x10^5$ CFU/mL	35.4±0.1	-	-	-	-
Campylobacter coli BAA-370	*Campylobacter*	$2x10^4$ CFU/mL	37.8±0.2	-	-	-	-
Campylobacter coli BAA-371	*Campylobacter*	$2x10^4$ CFU/mL	34.4±0.1	-	-	-	-
Campylobacter coli BAA-372	*Campylobacter*	$2x10^5$ CFU/mL	36.6±0.2	-	-	-	-
Campylobacter coli 33559	*Campylobacter*	$2x10^5$ CFU/mL	35.1±0.4	-	-	-	-
E. coli DEC10B (O26:H11)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	37.8±0.3
E. coli 97-3250 (O26:H11)	*stx 1 & stx 2*	$2x10^5$ CFU/mL	-	-	-	38.2±1.4	37.3±0.5
E. coli DA-21 (O45:H/NM)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	35.7±0.5
E. coli MI03-19 (O45:H2)	*stx1*	$2x10^5$ CFU/mL	-	-	-	-	36.5±0.6
E. coli MT#80 (O103:H2)	*stx1*	$1x10^5$ CFU/mL	-	-	-	-	37.5±1.3
E. coli 3215-99 (O111:H8)	*stx 1 & stx 2*	$2x10^4$ CFU/mL	-	-	-	37.7±0.8	36.6±0.8
E. coli RD8 (H111:O2)	*stx 2*	$2x10^5$ CFU/mL	-	-	-	36.6±0.2	-
E. coli 0201 9611 (O111:H11)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	36.8±0.4

ProGastro SSCS Analytical Reactivity Study Results							
Strain	**Target**	**Concentration Tested***	**SSC Mix**			**STEC Mix**	
			Campy. Mean Ct±SD	**Sal. Mean Ct±SD**	**Shi. Mean Ct±SD**	***stx2* Mean Ct±SD**	***stx1* Mean Ct±SD**
E. coli DA-5 (O121:H19)	*stx 2*	$2x10^5$ CFU/mL	-	-	-	37.3±1.0	-
E. coli DA-1 (O121:H/NM)	*stx 2*	$2x10^5$ CFU/mL	-	-	-	38.9±1.4	-
E. coli GS G5578620 (O145:H/NM)	*stx1*	$2x10^4$ CFU/mL	-	-	-	-	39.0±1.0
E. coli IH 16 (O145:H/NT)	*stx 2*	$2x10^5$ CFU/mL	-	-	-	36.3±0.4	-
E. coli 7:85 (O157:H/N)	*stx 1 & stx 2*	$2x10^5$ CFU/mL	-	-	-	36.9±1.3	36.5±0.7
E. coli 93-111 (O157:H7)	*stx 1 & stx 2*	$2x10^6$ CFU/mL	-	-	-	36.4±0.9	37.7±0.5
E. coli DA-34 (O157:H/NM)	*stx1*	$2x10^5$ CFU/mL	-	-	-	-	39.8±0.7
E. coli EDL933 (O157:H7)	*stx 1 & stx 2*	$2x10^6$ CFU/mL	-	-	-	36.5±0.5	35.9±0.6
E. coli 1:361 (O157:H7)	*stx 2*	$2x10^4$ CFU/mL	-	-	-	34.8±0.3	-
E. coli DA-54 (O157:H/NM)	*stx 2*	$2x10^5$ CFU/mL	-	-	-	36.0±0.4	-
E. coli O104:H4 Genomic DNA¥ (O104:H4)	*stx2*	0.5pg/µL (2.5pg/rxn)	-	-	-	34.5±0.1	-
E. coli O118:H16, EK36 TW08644	*stx1*	$1x10^4$ CFU/mL	-	-	-	-	36.8±0.5
E. coli O118:H-, RW2030 TW06407	*stx1*	$1x10^4$ CFU/mL	-	-	-	-	37.4±0.8
E. coli O118:H16, RW1191 TW07879	*stx1 and stx2*	$1x10^4$ CFU/mL	-	-	-	35.0±0.2	33.4±0.5

* If more than one concentration was tested, the lowest concentration that tested positive for 3/3 reactions was reported.

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

¥ Genomic DNA was used to test the reactivity of the SSC and STEC Mixes towards the enterohemorragic *E. coli* (EHEC) strain associated with the outbreak in Europe during the Spring/Summer of 2011.

In silico analysis of a single sequenced strain available for *Salmonella enterica* subsp. *Enterica* serotype Schwarzengrund demonstrated 100% homology of the ProGastro SSCS Assay *Salmonella* oligonucleotide primers and probes with the Schwarzengrund strain.

In conclusion, all of the strains used for this study with the exception of *Salmonella bongori*, *Salmonella enterica* subsp. *salamae*, *Salmonella enterica* subsp. *arizonae*, *Salmonella enterica* subsp. *diarizonae*, and *Salmonella enterica* subsp. *houtenae*

tested positive with the ProGastro SSCS Assay. All these exceptions are documented as non-reactive strains in the ProGastro SSCS Assay Instructions for Use (Package Insert) as limitations.

Shigella dysenteriae strains typically contain a Shiga Toxin, therefore this strain tested positive with both the STEC and SSC Mixes. This is documented in the ProGastro SSCS Assay Instructions for Use (Package Insert) as a limitation: "*Shigella dysenteriae* strains typically contain a Shiga Toxin, but not always. A positive call of STEC *stx*1/*stx*2 may be from either STEC or *Shigella dysenteriae*".

f. Analytical Specificity/Cross-reactivity Evaluation:

To determine the analytical specificity of the ProGastro SSCS Assay, an analytical cross-reactivity evaluation was carried out using cultured and titered strains of common gastrointestinal pathogens that are genetically related, cause similar disease states as the ProGastro SSCS Assay target organisms (*Salmonella*, *Shigella*, *Campylobacter* and Shiga Toxin producing *E. coli* (STEC)), or are commonly found in stool.

Analytical specificity is defined as an assay's ability to exclusively identify the target organisms while not cross-reacting with other organisms in a sample. The Analytical Specificity of the ProGastro SSCS Assay was determined with a panel of 54 organisms. *Cyclospora cayetanensis* was subject to an *in silico* analysis only because it was not available for "wet" testing.

All the ProGastro SSCS Assay target organisms (*Salmonella, Shigella, Campylobacter jejuni, Campylobacter coli,* and STEC) in the specificity panel were serially diluted in Stool Preservation and Transport Media (SPTM) and spiked into a ProGastro SSCS negative stool matrix pool at high concentrations of 10^6-10^7 CFU/mL. This was done to test the specificity of each mix for the target organisms and to also demonstrate that the assay is functioning as expected. The remaining members of the Specificity Panel were spiked into negative stool matrix pool at concentrations of $10^{3.5} – 10^{7.5}$ $TCID_{50}$/mL for viral targets and $10^6 – 8.8x10^8$ CFU/mL for bacterial and fungal targets. The Specificity Panel Organisms were not diluted prior to spiking into stool in order to test them at the highest concentration possible. Norovirus was only available in the form of a positive sample (raw stool) obtained from a Public Health Laboratory. This sample was diluted in SPTM according to the manufacturer's instructions and then processed according to the ProGastro SSCS Instructions for use.

Analytical Specificity Study results are presented in the table below. *In silico* analysis of the *Cyclospora cayetanensis* genome showed that each primer and probe included with the mixes had no similarity to the organism.

Analytical Specificity Study Results (Mean and Standard Deviation)						
Organism	**Conc. Tested**	***Campy.* Detection**	***Salmonella* Detection**	***Shigella* Detection**	***stx 1* Detection**	***stx 2* Detection**
Bacteria						
Salmonella Enteritidis	$1x10^6$ CFU/mL	-	29.6±0.1	-	-	-
Campylobacter jejuni	$1x10^6$ CFU/mL	28.3±0.1	-	-	-	-
Campylobacter coli	$1x10^6$ CFU/mL	31.5±0.1	-	-	-	-
Shigella sonnei	$1x10^6$ CFU/mL	-	-	27.9±0.3	-	-
STEC O157:H7 Strain 93-111	$1x10^7$ CFU/mL	-	-	-	26.7±0.1	27.8±0.1
Aeromonas hydrophila	$1.5x10^7$ CFU/mL	-	-	-	-	-
Bacillus cereus	$1.5x10^7$ CFU/mL	-	-	-	-	-
Bacteroides fragilis	$1.5x10^7$ CFU/mL	-	-	-	-	-
Campylobacter lari	$2.5x10^{-2}$ Dilution from Raw Stool Clinical Specimen*	-	-	-	-	-
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	-	-	21.6±0.1	-	-
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	-	-	-	-	-

Analytical Specificity Study Results (Mean and Standard Deviation)						
Organism	**Conc. Tested**	***Campy.* Detection**	***Salmonella* Detection**	***Shigella* Detection**	***stx 1* Detection**	***stx 2* Detection**
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	-	-	-	-	-
Viruses						
Adenovirus Type 40	$1.0x10^{5.5}$ $TCID_{50}$/mL	-	-	-	-	-
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**	-	-	-	-	-
Fungi						
Candida albicans	$1.66x10^7$ CFU/mL	-	-	-	-	-
Parasites						
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; nucleic acids from a positive clinical sample (Milwaukee City Public Health Lab Real Time PCR assay with a Ct value=20.5) were tested.

In conclusion, the ProGastro SSCS Assay did not react with any of the organisms listed in the table above, other than enteroinvasive *Escherichia coli* (EIEC). EIEC is

genetically very similar to *Shigella* and was detected by the SSC Mix as positive for *Shigella* as expected. This cross-reactivity is reported in the Package Insert as a limitation. No cross-reactivity was observed between the two Mixes (SSC and STEC). The ProGastro SSCS Assay demonstrates no cross reactivity with the organisms that are commonly found in stool, genetically related or cause similar disease states as the ProGastro SSCS Assay target organisms.

g. Assay cut-off:

To establish the cutoff threshold value of all targets of the ProGastro SSCS Assay on the Cepheid SmartCycler II, negative stool samples and spiked stool samples containing varying levels of *Salmonella, Shigella, Campylobacter jejuni, Campylobacter coli* and Shiga Toxin producing *E. coli* (STEC, Shiga Toxin 1 and Shiga Toxin 2 genes) were used. The ProGastro SSCS Assay is comprised of two PCR mixes, one used to detect and differentiate *Salmonella, Shigella* and *Campylobacter* (*C. jejuni* and *C. coli* only, not differentiated) (SSC Mix) while the other detects and differentiates the Shiga Toxin 1 (*stx1*) and Shiga Toxin 2 (*stx2*) genes in bacteria such as Shiga Toxin producing *E. coli* (STEC Mix). The accuracy of the chosen Relative Fluorescent Units (RFU) threshold for each channel was confirmed with retrospective clinical samples with the objective to obtain ≥ 90% positive and negative agreement to reference methods.

The "cutoff value" represents the signal level, reported in RFU, at which a "positive" reaction rises significantly above the background or baseline of a "negative" reaction. If a sample exceeds the threshold in a detection channel during PCR, the sample is considered positive for that channel. If the sample does not exceed the threshold for a detection channel by the last PCR cycle, the sample is considered negative for that channel.

Sources of variability incorporated into the Cutoff Determination and Confirmation Study included: operators, unique samples, multiple bacterial and control dilutions, extractions and reagent lots. Cutoff values were determined upon completion of the Cutoff Determination Study which included testing of spiked stool samples and controls. The cutoff values were then verified in the Cutoff Confirmation Study against a set of retrospective clinical samples and controls.

Cutoff Determination Study:

The cutoff value of the ProGastro SSCS Assay was determined with a panel of negative and spiked samples prepared from 82 individual negative stool clinical samples that were tested by each of the two operators (n=164). Individual samples (n = 12) were spiked with each ProGastro SSCS target bacteria (*Salmonella, Shigella, C. jejuni, C. coli, stx1* and *stx 2*) at three logs above the preliminary Limit of Detection (LoD) concentration representing high positive stool samples. Individual samples (n = 36) were spiked with each of the six ProGastro SSCS targets at two times the

preliminary LoD concentration representing low positive stool samples. Thirty four (34) samples were left un-spiked (negatives).

The high and low positive samples, as well as 30 of the negative samples were spiked with the Gastro Internal Control (GIC). The GIC monitors for PCR inhibition as well as any reagent, procedural or instrumentation failure. Four negative samples were not spiked with the GIC.

Replicates of the SSCS Control and *C. coli* Control (SSC Mix only) were included with each PCR run to test for global errors (absence of reagents, instrument failure, etc.). The SSCS Control and *C. coli* Positive Controls did not require nucleic acid isolation and were diluted in molecular grade water just prior to set up of the PCR reactions. Six replicates of each positive control were included during set-up of the PCR reactions (*C. coli* Control only used in conjunction with the SSC Mix). Six Negative Controls (NC) consisting of Stool Preservation and Transport Media (SPTM, Para-Pak C&S) spiked with GIC were also tested. The NC served to monitor for contamination during the testing procedure.

Samples were extracted using the bioMérieux NucliSENS easyMAG. The extracted nucleic acids and the ProGastro SSCS and *C. coli* Controls, as well as GIC diluted in water were run using two different lots of both the SSC and STEC Mixes contained in the ProGastro SSCS Assay on the Cepheid SmartCycler II.

