EVALUATION OF AUTOMATIC CLASS III DESIGNATION (DE NOVO) FOR xTAG® GASTROINTESTINAL PATHOGEN PANEL (GPP) DECISION SUMMARY

A. 510(k) Number: K121454

B. Purpose for Submission: Clearance of new assay

C. Measurand:

- *Campylobacter* (*C. jejuni, C. coli* and *C. lari* only)
- *Clostridium difficile* (*C. 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 – also known as *G. intestinalis* and *G. duodenalis*)
- Norovirus GI/GII
- Rotavirus A
- *Salmonella* (see Analytical Reactivity section for a list of serotypes detected)
- Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2
- *Shigella* (*S. boydii, S. sonnei, S. flexneri* and *S. dysenteriae*) in human stool samples.

D. Type of Test: Qualitative nucleic acid multiplex test

E. Applicant: Luminex Molecular Diagnostics, Inc.

F. Proprietary and Established Names: xTAG® Gastrointestinal Pathogen Panel (GPP)

G. Regulatory Information:

FDA identifies this generic type of device as:

A gastrointestinal microorganism multiplex nucleic acid-based assay is a qualitative *in vitro* diagnostic device intended to simultaneously detect and identify multiple gastrointestinal microbial nucleic acids extracted from human stool specimens. The device detects specific nucleic acid sequences for organism identification as well as for determining the presence of toxin genes. The detection and identification of a specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation and other laboratory findings. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

1. New Regulation Number:

   21 CFR 866.3990 – Gastrointestinal microorganism multiplex nucleic acid-based assay

2. Classification:

   Class II

3. Product code:

   PCH, NSU, JJH