Initial cutoff values used for the Cutoff Determination Study were based on preliminary testing during product development.

Data analysis of the Cutoff Determination Study included 1) calculation of lower limit of the acceptable range for cutoff threshold (RFU) based on negative samples; and 2) calculation of upper limit of the acceptable range for cutoff threshold (RFU) based on spiked positive samples. For a given detection, a range of possible cutoffs was generated by determining lower and upper boundaries of the threshold, based on final RFUs that would minimize false positives and false negatives. A Receiver Operator Characteristics (ROC) analysis was performed for all of the channels utilized by the ProGastro SSCS Assay's SSC and STEC Mixes. In addition, population distribution and statistics were analyzed to aid in selecting a cutoff threshold. Histograms of the final RFU values were used to assess the nature of the distributions and the percentile rankings.

Acceptable ranges for the potential cutoff were generated in the following manners:

- The distributions of the negatives and positives were first assessed for normality. If the population distributions were normal, the averages and standard deviations of the final RFUs were calculated for all detections. For the negatives, the lower limit of the acceptable range for cutoff threshold is defined as the average final RFU plus three standard deviations, that is, the point at which 99.7% of true negatives are expected to fall below assuming normality. Similarly, for the

positives, the upper limit of the acceptable range for cutoff threshold is defined as the average final RFU minus three standard deviations. This is the point at which at least 99.7% of true positives are expected to be greater than, assuming normality.

- Population statistics were also analyzed for each detection channel. The lower limit of the cutoff threshold range is defined minimally as the 99.5% percentile from the quantile analysis for negative samples. This is the RFU value at which at least 99.5% of true negatives are expected to be lower than. For the positives, the upper limit of the cutoff threshold range is defined maximally as the 0.5% percentile. This is the RFU value at which at least 99.5% of true positives are expected to be greater than.

- A ROC curve analysis was generated and assessed for all channels used with the ProGastro SSCS Assay's SSC and STEC Mixes. ROC curves for the SSC Mix (*Campylobacter* (FAM), *Salmonella* (TET), *Shigella* (TxRed), and GIC (CY5)) and the STEC Mix (*stx2* (FAM), *stx1* (TET), and GIC (CY5)) cutoffs were generated. The threshold range that yielded the maximum accuracy was identified.

- The cutoff threshold ranges determined by statistical analysis were used in conjunction with all data to select the cutoff settings. The following tables present the cutoff ranges and settings chosen from the Cutoff Determination Study for the SSC and STEC Mixes.

Preliminary Cutoff Threshold Values for SSC Mix

Channel	Target	EndPt Threshold (RFU)		
		Preliminary Cutoff Used*	Cutoff Range	Cutoff Value Chosen from the Cutoff Determination Study
FAM	*Campylobacter*	60	14-77	60
TET	*Salmonella*	30	27-357	35
TxR	*Shigella*	25	3-47	25
Cy5	Gastro Internal Control	32	15-54	35

*established during assay development

Preliminary Cutoff Threshold Values for STEC Mix

Channel	Target	EndPt Threshold (RFU)		
		Preliminary Cutoff Used*	Cutoff Range	Cutoff Value Chosen from the Cutoff Determination Study
FAM	*stx2*	70	12-314	70
TET	*stx1*	40	14-387	40
Cy5	Gastro Internal Control	35	3-143	35

*established during assay development

Cutoff Confirmation Study:

The Cutoff Confirmation Study goals were to achieve ≥90 Percent Positive and ≥90 Percent Negative Agreement of the ProGastro SSCS Assay using the preliminary cutoff values chosen after the Cutoff Determination Study as compared to the reference method results using retrospective positive and negative clinical stool samples.

Data analysis of the Cutoff Confirmation Study included 1) calculation of percent positive agreement for all retrospective ProGastro SSCS Assay positive clinical samples; and 2) calculation of percent negative agreement for all retrospective ProGastro SSCS Assay negative clinical samples.

The results of the Cutoff Confirmation Study are presented in the table below:

Cutoff Confirmation Study Final Results					
Mix	**Detection**	**% Positive Agreement**	**95% Confidence Interval**	**% Negative Agreement**	**95% Confidence Interval**
SSC Mix	*Campylobacter*	100%	83.9-100%	100%	94.3-100%
	Salmonella	100%	83.9-100%	98.4%	91.7-99.7%
	Shigella	100%	83.9-100%	100%	94.3-100%
STEC Mix	STEC	100%	83.9-100%	100%	94.3-100%

The following tables present the final settings chosen after the Cutoff Confirmation Study for the SSC and STEC Mixes:

Final Cutoff Threshold Values for SSC Mix

Channel	**Target**	**Final Cutoff Value (EndPt Threshold in RFU) after the Cutoff Confirmation Study**
FAM	*Campylobacter*	60
TET	*Salmonella*	35
TxR	*Shigella*	25
Cy5	Gastro Internal Control	35

Final Cutoff Threshold Values for STEC Mix

Channel	**Target**	**Final Cutoff Value (EndPt Threshold in RFU) after the Cutoff Confirmation Study**
FAM	*stx2*	70
TET	*stx1*	40
Cy5	Gastro Internal Control	35

Additional Cutoff Validation Studies:

The final cutoff values confirmed in the Cutoff Confirmation Study were further validated during the clinical studies and all subsequent analytical studies.

h. Internal Control Interference:

Competitive inhibition of the ProGastro SSCS Assay due to the presence of the Gastro RNA/DNA Internal Control (GIC) was assessed in an analytical study. Simulated samples were tested with and without the GIC to determine if the presence of the GIC affected the detection of the Assay target organisms. For this study, one cultured and titered strain each of *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Campylobacter coli*, and Shiga Toxin producing *Escherichia coli* (STEC, *stx1+/stx2+*) were diluted and individually spiked into a negative stool pool at 2x and at 1 log below each strain's Limit of Detection (LoD) as estimated in the Analytical Reactivity Study. *Shigella* was also generated and tested at 2 logs below LoD. All dilution series were extracted in triplicate on the bioMérieux NucliSENS easyMAG.

The strains used for the GIC Interference Study are listed below:

- *Salmonella* Enteritidis ATCC 6961 (stock concentration 1.06×10^9 CFU/mL)
- *Shigella flexneri* ATCC 12022 (stock concentration 9.0×10^9 CFU/mL)
- *Campylobacter jejuni* ATCC 29428 (stock concentration 1.10×10^8 CFU/mL)
- *Campylobacter coli* ATCC BAA-371 (stock concentration 7.20x108 CFU/mL)
- STEC (*stx1+*/*stx2+*) STEC Center Accession# TW07926 (stock concentration 1.75×10^9 CFU/mL)

The extracted nucleic acids were tested by PCR in triplicate using the ProGastro SSCS Assay reagents on the Cepheid SmartCyler II. This resulted in a total of nine data points for each strain at each concentration spiked with the GIC and nine data points for each strain at each concentration without the GIC.

For the GIC to be considered "non-interfering", the following criteria were required to be met:

- The LoD of samples without GIC must be within one log of the samples with GIC.
- The mean Cts for concentrations with and without IC must differ by less than 3.3 Cts for all concentrations tested.

All data were analyzed and Mean Cts, Standard Deviation, % Coefficient of Variance, and the number of positive results were calculated for each strain at each concentration. The Mean Ct difference between the samples with and without GIC at each concentration was also calculated (See the table below).

GIC Interference Results										
Strain	**Conc. CFU/mL**	**Samples containing GIC**				**Samples without GIC**				**Mean Ct Difference (GIC – No GIC)**
		No. Positive	**Mean Ct**	**SD**	**%CV**	**No. Positive**	**Mean Ct**	**SD**	**%CV**	
Salmonella Enteritidis ATCC 6961 $1.06x10^9$ CFU/mL	$2x10^4$	9/9	34.5	0.3	1.0	9/9	34.7	0.3	0.9	-0.2
	$1x10^3$	5/9	39.3	0.5	1.3	8/9	39.5	0.9	2.2	-0.2
Shigella flexneri ATCC 12022 $9.0x10^9$ CFU/mL	$2x10^4$	9/9	34.1	0.4	1.2	9/9	34.1	0.3	0.8	0.0
	$1x10^3$	0/9	NA	NA	NA	0/9	NA	NA	NA	NA
Campylobacter jejuni ATCC 29428 $1.10x10^8$ CFU/mL	$2x10^3$	9/9	37.2	0.3	0.7	9/9	37.6	0.3	0.9	-0.4
	$1x10^2$	7/9	40.7	0.8	1.9	8/9	41.2	0.6	1.5	-0.5
Campylobacter coli ATCC BAA-371 $7.20x10^8$ CFU/mL	$2x10^4$	9/9	34.0	0.4	1.1	9/9	34.4	0.3	0.8	-0.4
	$1x10^3$	9/9	37.4	0.7	2.0	9/9	37.6	0.5	1.3	-0.2
	$1x10^2$	4/9	40.0	0.5	1.4	8/9	40.3	0.9	2.3	-0.3
STEC (*stx1+/stx2+*) Acc# TW07926 $1.75x10^9$ CFU/mL ***stx 2* Detection**	$2x10^4$	9/9	35.3	0.3	0.9	9/9	36.3	0.6	1.7	-1.0
	$1x10^3$	8/9	39.5	1.2	3.0	6/9	39.9	0.7	1.8	-0.4
STEC (*stx1+/stx2+*) Acc# TW07926 $1.75x10^9$ CFU/mL ***stx 1* Detection**	$2x10^4$	9/9	35.4	0.6	1.8	9/9	35.6	0.6	1.7	-0.2
	$1x10^3$	7/9	39.6	2.0	5.0	6/9	40.1	1.4	3.4	-0.5

No competitive inhibition near the assay LoD was observed for detection of *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Campylobacter coli*, and STEC (*stx1+/stx2+*) in the presence GIC using the ProGastro SSCS Assay. The assay demonstrated equivalent detection of LoD concentrations for the different target strains with Mean Ct differences for each strain and concentration (with and without the GIC) ranging from -1.0 to 0.0.

i. Interfering Substances:

An analytical study was carried out to evaluate and assess the potential inhibitory effects of exogenous and endogenous substances that may be present in stool samples on the ProGastro SSCS Assay.

This study examined whether a panel of potential inhibitory endogenous and exogenous substances (The Interfering Substances Panel) affects the performance of the ProGastro SSCS Assay. The panel consisted of endogenous substances (blood, mucus, and fecal fats), over the counter and prescription medicines (antacids, laxatives, anti-diarrheal medications, antibiotics, etc.), and miscellaneous substances (such as spermicidal lubricant and moist towelette residue) that could potentially be found in stool samples. Twenty (20) aliquots of negative stool matrix were spiked with a single cultured and titered strain of *Campylobacter coli* (CC), 20 aliquots were spiked with a single strain of STEC (*stx1+/stx2+*), 20 aliquots were dual-spiked with a single cultured and titered strain of *Salmonella* (Sal) and a single strain of *Campylobacter jejuni* (CJ), and 20 aliquots were spiked with a single strain *Shigella* (Shi) only. All organisms were tested at a final concentration of 2x Limit of Detection (LoD). Each interfering substance was then spiked into a single aliquot each of CC spiked stool, STEC spiked stool, Sal/CJ spiked stool, and Shi spiked stool. Interfering

substances were added at high clinically relevant concentrations. One aliquot from each spiked sample type was not spiked with any interference substance for comparison purposes (control).

The Interfering Substances Panel samples and the controls were extracted on the bioMériuex NucliSENS easyMAG and samples tested in triplicate on a Cepheid Smartcycler II using one lot of ProGastro SSCS reagents.

Interfering Substances results are presented in the table below:

Mean/Standard Deviations of C_t Values for All Targets							
Substance #	**Substance Brand Name**	***C. coli*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***C. jejuni*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***Salmonella*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***Shigella*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***stx1*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***stx2*** Mean ± SD (Mean Ct Difference: Substance – No substance)
NS (No Substance Control)	NA	**34.6** ± 0.4	**37.1** ± 0.6	**35.0** ± 0.2	**34.9** ± 0.3	**35.8** ± 0.4	**36.3** ± 0.9
1	Mycostatin Vaginal Cream	**34.7** ± 0.1 (0.1)	**37.4** ± 0.1 (0.3)	**34.9** ± 0.4 (-0.1)	**35.0** ± 0.3 (0.1)	**35.5** ± 0.5 (-0.3)	**35.8** ± 0.4 (-0.5)
2	Cortizone 10	**34.4** ± 0.2 (-0.2)	**37.0** ± 0.4 (-0.1)	**34.8** ± 0.2 (-0.2)	**34.7** ± 0.4 (-0.2)	**36.1** ± 0.6 (0.3)	**36.0** ± 1.0 (-0.3)
3	Preparation H	**34.5** ± 0.1 (-0.1)	**37.4** ± 0.4 (0.3)	**34.9** ± 0.7 (-0.1)	**34.7** ± 0.4 (-0.2)	**36.4** ± 0.2 (0.6)	**36.2** ± 0.5 (-0.1)
4	TUMS	**34.5** ± 0.2 (-0.1)	**36.9** ± 0.2 (-0.2)	**35.3** ± 0.2 (0.3)	**34.5** ± 0.6 (-0.4)	**35.7** ± 0.7 (-0.1)	**36.7** ± 0.4 (0.4)
5	Amphojel Suspension	**34.6** ± 0.2 (0.0)	**37.9** ± 0.8 (0.8)	**35.5** ± 0.8 (0.5)	**34.9** ± 0.1 (0.0)	**36.2** ± 0.8 (0.4)	**36.4** ± 0.3 (0.1)
6	Phillips Milk of Magnesia	**34.0** ± 0.2 (-0.6)	**36.9** ± 0.4 (-0.2)	**34.5** ± 0.3 (-0.5)	**34.9** ± 0.1 (0.0)	**35.9** ± 0.4 (0.1)	**36.6** ± 0.9 (0.3)
7	CVS Mineral Oil Enema	**34.1** ± 0.2 (-0.5)	**36.8** ± 0.2 (-0.3)	**35.2** ± 0.1 (0.2)	**34.4** ± 0.3 (-0.5)	**36.1** ± 0.2 (0.3)	**35.9** ± 1.1 (-0.4)
8	Rowasa	**34.3** ± 0.2 (-0.3)	**37.0** ± 0.6 (-0.1)	**35.2** ± 0.7 (0.2)	**34.5** ± 0.3 (-0.4)	**35.5** ± 0.6 (-0.3)	**35.8** ± 0.6 (-0.5)
9	Ortho Options Contraceptive Gel	**34.3** ± 0.0 (-0.3)	**37.0** ± 0.5 (-0.1)	**35.5** ± 0.2 (0.5)	**34.3** ± 0.3 (-0.6)	**36.1** ± 0.4 (0.3)	**36.0** ± 0.5 (-0.3)
10	Imodium	**34.5** ± 0.1 (0.1)	**37.4** ± 0.2 (0.3)	**34.9** ± 0.3 (-0.1)	**34.4** ± 0.2 (-0.5)	**35.9** ± 0.3 (0.1)	**36.0** ± 0.6 (-0.3)
11	Pepto Bismol	**34.6** ± 0.1 (0.0)	**37.1** ± 0.4 (0.0)	**35.1** ± 0.3 (0.1)	**35.4** ± 0.6 (0.5)	**36.2** ± 0.3 (0.4)	**37.1** ± 0.7 (0.8)
12	Ex-Lax	**34.4** ± 0.2 (-0.2)	**37.0** ± 0.2 (-0.1)	**34.8** ± 0.7 (-0.2)	**35.5** ± 0.2 (0.6)	**35.9** ± 0.5 (0.1)	**36.0** ± 0.6 (-0.3)
13	Flagyl	**34.5** ± 0.1 (-0.1)	**37.2** ± 0.1 (0.1)	**35.1** ± 0.2 (0.1)	**34.9** ± 0.5 (0.0)	**36.3** ± 0.3 (0.5)	**35.9** ± 0.7 (-0.4)
14	Vancocin	**34.5** ± 0.1 (-0.1)	**37.2** ± 0.4 (0.1)	**35.5** ± 0.4 (0.5)	**34.9** ± 0.2 (0.0)	**35.1** ± 0.3 (-0.7)	**37.1** ± 1.0 (0.8)
15	Aleve	**34.9** ± 0.3 (0.3)	**37.6** ± 0.6 (0.5)	**36.0** ± 1.0 (1.0)	**34.7** ± 0.2 (-0.2)	**35.7** ± 0.4 (-0.1)	**35.8** ± 0.4 (-0.5)
16	Wet Ones	**34.4** ± 0.3 (-0.2)	**37.2** ± 0.2 (0.1)	**35.3** ± 0.3 (0.3)	**34.9** ± 0.5 (0.0)	**36.0** ± 0.2 (0.2)	**36.2** ± 0.7 (-0.1)
17	Palmitic/Stearic Acid	**34.8** ± 0.2 (0.2)	**37.0** ± 0.4 (-0.1)	**35.4** ± 0.4 (0.4)	**34.9** ± 0.4 (0.0)	**36.9** ± 1.1 (1.1)	**35.7** ± 0.5 (-0.6)
18	Blood	**34.8** ± 0.4 (0.2)	**37.4** ± 0.2 (0.3)	**35.8** ± 0.6 (0.8)	**35.8** ± 0.8 (0.9)	**36.6** ± 0.2 (0.8)	**36.5** ± 0.5 (0.2)

Mean/Standard Deviations of C_t Values for All Targets							
Substance #	**Substance Brand Name**	***C. coli*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***C. jejuni*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***Salmonella*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***Shigella*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***stx1*** Mean ± SD (Mean Ct Difference: Substance – No substance)	***stx2*** Mean ± SD (Mean Ct Difference: Substance – No substance)
19	Mucin	**34.4** ± 0.2 (-0.2)	**37.1** ± 0.2 (0.0)	**36.1** ± 0.5 (1.1)	**35.7** ± 0.3 (0.8)	**35.9** ± 0.5 (0.1)	**36.2** ± 0.9 (-0.1)

The ProGastro SSCS Assay did not appear to be affected by the presence of any endogenous or exogenous potential PCR inhibitors tested in this study.

j. Microbial Interference:

An analytical study was conducted to evaluate the ProGastro SSCS Assay for potential interference by microorganisms that are not detected by the assay using clinically relevant concentrations of potentially interfering microorganisms (same as those used in the Analytical Specificity Study).

Potentially interfering microorganisms (37 bacteria, six viruses, four parasites, and one yeast) were added to contrived positive sample groups (described below) at concentrations of 10^6 – 10^8 CFU/mL (bacteria and fungi) or at a minimum of 2.85 x 10^3 $TCID_{50}$/mL (viruses). The contrived positive samples were prepared from a pool of stool matrix that was confirmed negative by the ProGastro SSCS Assay.

The ProGastro SSCS target organisms were combined to form two contrived positive sample groups and tested at 2x Limit of Detection (LoD). One positive sample duo group contained *Salmonella* and *Campylobacter jejuni*, which have been found in co-infections the most frequently. *Shigella*, *Campylobacter coli*, and STEC (*stx1* and *stx2* positive) made up the second positive sample trio group. Each positive duo/trio sample group was aliquoted and spiked with a potential microbial interfering organism at high concentrations. Also, each duo/trio positive sample group was extracted without a microbial interferent to serve as a reference point. Final concentrations of each sample component can be found in the table below. Each sample was tested in replicates of three.

Microbial Interference Panel				
Sample	**Component 1 (Target Duo/Trio)**	**Component 1 Final Concentration**	**Component 2 (From Analytical Specificity Spiked Stool)**	**Component 2 Final Concentration**
SSCS MI 001	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	**NA**	**NA**
SSCS MI 002	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Aeromonas hydrophila*	$1.35x10^7$ CFU/mL
SSCS MI 003	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Bacillus cereus*	$1.35x10^7$ CFU/mL
SSCS MI 004	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Bacteroides fragilis*	$1.35x10^7$ CFU/mL
SSCS MI 005	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter upsaliensis*	$5.76x10^7$ CFU/mL

Microbial Interference Panel				
Sample	**Component 1 (Target Duo/Trio)**	**Component 1 Final Concentration**	**Component 2 (From Analytical Specificity Spiked Stool)**	**Component 2 Final Concentration**
SSCS MI 006	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter hyointestinalis*	6.70×10^{8} CFU/mL
SSCS MI 007	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter fetus*	4.86×10^{7} CFU/mL
SSCS MI 008	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter helveticus*	6.3×10^{7} CFU/mL
SSCS MI 009	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter gracilis*	2.16×10^{7} CFU/mL
SSCS MI 010	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter concisus*	9.0×10^{5} CFU/mL
SSCS MI 011	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter curvus*	4.05×10^{6} CFU/mL
SSCS MI 012	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter sputorum*	3.20×10^{7} CFU/mL
SSCS MI 013	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter rectus*	1.8×10^{7} CFU/mL
SSCS MI 014	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter showae*	3.87×10^{6} CFU/mL
SSCS MI 015	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Campylobacter mucosalis*	3.78×10^{6} CFU/mL
SSCS MI 016	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Citrobacter freundii*	4.32×10^{8} CFU/mL
SSCS MI 017	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Clostridium difficile*	1.35×10^{7} CFU/mL
SSCS MI 018	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Clostridium perfringens*	1.35×10^{7} CFU/mL
SSCS MI 019	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Enterobacter cloacae*	1.35×10^{7} CFU/mL
SSCS MI 020	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Enterococcus faecalis*	1.35×10^{7} CFU/mL
SSCS MI 021	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Escherichia coli* Non-STEC	1.35×10^{7} CFU/mL
SSCS MI 022	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Escherichia coli* (enteroinvasive)	1.98×10^{8} CFU/mL
SSCS MI 023	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Escherichia fergusonii*	1.8×10^{8} CFU/mL
SSCS MI 024	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Escherichia hermannii*	7.92×10^{8} CFU/mL
SSCS MI 025	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Helicobacter pylori*	5.04×10^{7} CFU/mL
SSCS MI 026	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Klebsiella pneumoniae*	1.35×10^{7} CFU/mL
SSCS MI 027	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Lactococcus lactis*	1.03×10^{8} CFU/mL
SSCS MI 028	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Listeria monocytogenes*	3.78×10^{6} CFU/mL
SSCS MI 029	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Peptostreptococcus anaerobius*	2.88×10^{7} CFU/mL
SSCS MI 030	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Plesiomonas shigelloides*	1.62×10^{8} CFU/mL
SSCS MI 031	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Proteus vulgaris*	1.35×10^{7} CFU/mL
SSCS MI 032	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Pseudomonas aeruginosa*	1.35×10^{7} CFU/mL
SSCS MI 033	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Pseudomonas fluorescens*	5.04×10^{8} CFU/mL

Microbial Interference Panel				
Sample	**Component 1 (Target Duo/Trio)**	**Component 1 Final Concentration**	**Component 2 (From Analytical Specificity Spiked Stool)**	**Component 2 Final Concentration**
SSCS MI 034	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Serratia marcescens*	$7.74x10^8$ CFU/mL
SSCS MI 035	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Staphylococcus aureus*	$1.35x10^7$ CFU/mL
SSCS MI 036	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Staphylococcus epidermidis*	$1.35x10^7$ CFU/mL
SSCS MI 037	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Vibrio parahaemolyticus*	$1.35x10^7$ CFU/mL
SSCS MI 038	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Yersinia enterocolitica*	$2.97x10^7$ CFU/mL
SSCS MI 039	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	Adenovirus Type 40	$2.85x10^5$ $TCID_{50}$/mL
SSCS MI 040	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	Adenovirus Type 41	$1.42x10^5$ $TCID_{50}$/mL
SSCS MI 041	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	Coxsackievirus B5/10/2006	$2.85x10^6$ $TCID_{50}$/mL
SSCS MI 042	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	Echovirus 11	$2.85x10^7$ $TCID_{50}$/mL
SSCS MI 043	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	Rotavirus	$2.85x10^3$ $TCID_{50}$/mL
SSCS MI 044	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	Norovirus	$2.25x10^{-2}$ Dilution
SSCS MI 045	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Candida albicans*	$1.49x10^7$ CFU/mL
SSCS MI 046	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Blastocystis hominis*	$9x10^{-2}$ Dilution
SSCS MI 047	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Giardia lamblia*	$9x10^{-2}$ Dilution
SSCS MI 048	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Cryptosporidium parvum*	$9x10^{-2}$ Dilution
SSCS MI 049	*Salmonella* Enteritidis & *Campylobacter jejuni*	2X LoD	*Entamoeba histolytica*	$9x10^{-2}$ Dilution
SSCS MI 050	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	**NA**	**NA**
SSCS MI 051	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Aeromonas hydrophila*	$1.35x10^7$ CFU/mL
SSCS MI 052	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Bacillus cereus*	$1.35x10^7$ CFU/mL
SSCS MI 053	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Bacteroides fragilis*	$1.35x10^7$ CFU/mL
SSCS MI 054	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter upsaliensis*	$5.76x10^7$ CFU/mL
SSCS MI 055	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter hyointestinalis*	$6.70x10^8$ CFU/mL
SSCS MI 056	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter fetus*	$4.86x10^7$ CFU/mL
SSCS MI 057	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter helveticus*	$6.3x10^7$ CFU/mL
SSCS MI 058	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter gracilis*	$2.16x10^7$ CFU/mL
SSCS MI 059	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter concisus*	$9.0x10^5$ CFU/mL
SSCS MI 060	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter curvus*	$4.05x10^6$ CFU/mL
SSCS MI 061	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter sputorum*	$3.20x10^7$ CFU/mL

Microbial Interference Panel				
Sample	**Component 1 (Target Duo/Trio)**	**Component 1 Final Concentration**	**Component 2 (From Analytical Specificity Spiked Stool)**	**Component 2 Final Concentration**
SSCS MI 062	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter rectus*	$1.8x10^7$ CFU/mL
SSCS MI 063	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter showae*	$3.87x10^6$ CFU/mL
SSCS MI 064	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Campylobacter mucosalis*	$3.78x10^6$ CFU/mL
SSCS MI 065	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Citrobacter freundii*	$4.32x10^8$ CFU/mL
SSCS MI 066	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Clostridium difficile*	$1.35x10^7$ CFU/mL
SSCS MI 067	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Clostridium perfringens*	$1.35x10^7$ CFU/mL
SSCS MI 068	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Enterobacter cloacae*	$1.35x10^7$ CFU/mL
SSCS MI 069	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Enterococcus faecalis*	$1.35x10^7$ CFU/mL
SSCS MI 070	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Escherichia coli* Non-STEC	$1.35x10^7$ CFU/mL
SSCS MI 071	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Escherichia coli* (enteroinvasive)	$1.98x10^8$ CFU/mL
SSCS MI 072	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Escherichia fergusonii*	$1.8x10^8$ CFU/mL
SSCS MI 073	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Escherichia hermannii*	$7.92x10^8$ CFU/mL
SSCS MI 074	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Helicobacter pylori*	$5.04x10^7$ CFU/mL
SSCS MI 075	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Klebsiella pneumoniae*	$1.35x10^7$ CFU/mL
SSCS MI 076	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Lactococcus lactis*	$1.03x10^8$ CFU/mL
SSCS MI 077	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Listeria monocytogenes*	$3.78x10^6$ CFU/mL
SSCS MI 078	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Peptostreptococcus anaerobius*	$2.88x10^7$ CFU/mL
SSCS MI 079	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Plesiomonas shigelloides*	$1.62x10^8$ CFU/mL
SSCS MI 080	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Proteus vulgaris*	$1.35x10^7$ CFU/mL
SSCS MI 081	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Pseudomonas aeruginosa*	$1.35x10^7$ CFU/mL
SSCS MI 082	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Pseudomonas fluorescens*	$5.04x10^8$ CFU/mL
SSCS MI 083	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Serratia marcescens*	$7.74x10^8$ CFU/mL
SSCS MI 084	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Staphylococcus aureus*	$1.35x10^7$ CFU/mL
SSCS MI 085	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Staphylococcus epidermidis*	$1.35x10^7$ CFU/mL
SSCS MI 086	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Vibrio parahaemolyticus*	$1.35x10^7$ CFU/mL
SSCS MI 087	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Yersinia enterocolitica*	$2.97x10^7$ CFU/mL
SSCS MI 088	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	Adenovirus Type 40	$2.85x10^5$ $TCID_{50}$/mL
SSCS MI 089	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	Adenovirus Type 41	$1.42x10^5$ $TCID_{50}$/mL

Microbial Interference Panel				
Sample	**Component 1 (Target Duo/Trio)**	**Component 1 Final Concentration**	**Component 2 (From Analytical Specificity Spiked Stool)**	**Component 2 Final Concentration**
SSCS MI 090	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	Coxsackievirus B5/10/2006	$2.85x10^6$ $TCID_{50}$/mL
SSCS MI 091	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	Echovirus 11	$2.85x10^7$ $TCID_{50}$/mL
SSCS MI 092	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	Rotavirus	$2.85x10^3$ $TCID_{50}$/mL
SSCS MI 093	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	Norovirus	$2.25x10^{-2}$ Dilution
SSCS MI 094	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Candida albicans*	$1.49x10^7$ CFU/mL
SSCS MI 095	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Blastocystis hominis*	$9x10^{-2}$ Dilution
SSCS MI 096	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Giardia lamblia*	$9x10^{-2}$ Dilution
SSCS MI 097	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Cryptosporidium parvum*	$9x10^{-2}$ Dilution
SSCS MI 098	*Shigella sonnei*, *Campylobacter coli*, and STEC	2X LoD	*Entamoeba histolytica*	$9x10^{-2}$ Dilution

The Mean Ct and Standard Deviations (SD) for each positive sample, and the Mean Ct difference (calculated by taking the average Ct for the sample containing only the duo/trio targets and subtracting the mean Ct from the sample containing the duo/trio targets with each Microbial Interference Panel Member added) are presented in tables below. A Microbial Interference Panel Member with a Mean Ct difference of over 3.3 is considered interfering.

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – SSC Mix					
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	**Campy/FAM Mean Ct±SD**	**Sal/TET Mean Ct±SD**	**Shi/TxR Mean Ct±SD**
SSCS MI 001	**NA**	N/A	38.6±0.6	34.9±0.5	N/A
SSCS MI 002	*Aeromonas hydrophila*	FAM: 0.2 TET: -0.3	38.4±0.3	35.2±0.3	N/A
SSCS MI 003	*Bacillus cereus*	FAM: 0.2 TET: 0.0	38.4±0.3	34.9±0.3	N/A
SSCS MI 004	*Bacteroides fragilis*	FAM: 0.3 TET: -0.4	38.3±0.5	35.3±0.4	N/A
SSCS MI 005	*Campylobacter upsaliensis*	FAM: 0.1 TET: 0.1	38.5±0.3	34.8±0.3	N/A
SSCS MI 006	*Campylobacter hyointestinalis*	FAM: 0.5 TET: 0.5	38.1±0.4	34.4±0.5	N/A
SSCS MI 007	*Campylobacter fetus*	FAM: 1.2 TET: 1.0	37.4±0.7	33.9±0.2	N/A
SSCS MI 008	*Campylobacter helveticus*	FAM: 1.1 TET: 0.6	37.5±0.5	34.3±0.3	N/A
SSCS MI 009	*Campylobacter gracilis*	FAM: -0.1 TET: 0.3	38.7±0.3	34.6±0.4	N/A
SSCS MI 010	*Campylobacter concisus*	FAM: -1.6 TET: 0.4	40.2±2.2	34.5±0.7	N/A

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – SSC Mix					
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	**Campy/FAM Mean Ct±SD**	**Sal/TET Mean Ct±SD**	**Shi/TxR Mean Ct±SD**
SSCS MI 011	*Campylobacter curvus*	FAM: 0.6 TET: 0.1	38.0±0.3	34.8±0.3	N/A
SSCS MI 012	*Campylobacter sputorum*	FAM: 0.2 TET: 0.1	38.4±0.5	34.8±0.4	N/A
SSCS MI 013	*Campylobacter rectus*	FAM: 0.8 TET: 0.1	37.8±0.4	35.0±0.3	N/A
SSCS MI 014	*Campylobacter showae*	FAM: 0.6 TET: -0.1	38.0±0.3	35.0±0.3	N/A
SSCS MI 015	*Campylobacter mucosalis*	FAM: 0.4 TET: -0.1	38.2±0.2	35.0±0.4	N/A
SSCS MI 016	*Citrobacter freundii*	FAM: -0.4 TET: 0.0	39.0±0.5	34.9±0.4	N/A
SSCS MI 017	*Clostridium difficile*	FAM: -0.4 TET: -0.4	39.0±0.6	35.3±0.4	N/A
SSCS MI 018	*Clostridium perfringens*	FAM: -0.7 TET: 0.1	39.3±1.8	34.8±0.2	N/A
SSCS MI 019	*Enterobacter cloacae*	FAM: 0.3 TET: 0.0	38.3±0.8	34.9±0.3	N/A
SSCS MI 020	*Enterococcus faecalis*	FAM: 1.4 TET: 0.8	37.2±0.2	34.1±0.5	N/A
SSCS MI 021	*Escherichia coli* Non-STEC	FAM: 0.7 TET: 0.2	37.9±0.5	34.7±0.3	N/A
SSCS MI 022	*Escherichia coli* (enteroinvasive)	FAM: 1.1 TET: 0.3	37.5±0.6	34.6±0.6	22.4±0.2*
SSCS MI 023	*Escherichia fergusonii*	FAM: 0.9 TET: 0.2	37.7±0.1	34.7±0.3	N/A
SSCS MI 024	*Escherichia hermannii*	FAM: 0.2 TET: -1.1	38.4±0.1	36.0±2.2	N/A
SSCS MI 025	*Helicobacter pylori*	FAM: -0.7 TET: -0.1	39.3±0.6	35.0±0.8	N/A
SSCS MI 026	*Klebsiella pneumoniae*	FAM: -0.3 TET: 0.1	38.9±0.3	34.8±0.5	N/A
SSCS MI 027	*Lactococcus lactis*	FAM: 0.6 TET: 0.2	38.0±0.7	34.7±0.5	N/A
SSCS MI 028	*Listeria monocytogenes*	FAM: 0.4 TET: 0.6	38.2±0.8	34.3±0.1	N/A
SSCS MI 029	*Peptostreptococcus anaerobius*	FAM: 0.5 TET: 0.4	38.1±0.7	34.5±0.3	N/A
SSCS MI 030	*Plesiomonas shigelloides*	FAM: 0.6 TET: 0.3	38.0±0.7	34.6±0.2	N/A
SSCS MI 031	*Proteus vulgaris*	FAM: 0.7 TET: 0.5	37.9±0.5	34.4±0.3	N/A
SSCS MI 032	*Pseudomonas aeruginosa*	FAM: 0.5 TET: 0.5	38.1±0.4	34.4±0.2	N/A
SSCS MI 033	*Pseudomonas fluorescens*	FAM: 0.8 TET: -0.3	37.8±0.3	35.2±0.7	N/A
SSCS MI 034	*Serratia marcescens*	FAM: 0.1 TET: -0.1	38.5±0.3	35.0±0.1	N/A

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – SSC Mix					
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	**Campy/FAM Mean Ct±SD**	**Sal/TET Mean Ct±SD**	**Shi/TxR Mean Ct±SD**
SSCS MI 035	*Staphylococcus aureus*	FAM: 0.3 TET: 0.1	38.3±0.4	35.0±0.6	N/A
SSCS MI 036	*Staphylococcus epidermidis*	FAM: 0.4 TET: 0.0	38.2±0.5	34.9±0.5	N/A
SSCS MI 037	*Vibrio parahaemolyticus*	FAM: -0.4 TET: 0.0	39.0±0.4	34.9±0.3	N/A
SSCS MI 038	*Yersinia enterocolitica*	FAM: -0.1 TET: 0.6	38.7±0.5	34.3±0.3	N/A
SSCS MI 039	Adenovirus Type 40	FAM: 0.2 TET: 0.1	38.4±0.4	35.0±0.2	N/A
SSCS MI 040	Adenovirus Type 41	FAM: -0.3 TET: -0.7	38.9±0.2	35.6±0.5	N/A
SSCS MI 041	Coxsackievirus B5/10/2006	FAM: -0.8 TET: -0.3	39.4±0.1	35.2±0.3	N/A
SSCS MI 042	Echovirus 11	FAM: 0.0 TET: 0.0	38.6±0.4	34.9±0.6	N/A
SSCS MI 043	Rotavirus	FAM: -0.4 TET: 0.1	39.0±0.3	34.8±0.2	N/A
SSCS MI 044	Norovirus	FAM: -0.9 TET: 0.3	39.5±0.4	34.6±0.1	N/A
SSCS MI 045	*Candida albicans*	FAM: -0.2 TET: -0.3	38.8±0.4	35.2±0.9	N/A
SSCS MI 046	Blastocystis hominis	FAM: -0.3 TET: 0.2	38.9±0.2	34.7±0.4	N/A
SSCS MI 047	Giardia lamblia	FAM: -0.8 TET: 0.1	39.4±0.3	34.8±0.4	N/A
SSCS MI 048	Cryptosporidium parvum	FAM: 0.1 TET: 0.5	38.5±0.6	34.4±0.3	N/A
SSCS MI 049	Entamoeba histolytica	FAM: -0.1 TET: 0.2	38.7±0.3	34.7±0.5	N/A
SSCS MI 050	**NA**	N/A	34.8±0.5	N/A	34.9±0.5
SSCS MI 051	*Aeromonas hydrophila*	FAM: 0.1 TxR: -0.8	34.7 ±0.2	N/A	35.7±0.3
SSCS MI 052	*Bacillus cereus*	FAM: 0.1 TxR: 0.1	34.9±0.3	N/A	34.8±0.4
SSCS MI 053	*Bacteroides fragilis*	FAM: 0.2 TxR: 0.2	34.6±0.2	N/A	34.7±0.3
SSCS MI 054	*Campylobacter upsaliensis*	FAM: 0.3 TxR: -0.2	34.5±0.0	N/A	35.1±0.5
SSCS MI 055	*Campylobacter hyointestinalis*	FAM: 0.1 TxR: -0.6	34.9±0.2	N/A	35.5±0.5
SSCS MI 056	*Campylobacter fetus*	FAM: 0.1 TxR: 0.2	34.7±0.1	N/A	34.7±0.6
SSCS MI 057	*Campylobacter helveticus*	FAM: -0.1 TxR: 0.3	34.9±0.2	N/A	35.2±0.1
SSCS MI 058	*Campylobacter gracilis*	FAM: -0.2 TxR: -0.2	35.0±0.1	N/A	35.1±0.3

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – SSC Mix					
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	**Campy/FAM Mean Ct±SD**	**Sal/TET Mean Ct±SD**	**Shi/TxR Mean Ct±SD**
SSCS MI 059	*Campylobacter concisus*	FAM: 0.4 TxR: 0.0	35.2±0.1	N/A	34.9±0.8
SSCS MI 060	*Campylobacter curvus*	FAM: 0.1 TxR: -0.9	34.7±0.5	N/A	35.8±0.9
SSCS MI 061	*Campylobacter sputorum*	FAM: -0.1 TxR: -0.7	34.9±0.4	N/A	35.6±0.7
SSCS MI 062	*Campylobacter rectus*	FAM: 1.2 TxR: 1.4	36.0±0.4	N/A	36.3±02
SSCS MI 063	*Campylobacter showae*	FAM: 0.9 TxR: -1.5	35.7±0.3	N/A	36.4±0.2
SSCS MI 064	*Campylobacter mucosalis*	FAM: -0.7 TxR: -0.8	35.5±0.1	N/A	35.7±0.2
SSCS MI 065	*Citrobacter freundii*	FAM: -1.0 TxR: -1.4	35.8±0.3	N/A	36.3±0.4
SSCS MI 066	*Clostridium difficile*	FAM: 0.6 TxR: -1.4	35.4±0.2	N/A	36.3±0.7
SSCS MI 067	*Clostridium perfringens*	FAM: -0.4 TxR: -0.6	35.2±0.6	N/A	35.5±0.4
SSCS MI 068	*Enterobacter cloacae*	FAM: -0.9 TxR: 1.0	35.7±0.3	N/A	35.9±0.5
SSCS MI 069	*Enterococcus faecalis*	FAM: 1.1 TxR: -1.4	35.9±0.2	N/A	36.3±0.6
SSCS MI 070	*Escherichia coli* Non-STEC	FAM: 1.0 TxR: 1.5	35.8±0.2	N/A	36.4±0.5
SSCS MI 071	*Escherichia coli* (enteroinvasive)	FAM: 0.4 TxR: 12.2*	35.2±0.2	N/A	22.7±0.4*
SSCS MI 072	*Escherichia fergusonii*	FAM: -1.0 TxR: -1.0	35.8±0.4	N/A	35.9±0.3
SSCS MI 073	*Escherichia hermannii*	FAM: -0.4 TxR: -0.1	35.1±0.1	N/A	35.0±0.1
SSCS MI 074	*Helicobacter pylori*	FAM: 1.3 TxR: -1.5	36.1±0.4	N/A	36.4±0.2
SSCS MI 075	*Klebsiella pneumoniae*	FAM: -0.9 TxR: -1.3	35.7±0.4	N/A	36.2±0.1
SSCS MI 076	*Lactococcus lactis*	FAM: -1.2 TxR: 1.2	36.0±0.4	N/A	36.1±0.2
SSCS MI 077	*Listeria monocytogenes*	FAM: -0.6 TxR: -0.6	35.4±0.4	N/A	35.5±0.2
SSCS MI 078	*Peptostreptococcus anaerobius*	FAM: -1.1 TxR: -0.8	35.9±0.1	N/A	35.7±0.3
SSCS MI 079	*Plesiomonas shigelloides*	FAM: 1.0 TxR: -1.2	35.8±0.5	N/A	36.1±0.4
SSCS MI 080	*Proteus vulgaris*	FAM: 0.8 TxR: -1.1	35.6±0.2	N/A	36.0±0.2
SSCS MI 081	*Pseudomonas aeruginosa*	FAM: -1.3 TxR: -1.2	36.1±0.2	N/A	36.1±0.4
SSCS MI 082	*Pseudomonas fluorescens*	FAM: -0.3 TxR: -0.3	35.1±0.3	N/A	35.2±0.2

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – SSC Mix					
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	**Campy/FAM Mean Ct±SD**	**Sal/TET Mean Ct±SD**	**Shi/TxR Mean Ct±SD**
SSCS MI 083	*Serratia marcescens*	FAM: -0.2 TxR: -0.3	35.0±0.6	N/A	35.2±0.3
SSCS MI 084	*Staphylococcus aureus*	FAM: 0.9 TxR: -0.9	35.7±0.4	N/A	35.8±0.1
SSCS MI 085	*Staphylococcus epidermidis*	FAM: -0.7 TxR: 0.4	35.5±0.2	N/A	35.3±0.4
SSCS MI 086	*Vibrio parahaemolyticus*	FAM: -0.3 TxR: -0.7	35.1±0.2	N/A	35.6±0.3
SSCS MI 087	*Yersinia enterocolitica*	FAM: 0.9 TxR: -1.3	35.7±0.4	N/A	36.2±0.2
SSCS MI 088	Adenovirus Type 40	FAM: -0.3 TxR: -1.3	35.1±0.3	N/A	36.2±0.5
SSCS MI 089	Adenovirus Type 41	FAM: -0.5 TxR: 0.4	35.3±0.4	N/A	35.3±0.4
SSCS MI 090	Coxsackievirus B5/10/2006	FAM: -0.9 TxR: -0.5	35.7±0.6	N/A	35.4±0.4
SSCS MI 091	Echovirus 11	FAM: -0.4 TxR: 0.1	35.2±0.2	N/A	35.0±0.3
SSCS MI 092	Rotavirus	FAM: -0.2 TxR: -0.3	35.0±0.3	N/A	35.2±0.2
SSCS MI 093	Norovirus	FAM: -0.3 TxR: -0.5	35.1±0.3	N/A	35.4±0.2
SSCS MI 094	*Candida albicans*	FAM: -0.5 TxR: -0.6	35.3±0.1	N/A	35.5±0.3
SSCS MI 095	*Blastocystis hominis*	FAM: -0.4 TxR: -0.1	35.2±0.3	N/A	35.0±0.4
SSCS MI 096	*Giardia lamblia*	FAM: 0.1 TxR: -0.9	34.9±0.4	N/A	35.8±0.7
SSCS MI 097	*Cryptosporidium parvum*	FAM: -0.2 TxR: -0.8	35.0±0.1	N/A	35.7±1.2
SSCS MI 098	*Entamoeba histolytica*	FAM: -0.3 TxR: 0.3	35.1±0.2	N/A	34.6±0.6

* Sample contains a high concentration of EIEC; therefore the Ct value is very low

Enteroinvasive *Escherichia coli* (EIEC) is genetically very similar to *Shigella* and was detected by the SSC Mix as positive for *Shigella* as expected. This cross-reactivity is reported in the Package Insert as a limitation.

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – STEC Mix				
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	***stx2*/FAM Mean Ct±SD**	***stx1*/TET Mean Ct±SD**
SSCS MI 050	**NA**	N/A	37.1±0.3	36.3±0.8
SSCS MI 051	*Aeromonas hydrophila*	*stx2*: 0.5 *stx1*: 0.7	36.6±0.3	35.6±0.2

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – STEC Mix				
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	***stx2*/FAM Mean Ct±SD**	***stx1*/TET Mean Ct±SD**
SSCS MI 052	*Bacillus cereus*	*stx2*: 0.5 *stx1*: 0.1	36.6±0.4	36.2±0.5
SSCS MI 053	*Bacteroides fragilis*	*stx2*: 1.0 *stx1*: 0.5	36.1±0.6	35.8±0.3
SSCS MI 054	*Campylobacter upsaliensis*	*stx2*: 1.0 *stx1*: 0.1	36.1±0.5	36.2±0.7
SSCS MI 055	*Campylobacter hyointestinalis*	*stx2*: 1.0 *stx1*: 0.6	36.1±0.3	35.7±0.5
SSCS MI 056	*Campylobacter fetus*	*stx2*: 0.8 *stx1*: 0.7	36.3±0.1	35.6±0.8
SSCS MI 057	*Campylobacter helveticus*	*stx2*: 0.0 *stx1*: 0.6	37.1±0.8	35.7±0.4
SSCS MI 058	*Campylobacter gracilis*	*stx2*: 0.1 *stx1*: 0.8	37.0±0.5	35.5±0.2
SSCS MI 059	*Campylobacter concisus*	*stx2*: 0.7 *stx1*: 0.8	36.4±0.2	35.5±1.0
SSCS MI 060	*Campylobacter curvus*	*stx2*: -0.4 *stx1*: -0.5	37.5±2.8	36.8±1.0
SSCS MI 061	*Campylobacter sputorum*	*stx2*: 1.1 *stx1*: 0.8	36.0±0.2	35.5±0.3
SSCS MI 062	*Campylobacter rectus*	*stx2*: 0.9 *stx1*: 1.3	36.2±0.9	35.0±0.3
SSCS MI 063	*Campylobacter showae*	*stx2*: 1.3 *stx1*: 1.0	35.8±0.2	35.3±0.5
SSCS MI 064	*Campylobacter mucosalis*	*stx2*: 1.4 *stx1*: 1.7	35.7±0.2	34.6±0.3
SSCS MI 065	*Citrobacter freundii*	*stx2*: 0.9 *stx1*: 1.4	36.2±0.4	34.9±0.5
SSCS MI 066	*Clostridium difficile*	*stx2*: 0.5 *stx1*: -0.3	36.6±0.5	36.6±0.3
SSCS MI 067	*Clostridium perfringens*	*stx2*: 0.1 *stx1*: 0.3	37.0±0.2	36.0±0.5
SSCS MI 068	*Enterobacter cloacae*	*stx2*: 0.6 *stx1*: 0.0	36.5±0.2	36.3±0.9
SSCS MI 069	*Enterococcus faecalis*	*stx2*: 0.9 *stx1*: -1.0	36.2±0.5	37.3±0.3
SSCS MI 070	*Escherichia coli* Non-STEC	*stx2*: 0.7 *stx1*: -0.5	36.4±0.3	36.8±0.4
SSCS MI 071	*Escherichia coli* (enteroinvasive)	*stx2*: -0.1 *stx1*: -0.3	37.1±0.7	36.6±0.6
SSCS MI 072	*Escherichia fergusonii*	*stx2*: 0.8 *stx1*: 0.0	36.3±0.3	36.3±0.1
SSCS MI 073	*Escherichia hermannii*	*stx2*: 0.7 *stx1*: 0.2	36.4±0.4	36.1±0.2
SSCS MI 074	*Helicobacter pylori*	*stx2*: 1.2 *stx1*: 1.3	35.9±0.3	35.0±0.1

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – STEC Mix				
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	***stx2*/FAM Mean Ct±SD**	***stx1*/TET Mean Ct±SD**
SSCS MI 075	*Klebsiella pneumoniae*	*stx2*: 1.4 *stx1*: 1.1	35.7±0.4	35.2±0.8
SSCS MI 076	*Lactococcus lactis*	*stx2*: 0.7 *stx1*: 0.8	36.4±0.1	35.5±0.2
SSCS MI 077	*Listeria monocytogenes*	*stx2*: 0.6 *stx1*: 0.5	36.5±0.6	35.8±0.4
SSCS MI 078	*Peptostreptococcus anaerobius*	*stx2*: 0.6 *stx1*: 1.1	36.5±0.2	35.2±0.3
SSCS MI 079	*Plesiomonas shigelloides*	*stx2*: 0.2 *stx1*: 0.6	36.9±0.8	35.7±0.5
SSCS MI 080	*Proteus vulgaris*	*stx2*: 0.9 *stx1*: -0.1	36.2±0.6	36.4±0.8
SSCS MI 081	*Pseudomonas aeruginosa*	*stx2*: 0.8 *stx1*: 0.6	36.3±0.5	35.7±0.5
SSCS MI 082	*Pseudomonas fluorescens*	*stx2*: 0.6 *stx1*: 0.0	36.5±0.3	36.3±0.7
SSCS MI 083	*Serratia marcescens*	*stx2*: 0.6 *stx1*: 0.4	36.5±0.5	35.9±0.2
SSCS MI 084	*Staphylococcus aureus*	*stx2*: 1.4 *stx1*: 0.4	35.7±0.3	35.9±0.3
SSCS MI 085	*Staphylococcus epidermidis*	*stx2*: 0.5 *stx1*: 0.8	36.5±0.5	35.5±0.1
SSCS MI 086	*Vibrio parahaemolyticus*	*stx2*: 0.6 *stx1*: 0.1	36.5±0.8	36.2±1.1
SSCS MI 087	*Yersinia enterocolitica*	*stx2*: 0.1 *stx1*: 0.4	37.0±0.3	35.9±0.5
SSCS MI 088	Adenovirus Type 40	*stx2*: 0.6 *stx1*: 0.8	36.5±0.4	35.5±0.5
SSCS MI 089	Adenovirus Type 41	*stx2*: 1.2 *stx1*: 0.5	35.9±0.5	35.8±0.4
SSCS MI 090	Coxsackievirus B5/10/2006	*stx2*: 0.4 *stx1*: 0.2	36.7±0.5	36.1±0.2
SSCS MI 091	Echovirus 11	*stx2*: 0.6 *stx1*: 0.3	36.5±0.6	36.0±0.3
SSCS MI 092	Rotavirus	*stx2*: 0.5 *stx1*: 0.2	36.6±0.8	36.1±0.1
SSCS MI 093	Norovirus	*stx2*: 0.8 *stx1*: 0.2	36.3±0.4	36.1±0.6
SSCS MI 094	*Candida albicans*	*stx2*: 0.9 *stx1*: 0.3	36.2±0.2	36.0±0.7
SSCS MI 095	*Blastocystis hominis*	*stx2*: 1.1 *stx1*: 0.7	36.0±0.3	35.6±0.1
SSCS MI 096	*Giardia lamblia*	*stx2*: 0.6 *stx1*: 0.4	36.5±1.1	35.9±0.7
SSCS MI 097	*Cryptosporidium parvum*	*stx2*: 0.6 *stx1*: -0.1	36.5±0.2	36.4±0.9

Microbial Interference Ct Averages and Standard Deviations for Positive Samples – STEC Mix				
Sample ID	**MI Panel Member**	**CT Difference (Target only Ct – interferent sample Ct)**	***stx2*/FAM Mean Ct±SD**	***stx1*/TET Mean Ct±SD**
SSCS MI 098	*Entamoeba histolytica*	*stx2*: 1.5 *stx1*: 1.2	35.6±0.2	35.1±0.5

In conclusion, the presence of high clinically relevant concentrations of potentially interfering microorganisms did not appear to affect the performance of the ProGastro SSCS Assay detecting low concentrations (close to the LoD concentrations) of assay target organisms.

k. Competitive Interference

To evaluate the effects of Competitive Interference on the ProGastro SSCS Assay's SSC Mix and STEC Mix when various combinations of the Assay's target organisms were present in a single sample, an analytical study was carried out.

Competitive Interference may occur when the detection of one target in a multiplex PCR Mix is outcompeted by another target(s) in that same multiplex PCR Mix for essential reaction components (dNTPs, Mg^{2+} etc.). 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. This study utilized one cultured and titered strain each of *Salmonella*, *Shigella*, *Campylobacter jejuni*, and *Campylobacter coli* for use with the SSC Mix. For the STEC Mix testing, two Shiga Toxin producing *Escherichia coli* (STEC) strains (*stx1*+/*stx2*- and *stx1*-/*stx2*+) were used. The bacterial strains were diluted in Stool Preservation and Transport Media (SPTM, ParaPak C&S) and spiked into aliquots of a ProGastro SSCS negative stool matrix pool with one or two of the targets at 3 logs above their particular LoD and a second or third target analyzed close to the LoD depending on the particular sample. (See tables below).

Description of Competitive Interference Samples – SSC Mix			
***Salmonella* Concentration**	***Shigella* Concentration**	***C. coli* Concentration**	***C. jejuni* Concentration**
2x LoD	-	-	-
-	2x LoD	-	-
-	10x LoD	-	-
-	-	2x LoD	-
-	-	-	2x LoD
2x LoD	3 logs above LoD	3 logs above LoD	-
2x LoD	3 logs above LoD	-	3 logs above LoD
-	2x LoD	3 logs above LoD	-
-	2x LoD	-	3 logs above LoD
3 logs above LoD	2x LoD	-	-
-	3 logs above LoD	2x LoD	-
-	3 logs above LoD	-	2x LoD
3 logs above LoD	-	2x LoD	-

Description of Competitive Interference Samples – SSC Mix			
***Salmonella* Concentration**	***Shigella* Concentration**	***C. coli* Concentration**	***C. jejuni* Concentration**
3 logs above LoD	-	-	2x LoD
3 logs above LoD	2x LoD	-	-

Description of Competitive Interference Samples – STEC Mix	
***Stx1* Concentration**	***Stx2* Concentration**
2x LoD	-
-	2x LoD
2x LoD	3 logs above LoD
2x LoD	3 logs above LoD
3 logs above LoD	2x LoD

The Competitive Interference Panel samples and the required Negative Controls were extracted on the bioMériuex NucliSENS easyMAG and samples tested in triplicate (Positive and Negative Controls in a single replicate per PCR Run) on a Cepheid Smartcycler II using one lot of ProGastro SSCS reagents.

The results were analyzed and any organism(s) that prevented the detection of any of the target organisms were reported as a competitive interferent of the ProGastro SSCS Assay.

The Competitive Interference summary data stratified by the SSC Mix and the STEC Mix is presented in the two tables below. The tables present a summary of the data generated in the study, highlighting (in yellow) the organism combinations that exhibited competitive interference.

SSC Mix Competitive Interference (Multiple-Infection) Summary

***Salmonella* Conc.**	***Shigella* Conc.**	***C. coli* Conc.**	***C. jejuni* Conc.**	***Salmonella* Detected/Mean Ct**	***Shigella* Detected/ Mean Ct**	***C. coli* Detected/ Mean Ct**	***C. jejuni* Detected/ Mean Ct**	**Avg Ct Difference (Low Conc. Analyte Alone - Low Conc. Analyte w/ High Conc, Competitive Analyte)**
2x LoD	-	-	-	YES/35.8	-	-	-	N/A
-	2x LoD	-	-	-	YES/31.4	-	-	N/A
	10x LoD			-	YES/29.2	-	-	N/A
-	-	2x LoD	-	-	-	YES/35.3	-	N/A
-	-	-	2x LoD	-	-	-	YES/38.0	N/A
2x LoD	3 logs above LoD	3 logs above LoD	-	YES/35.7	YES/21.5	YES/25.5	-	0.1
2x LoD	3 logs above LoD	-	3 logs above LoD	YES/40.8	YES/21.7	-	YES/27.6	-5.0
-	2x LoD	3 logs above LoD	-	-	YES/31.6	YES/24.8	-	-0.2

Salmonella Conc.	*Shigella* Conc.	*C. coli* Conc.	*C. jejuni* Conc.	*Salmonella* Detected/Mean Ct	*Shigella* Detected/ Mean Ct	*C. coli* Detected/ Mean Ct	*C. jejuni* Detected/ Mean Ct	Avg Ct Difference (Low Conc. Analyte Alone - Low Conc. Analyte w/ High Conc, Competitive Analyte)
-	**2x LoD**	**-**	**3 logs above LoD**	**-**	**NO (only 1 of 3 reps detected)**	**-**	**YES/25.3**	**N/A**
-	**10x LoD***	**-**	**3 logs above LoD**	**-**	**YES/32.7**	**-**	**YES/25.4**	**-3.5**
3 logs above LoD	2x LoD	-	-	YES/25.9	YES/32.0	-	-	-0.6
-	3 logs above LoD	2x LoD	-	-	YES/21.8	YES/34.0	-	1.3
-	3 logs above LoD	-	2x LoD	-	YES/22.0	-	YES/37.7	0.3
3 logs above LoD	**-**	**2x LoD**	**-**	**YES/25.7**	**-**	**NO**	**-**	**N/A**
2 logs above LoD**	**-**	**2x LoD**	**-**	**YES/30.0**	**-**	**YES/34.6**	**-**	**-0.1**
3 logs above LoD	**-**	**-**	**2x LoD**	**YES/25.7**	**-**	**-**	**NO**	**N/A**
2 logs above LoD**	**-**	**-**	**2x LoD**	**YES/30.2**	**-**	**-**	**YES/42.8**	**-4.4**
3 logs above LoD	2x LoD	-	-	YES/25.9	YES/32.0	-	-	-0.6

Bold: Samples that exhibited competitive interference.
*Tested at a higher "low" concentration due to low analyte not detected
**Tested at a lower "high" concentration due to low analyte not detected

STEC Mix Competitive Interference (Multiple-Infection) Summary

***Stx1* Concentration**	***Stx2* Concentration**	***Stx1* Detected/Mean Ct**	***Stx2* Detected/Mean Ct**	**Avg Ct Difference (Low ConcAnalyte Alone - Low ConcAnalyte w/ Competition)**
2x LoD	-	YES/38.7	-	N/A
-	2x LoD	-	YES/35.3	N/A
2x LoD	3 logs above LoD	YES/41.2	YES/25.8	-2.5
3 logs above LoD	2x LoD	YES/27.0	YES/36.0	-0.7

Competitive Interference was observed for several samples using the ProGastro SSCS Assay's SSC Mix. 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. These limitations are added to the ProGastro SSCS Assay Instructions for Use.

Competitive Interference for STEC strains was not observed using the ProGastro SSCS Assay's STEC Mix.

l. Carry-Over Contamination:

A Carry-Over Contamination Study was conducted to demonstrate the degree of Carry-Over/Cross-Contamination that may occur when High Positive (HP) samples were processed alongside True Negative (TN) samples during nucleic acid extraction on the bioMérieux NucliSENS easyMAG and during subsequent PCR setup of the ProGastro SSCS Assay on the Cepheid SmartCycler II.

This study assessed the level of carry-over contamination with the ProGastro SSCS Assay by testing simulated dual positive *Shigella* (*Shigella flexneri* ATCC12022) and Shiga Toxin producing *E. Coli* (STEC) (*E. coli* Strain 3215-99 O111:H8, *stx1+*/*stx2+*) high positive stool samples run in series with negative stool samples over the course of five runs (one run/day). This study used bacterial targets that are typically found at the highest level in clinical samples, which based on development and analytical studies were *Shigella* and STEC. The High Positive (HP) samples consisted of negative stool matrix spiked with a concentration of 5 logs above the Limit of Detection (LoD) for *Shigella* and 4 logs above the LoD for STEC. These concentrations were used to represent the earlier Ct range for these organisms as observed in the Cutoff Determination and Confirmation Study. True Negative (TN) samples consisted of negative stool matrix. The samples were processed and extracted in a HP/TN alternating fashion (i.e. checkerboard pattern) on the extraction instrument and likewise processed and run using both the SSC and STEC Mixes on the Cepheid SmartCycler II instrument in an alternating fashion.

Eleven (11) HP, 11 TN samples, and a Negative Control were extracted per run on the easyMAG for a total of 55 HP samples and 55 TN samples over five runs. The table below presents the order of samples run on the easyMAG. Sample cartridges were placed in a linear array on the easyMAG instrument such that Cartridge A / Vessel 8 (TN 4) was adjacent to Cartridge B / Vessel 1 (HP 5) and Cartridge B / Vessel 8 (TN 8) was adjacent to Cartridge C / Vessel 1 (HP 9), maintaining the alternating pattern.

Sample Order for Extraction Using the easyMAG

Sample Cartridge A							
Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Vessel 7	Vessel 8
HP 1	TN 1	HP 2	TN 2	HP 3	TN 3	HP 4	TN 4
Sample Cartridge B							
Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Vessel 7	Vessel 8
HP 5	TN 5	HP 6	TN 6	HP 7	TN 7	HP 8	TN 8
Sample Cartridge C							
Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Vessel 7	Vessel 8
HP 9	TN 9	HP 10	TN 10	HP 11	TN 11	NC	NA

Mean Ct values and standard deviation for the *Shigella* (TxRed, HP samples, SSC Mix), *stx1* (TET, HP samples, STEC Mix), *stx2* (FAM, HP samples, STEC Mix), SSC IC (CY5, TN samples) and STEC IC (CY5, TN samples) Cts are reported in the table below:

Average Ct Values and Standard Deviation for High Positive and True Negative Samples – Both Mixes*										
	SSC Mix				STEC Mix					
	Shigella		IC (TN)		*stx1*		*stx2*		IC (TN)	
Sample ID	**Average Ct Value**	**SD**	**Average Ct Value**	**SD**	**Average Ct Value**	**SD**	**Average Ct Value**	**SD**	**Average Ct Value**	**SD**
High Positive	17.5	0.3	N/A	N/A	22.9	0.4	24.3	0.3	N/A	N/A
True Negative	N/A	N/A	33.0	0.5	N/A	N/A	N/A	N/A	32.7	0.4

The number of correct samples is reported in the table below. The results are broken out by Mix (SSC Mix and STEC Mix) and by the Assay as a whole, including the total number and percent correct.

Number of Correct Samples after Initial Testing and Retesting					
	Initial Testing			Retesting (including Original Data as described)	
	SSC Mix	STEC Mix	Combined	SSC Mix	Combined
High Positive	54/55* (98.2%)	55/55 (100%)	109/110 (99.1%)	77/78 (98.7%)	132/133 (99.2%)
True Negative	54/55** (98.2%)	55/55 (100%)	109/110 (99.1%)	77/78 (98.7%)	132/133 (99.2%)

* Sample HP 5 false positive for *Campylobacter* (SSC Mix), results from repeat testing from nucleic acid in duplicate were negative for *Campylobacter*

**Sample TN 40 false positive for *Salmonella* (SSC Mix), results from repeat testing from nucleic acid in duplicate were negative for *Salmonella*

The results of this study demonstrate no evidence of Carry-Over Contamination over a five day course of processing High Positive *Shigella* and STEC samples alongside True Negative samples using the ProGastro SSCS Assay. 100% of the True Negative samples were negative for *Shigella*, *stx1* and *stx2*.

The false positive *Campylobacter* and *Salmonella* results in the samples tested indicates that environmental contamination can occur during PCR set up, most likely from the SSCS Positive Control, as these organisms were not used for this study. The following statement is included in the "Limitations" section of the product package insert: "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".

m. Stool Preservation Transport Medium (SPTM) Comparison Study:

An analytical study was carried out to demonstrate equivalent performance of the two stool preservation media, Meridian ParaPak C&S (C&S) and the Remel Cary Blair (CB) Transport Medium, used for collection and transport of stool samples for use with the ProGastro SSCS Assay.

Meridian Para-Pak (C&S) and Remel Cary Blair Transport Medium with Indicator Transport Media (CB) are the two most utilized Stool Preservation and Transport Media (SPTM) in clinical laboratory settings. The intended use of C&S and CB is for the routine collection, transportation, preservation, and culture of clinical stool specimens for bacterial enteric pathogens. The SPTMs have essentially the same ingredients and it was anticipated that there would be minimal differences in assay performance using either of the materials (see the table below).

Characteristics of SPTM		
	Meridian ParaPak C&S Cat#900612	**Remel Cary Blair Transport Medium with Indicator Cat#R21610 or R21925**
Components	Agar Phosphate Buffer Sodium Chloride Thioglycolic Acid Sodium Salt Calcium Chloride Phenol Red Water	Sodium Chloride Agar Sodium Thioglycollate Disodium Phosphate Calcium Chloride Phenol Red Indicator Demineralized water
pH	Not stated in the Package Insert	8.0 ± 0.5 @ 25ºC
Storage Temperature	Room Temperature	Room Temperature
Volume per Vial	15 mL	15 mL

Five cultured and titered bacterial strains, *Salmonella*, *Shigella*, *Campylobacter jejuni* (CJ), *Campylobacter coli* (CC), and Shiga Toxin producing *E. coli*, (STEC), were diluted in PBS and were spiked at 1 log above, at, 1 log below, and 2 logs below the limit of detection (LoD) in each of the two media. The table below contains the strains and concentrations tested in this study:

Strains and Concentrations Tested		
Bacterial Strain	**Limit of Detection***	**Concentrations Tested**
Salmonella Enteritidis ATCC 6961	$1x10^4$ CFU/mL	$1x10^5$ CFU/mL
		$1x10^4$ CFU/mL
		$1x10^3$ CFU/mL
		$1x10^2$ CFU/mL
Shigella sonnei ATCC 29029	$1x10^3$ CFU/mL	$1x10^4$ CFU/mL
		$1x10^3$ CFU/mL
		$1x10^2$ CFU/mL
		$1x10^1$ CFU/mL
Campylobacter jejuni ATCC 33291	$1x10^3$ CFU/mL	$1x10^4$ CFU/mL
		$1x10^3$ CFU/mL
		$1x10^2$ CFU/mL

Strains and Concentrations Tested		
Bacterial Strain	**Limit of Detection***	**Concentrations Tested**
		$1x10^{1}$ CFU/mL
		$1x10^{0}$ CFU/mL
		$1x10^{-1}$ CFU/mL
Campylobacter coli ATCC BAA-371	$1x10^{4}$ CFU/mL	$1x10^{5}$ CFU/mL
		$1x10^{4}$ CFU/mL
		$1x10^{3}$ CFU/mL
		$1x10^{2}$ CFU/mL
		$1x10^{1}$ CFU/mL
		$1x10^{0}$ CFU/mL
STEC O111:H8 (*stx1+/stx2+*) Strain 3215-99	$1x10^{4}$ CFU/mL	$1x10^{5}$ CFU/mL
		$1x10^{4}$ CFU/mL
		$1x10^{3}$ CFU/mL
		$1x10^{2}$ CFU/mL

*****LoD as estimated in stool matrix in the Analytical Reactivity Study
Concentrations **in bold** are additional concentrations that required retesting due to an LoD not being achieved during initial SPTM comparison testing.

Each sample was extracted using the bioMérieux NucliSENS easyMAG in triplicate and run in a single replicate using one lot of ProGastro SSCS reagents on the Cepheid SmartCycler II.

Ct values for the triplicate extractions were averaged and the average, standard deviation, and % CV are shown in the table below. The LoD estimate for each strain using each media is **bolded** below in the table.

Average Ct, Standard Deviation (SD), and % Coefficient of Variance (CV) for Target Strains*									
	Conc. CFU/mL	**C&S**				**CB**			
		#Pos	**Avg Ct**	**Ct SD**	**% CV**	**#Pos**	**Avg Ct**	**Ct SD**	**% CV**
Salmonella	$1x10^{5}$	3/3	32.4	0.4	1.1	3/3	32.5	0.6	1.8
	$1x10^{4}$	3/3	36.1	0.1	0.3	**3/3**	**35.6**	**0.2**	**0.6**
	$1x10^{3}$	**3/3**	**38.5**	**0.8**	**1.9**	2/3	39.0	1.1	2.7
	$1x10^{2}$	1/3	39.5	NA	NA	0/3	NA	NA	NA
Shigella	$1x10^{4}$	3/3	32.2	0.1	0.3	**3/3**	**32.9**	**0.6**	**1.8**
	$1x10^{3}$	**3/3**	**35.5**	**0.6**	**1.6**	2/3	36.0	0.8	2.2
	$1x10^{2}$	0/3	NA	NA	NA	0/3	NA	NA	NA
	$1x10^{1}$	0/3	NA	NA	NA	0/3	NA	NA	NA
C jejuni	$1x10^{4}$	3/3	31.4	0.1	0.4	3/3	31.7	0.1	0.3
	$1x10^{3}$	3/3	35.1	0.2	0.6	3/3	34.8	0.2	0.6
	$1x10^{2}$	3/3	38.3	0.4	0.9	**3/3**	**38.2**	**0.3**	**0.8**
	$1x10^{1}$	**3/3**	**42.8**	**0.8**	**1.8**	2/3	41.7	0.7	1.7
	$1x10^{0}$	1/3	40.3	NA	NA	1/3	42.7	NA	NA
	$1x10^{-1}$	0/3	NA	NA	NA	1/3	43.5	NA	NA
C. coli	$1x10^{5}$	3/3	29.6	0.5	1.5	3/3	30.1	0.3	1.1
	$1x10^{4}$	3/3	33.0	0.3	0.9	3/3	33.7	0.5	1.4
	$1x10^{3}$	3/3	36.4	0.1	0.2	3/3	36.8	0.4	1.1
	$1x10^{2}$	**3/3**	**40.0**	**1.0**	**2.6**	**3/3**	**39.4**	**0.8**	**2.0**
	$1x10^{1}$	1/3	40.6	NA	NA	0/3	NA	NA	NA
	$1x10^{0}$	0/3	NA	NA	NA	0/3	NA	NA	NA
STEC *stx1+/stx2+*	***stx1* Detection**								
	$1x10^{5}$	3/3	32.5	0.5	1.5	3/3	33.5	0.2	0.6
	$1x10^{4}$	**3/3**	**36.5**	**0.6**	**1.6**	**3/3**	**38.1**	**1.7**	**4.4**
	$1x10^{3}$	1/3	37.6	NA	NA	1/3	39.5	NA	NA
	$1x10^{2}$	0/3	NA	NA	NA	0/3	NA	NA	NA
	***stx2* Detection**								
	$1x10^{5}$	3/3	33.7	0.2	0.6	3/3	34.5	0.3	0.9

Average Ct, Standard Deviation (SD), and % Coefficient of Variance (CV) for Target Strains*									
	Conc. CFU/mL	C&S				CB			
		#Pos	Avg Ct	Ct SD	% CV	#Pos	Avg Ct	Ct SD	% CV
	$1x10^4$	**3/3**	**36.6**	**1.0**	**2.8**	**3/3**	**38.0**	**1.6**	**4.1**
	$1x10^3$	1/3	38.5	NA	NA	2/3	39.3	0.5	1.3
	$1x10^2$	2/3	39.4	0.1	0.2	1/3	40.7	NA	NA

For the CB to be considered equivalent to C&S:

- All three replicates for at least one concentration must test positive and within one log of the lowest concentration in which all 3 replicates of C&S are positive.
- The average Ct values at all concentrations in which 3 of 3 replicates are positive shall not differ by more than 3.3 Cts from the average Ct of that concentration diluted in C&S. If the sample differs by more than 3.3 Cts the media will not be considered equivalent.

The Mean Ct difference between the two media for the average of each strain at each concentration is shown the table below:

Ct Difference and Data Acceptance for CB in Comparison to ParaPak C&S					
Strain	**LoD Estimate (CFU/mL) in C&S**	**LoD Estimate (CFU/mL) in CB**	**Concentration (CFU/mL)**	**Ct Difference* (CB – C&S)**	**Equivalent?**
Salmonella	$1x10^3$	$1x10^4$	$1x10^5$	0.1	YES
			$1x10^4$	-0.5	
			$1x10^3$	NA	
			$1x10^2$	NA	
Shigella	$1x10^3$	$1x10^4$	$1x10^4$	0.7	YES
			$1x10^3$	NA	
			$1x10^2$	NA	
			$1x10^1$	NA	
C jejuni	$1x10^1$	$1x10^2$	$1x10^4$	0.3	YES
			$1x10^3$	-0.3	
			$1x10^2$	-0.1	
			$1x10^1$	NA	
			$1x10^0$	NA	
			$1x10^{-1}$	NA	
C. coli	$1x10^2$	$1x10^2$	$1x10^5$	0.5	YES
			$1x10^4$	0.7	
			$1x10^3$	0.4	
			$1x10^2$	-0.6	
			$1x10^1$	NA	
			$1x10^0$	NA	
STEC ***stx1+/stx2+***	***stx1* Detection**				
	$1x10^4$	$1x10^4$	$1x10^5$	1.0	YES
			$1x10^4$	1.6	
			$1x10^3$	NA	
			$1x10^2$	NA	
	***stx2* Detection**				
	$1x10^4$	$1x10^4$	$1x10^5$	0.8	YES
			$1x10^4$	1.4	
			$1x10^3$	NA	
			$1x10^2$	NA	

In conclusion, both the CB and C&S media resulted in 100% detection at the same lowest concentration tested for *C. coli* and STEC. *Salmonella*, *Shigella*, and *C. jejuni* in CB medium resulted in 100% detection at a concentration 1 log higher than in C&S

medium. In addition, none of the organisms revealed a difference in average Ct value of more than 3.3 between the different media at any concentration tested. Therefore, the performance is considered equivalent. Stool samples may be collected in CB and/or C&S for use with the ProGastro SSCS Assay.

n. Comparator Assays Analytical Validation Studies

The ProGastro SSCS Assay is a Real Time PCR Assay comprised of two Mixes, one used to detect and differentiate *Salmonella*, *Shigella*, and *Campylobacter* (*C. jejuni* and *C. coli* only, not differentiated, SSC Mix) while the other Mix detects and differentiates the Shiga Toxin 1 (*stx1*) and Shiga Toxin 2 (*stx2*) genes in bacteria such as Shiga Toxin producing *E. coli* (STEC Mix). The ProGastro SSCS Assay Clinical Study established performance by comparing ProGastro SSCS results to broth enrichment/EIA for Shiga Toxin producing bacteria. The broth enrichment/EIA Kit (Meridian Biosciences Premier EHEC EIA Kit) does not differentiate between *stx1* and *stx2* positive STEC samples and as a result, a sequence reference method (SRM) for the detection and differentiation of *stx1* and *stx2* was developed. Any STEC positive sample via one or both testing methods (EIA or ProGastro SSCS STEC Mix) was further tested for *stx1* and *stx2* using these sequencing assays. The SRM is comprised of two different PCR mixes, one targeting a different area of the *stx1* gene than the ProGastro SSCS Assay's STEC Mix and the second targeting a different area of the *stx2* gene than the STEC Mix. Samples yielding a PCR product from one or both of the SRM PCR Mixes were sent to an external facility for bi-directional sequencing.

Analytical Sensitivity, Reactivity, and Specificity studies were conducted to verify the analytical performance of these SRM *stx1* and *stx2* sequencing assays.

<u>Limit of Detection</u>

The limit of detection (LoD) of the STEC SRM was determined using previously titered (CFU/ml) cultures of STEC, including a single strain of STEC O157:H7 (*stx1*+/*stx2*-) for the *stx1* SRM Mix and STEC Non-O157 (*stx1*-/*stx2*+) for the *stx2* SRM Mix. Two strains of previously cultured and titered bacteria were serially diluted, spiked into a ProGastro SSCS Assay negative stool matrix pool, and tested at three concentrations: 1 log above, at, and 1 log below the tentative LoD. Each dilution series was extracted on the bioMérieux NucliSENS easyMAG in a single replicate and tested in five replicate PCR reactions using the required STEC SRM Mix depending on the strain (*stx1*+ strain with the *stx1* Mix and *stx2*+ strain with the *stx2* Mix). PCR products generated with the SRM Mixes were analyzed using the Qiagen QIAxcel capillary electrophoresis instrument. LoD was determined to be the lowest concentration of bacteria detected $\geq$95% of the time (5/5 replicates). The LoD for each strain was confirmed by the generation of 20 independent extraction data points using the specific spiked stool concentration utilized during the LoD Determination portion of this study. The required spiked sample was used to perform 20 independent extractions (per strain) on the easyMAG with each purified nucleic acid sample run in

a single replicate using a single lot of the required STEC SRM Mix depending on the strain. Subsequent to capillary electrophoresis during the LoD Confirmation portion of the study, one representative sample containing the least concentrated product for each strain was confirmed for the correct gene target by bi-directional genetic sequencing and NCBI BLAST Analysis (www.ncbi.nlm.nih.gov/blast/BLAST.cgi). At least 95% of the 20 replicates were required to test positive to confirm the LoD for each bacterial strain.

The results of the LoD estimation portion of the study are presented in the following table. Concentrations that are **bolded** were used for the subsequent Confirmation portion of the study.

SRM Assay LoD Estimation Results					
STEC O Antigen: H Type	**STEC Center Accession#**	**Strain Name**	***stx1/stx2* Designation**	**Concentration Tested (CFU/ml)**	**Replicates Detected**
STEC O157:H7	TW00975	2886-75	(*stx1*+/*stx2*-)	$1x10^5$	5/5
				$1x10^4$	**5/5**
				$1x10^3$	3/5
STEC O26:HNM	TW08569	CB7776	(*stx1*-/*stx2*+)	$1x10^5$	5/5
				$1x10^4$	**5/5**
				$1x10^3$	1/5

The table below summarizes the confirmed LoD for the strains tested.

SRM Assay LoD Confirmation Results						
STEC O Antigen: H Type	**STEC Center Accession#**	**Strain Name**	***stx1/stx2* Designation**	**Concentration Tested (CFU/ml)**	**Replicates Detected**	**% Detected**
STEC O157:H7	TW00975	2886-75	(*stx1*+/*stx2*-)	$1x10^4$	20/20	100%
STEC O26:HNM	TW08569	CB7776	(*stx1*-/*stx2*+)	$1x10^4$	20/20	100%

The LoD comparison between the ProGastro SSCS Assay (STEC Mix) and the STEC SRM is presented in the table below. The LoD for the SRM is comparable to the LoD confirmed for these two strains using the ProGastro SSCS Assay.

Comparison of LoD between SRM Assays and ProGastro SSCS Assay					
STEC O Antigen: H Type	**STEC Center Accession#**	**Strain Name**	***stx1/stx2* Designation**	**LoD STEC SRM**	**LoD ProGastro SSCS Assay**
STEC O157:H7	TW00975	2886-75	(*stx1*+/*stx2*-)	$1x10^4$ CFU/mL	1.66×10^4 CFU/mL
STEC O26:HNM	TW08569	CB7776	(*stx1*-/*stx2*+)	$1x10^4$ CFU/mL	9.27×10^3 CFU/mL

Analytical Reactivity

Each STEC strain analyzed in this study tested positive by the STEC SRM Assay for its respective *stx* gene (*stx1* and/or *stx2*, depending on the strain). *Shigella dysenteriae* ATCC# 29027 was found to be non-reactive with the STEC SRM Assay (both *stx1* and *stx2*) as expected. The following table summarizes the results.

SRM Assay Analytical Reactivity Results Summary					
Strain	**Concentration Tested**	***stx1***	***stx1* SRM Mix BLAST Result**	***stx2***	***stx2* SRM Mix BLAST Result**
STEC DEC10B	$2x10^3$ CFU/ml	+	**Positive**	-	Negative
97-3250	$2x10^4$ CFU/ml	+	**Positive**	+	**Positive**
DA-21	$2x10^4$ CFU/ml	+	**Positive**	-	Negative
MI03-19	$2x10^4$ CFU/ml	+	**Positive**	-	Negative
STEC MT#80	$2x10^3$ CFU/ml	+	**Positive**	-	Negative
3215-99	$2x10^4$ CFU/ml	+	**Positive**	+	**Positive**
RD8	$2x10^4$ CFU/ml	-	Negative	+	**Positive**
STEC 0201 9611	$2x10^3$ CFU/ml	+	**Positive**	-	Negative
STEC DA-5	$2x10^3$ CFU/ml	-	Negative	+	**Positive**
STEC DA-1	$2x10^3$ CFU/ml	-	Negative	+	**Positive**
STEC GS G5578620	$2x10^3$ CFU/ml	+	**Positive**	-	Negative
IH 16	$2x10^4$ CFU/ml	-	Negative	+	**Positive**
STEC 7:85	$2x10^4$ CFU/ml (*stx1*)	+	**Positive**	+	NA*
STEC 7:85	$2x10^3$ CFU/ml (*stx2*)	+	NA*	+	**Positive**
STEC 93-111	$2x10^3$ CFU/ml	+	**Positive**	+	**Positive**
DA-34	$2x10^4$ CFU/ml	+	**Positive**	-	Negative
STEC EDL933	$2x10^3$ CFU/ml	+	**Positive**	+	**Positive**
1:361	$2x10^4$ CFU/ml	-	Negative	+	**Positive**
DA-54	$2x10^4$ CFU/ml	-	Negative	+	**Positive**
Shigella dysenteriae	$2x10^3$ CFU/ml	+	**Positive**	-	Negative
Shigella dysenteriae ATCC 29027	$1.03x10^3$ CFU/ml	-	Negative	-	Negative

Analytical Specificity

The STEC SRM Assay did not react with any of the non-target organisms included in this study, other than *Escherichia hermannii*. This strain produced a band with interpretable sequencing results with the *stx2* Mix which was identified as *E. coli* by BLAST analysis, but further analysis revealed no positive hits for *stx2*, so it is not in fact reactive with the STEC SRM assay. The *stx1* target strain was detected using the *stx1* Mix, but negative with the *stx2* Mix. The *stx2* target strain was detected using the *stx2* Mix, but negative with the *stx1* Mix. The STEC SRM Assay demonstrates no cross reactivity with the organisms that are commonly found in stool, genetically related or cause similar disease states as the ProGastro SSCS Assay target organisms. *In silico* analysis of the *Cyclospora cayetanensis* genome showed that each primer and probe included with the SRM Mixes had no similarity to the organism.

In conclusion, the STEC SRM demonstrated similar analytical sensitivity (limit of detection, LoD) and reactivity as compared to the ProGastro SSCS Assay STEC Mix. The STEC SRM was also reactive to the same set of STEC used in the Analytical Specificity Study for the ProGastro SSCS Assay STEC Mix. The STEC SRM was not cross-reactive with any common stool organisms or organisms that cause similar disease states as the ProGastro SSCS Assay target organisms. Based on these

analytical studies performed, the STEC SRM was acceptable to use as part of the reference method to differentiate *stx*1 from *stx*2 for positive samples found by the broth enrichment/EIA Kit (Meridian Biosciences Premier EHEC EIA Kit) reference method or the ProGastro SSCS Assay STEC Mix.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Refer to the Clinical Studies Section of this document.

b. Matrix comparison:

Not applicable

3. Clinical studies:

Prospective Clinical Study

The prospective clinical performance study was conducted at four U.S. clinical laboratory sites using prospectively collected samples. All four sites enrolled and tested prospective samples that were collected from July 2011 through November 2011. One of the four sites also enrolled and tested approximately 250 prospective samples collected in May 2012 – July 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%)

Leftover stool specimens collected from symptomatic patients were split and one portion was tested using each site's routine culture/EIA procedures. The second portion of the samples underwent nucleic acid extraction using the bioMérieux NucliSens easyMAG Instrument and reagents. Purified nucleic acids were tested with

the ProGastro SSCS Assay using both the SSC and STEC Mixes on the Cepheid SmartCycler II.

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*, STEC). Samples positive for STEC by broth/EIA and/or the ProGastro SSCS Assay underwent PCR/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. Sixty-one patient/samples were excluded from the performance calculations due to deviations from the clinical study protocol. Fourteen 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.

The prospective study performance data (all sites combined) are presented in the following tables stratified by analyte:

***Campylobacter (C. jejuni / C. coli*), Prospective Samples**

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

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

***Salmonella*, Prospective Samples**

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

[a] Ten samples were positive for *Salmonella* by bi-directional sequence analysis.
[b] Sample was positive for *Salmonella* by bi-directional sequence analysis.

***Shigella*, Prospective Samples**

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

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

STEC, Prospective Samples

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

[a] Six samples positive for *stx1*, one sample positive for *stx2*, and two samples positive for *stx1* and *stx2* by bi-directional sequence analysis.
[b] Six samples positive for *stx1* and three samples positive for *stx2* by bi-directional sequence analysis.

***Stx* 1, Prospective Samples (Percent Agreement between ProGastro SSCS and Composite Reference Method for *stx1*)**

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

[a] Five samples were positive by bi-directional sequencing for *stx*1, but were negative by Broth Enrichment/EIA.

***Stx2*, Prospective Samples (Percent Agreement between ProGastro SSCS and Composite Reference Method for *stx2*)**

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

[a] Three samples were positive by bi-directional sequencing for stx2, but were negative by Broth Enrichment/EIA.

Prospective Clinical Study Mixed Infection Analysis

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 infections sample was double infections 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			**Total Co-infections**	**Number of Discrepant Co-infections[a]**	**Discrepant Analyte(s)[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 or discrepant analyte 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 Clinical Study

In addition to the prospective clinical study, two of the four 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

The retrospective study performance data (both sites combined) are presented in the following tables stratified by analyte:

***Campylobacter (C. jejuni / C. coli*), Retrospective Samples**

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

***Salmonella*, Retrospective Samples**

ProGastro SSCS Assay SSC Mix	**Culture**		
	Positive	**Negative**	**Total**
Positive	3	0	3
Negative	0	102	102
Total	3	102	105
Positive Percent Agreement y: 100.0% (43.9% - 100.0%)			
Negative Percent Agreement: 100.0% (96.4% - 100.0%)			

***Shigella*, Retrospective Samples**

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

STEC, Retrospective Samples

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

[a] Five samples positive for *stx1*, five samples positive for *stx2*, and nine samples positive for *stx1* and *stx2*.

Stx* 1, Retrospective Samples (Percent Agreement between ProGastro SSCS and Composite Reference Method for *stx1

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

Stx2*, Prospective Samples (Percent Agreement between ProGastro SSCS and Composite Reference Method for *stx2

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

ProGastro SSCS Assay Failure Rate Due to Controls (Prospective and Retrospective Studies Combined)

There were a total of four ProGastro SSCS runs out of 136 (75 SSC and 61 STEC) runs that were invalid, three from the prospective study and one from the retrospective study. Two of the run failures were due to Negative Control contamination. Another invalid run was due to the PC1 failure for STEC and it is hypothesized that the SSCS PC1 and the *C. coli* PC2 were mistakenly switched in the SmartCyler II. The final

invalid run was due to 3084/3089 error with an SSC run, where the SmartCycler II run is started before the I-CORE Lid has been closed. For each initially invalid run, all samples, the Negative Control, and the PMC (Extraction Control) were re-run using the ProGastro SSCS Assay starting from the purified nucleic acid and repeat test results were valid and used in analysis.

"Unresolved" Samples (Prospective and Retrospective Studies Combined)
An "Unresolved" result is generated when the Gastro 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). According to the Assay Instructions for Use and the ProGastro SSCS Clinical Study Protocol all "Unresolved" samples were to be retested starting from the purified nucleic acids. (Note: Detection of the GIC in the Cy5 detection channel is not required for reporting negative results for a sample when at least one analyte is detected in the sample in the proper detection channel, since high bacterial load can lead to reduced or absent Internal Control signal.) Samples with a repeat "Unresolved" result were excluded from the final data analysis. Valid repeat negative and positive results were included in the final analysis of the Clinical Study. The following table summarizes the ProGastro SSCS Assay "Unresolved" samples from the prospective and the retrospective study.

Summary of "Unresolved" ProGastro SSCS Clinical Trial Samples						
SSC Mix	**Site 1**	**Site 2**	**Site 4**	**Site 5**	**Site 6**	**Overall**
Number of Samples Run	345	400	340	123	50	1258
Unresolved upon initial testing	3	20	0	2	0	25
Unresolved upon repeat testing	1	12	0	1	N/A	14
% Unresolved	0.3%	3.0%	0.0%	0.8%	0.0%	1.1%
Negative upon repeat testing	2	8	0	0	N/A	10
Positive upon repeat testing	0	0	0	1	N/A	1
STEC Mix						
Number of Samples Run	345	400	340	123	50	1258
Unresolved upon initial testing	2	20	3	1	0	26
Unresolved upon repeat testing	2	11	0	1	N/A	14
% Unresolved	0.6%	2.8%	0.0%	0.8%	0.0%	1.1%
Negative upon repeat testing	0	9	3	0	N/A	12
Positive upon repeat testing	0	0	0	0	N/A	0

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

In the ProGastro SSCS Assay prospective 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, stratified by age group, are presented in the following table:

Age Group	**Total # SSC Mix/Total # STEC Mix**	**#*Campylobacter* Positive (Observed Positivity Rate)**	**#*Salmonella* Positive (Observed Positivity Rate)**	**#*Shigella* Positive (Observed Positivity Rate)**	**#STEC Positive (Observed Positivity Rate)**
< 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.

N. Instrument Name:

Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b or 3.0a/b

NucliSENS® easyMAG™ System (bioMérieux)

O. System Descriptions:

1. Modes of Operation:

   The bioMerieux NucliSENS easyMAG system is used for nucleic acid isolation from stool samples.

   The Cepheid SmartCycler II Real Time instrument with Dx software version 1.7b or 3.0a/b is used to perform PCR amplification and detection of nucleic acid.

   Several other Prodesse Assays have been cleared for use with the above instrumentation systems: Prodesse ProFlu+ (k110968), Pro hMPV+ (k082688), ProGastro Cd (k090239), ProParaflu+ (k091053), ProFAST+ (k101855) and ProAdeno+ (k102952).

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

User enters Patient ID/Sample ID by typing it in.

4. Specimen Sampling and Handling:

   Not applicable

5. Calibration:

   Not applicable

6. Quality Control:

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

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.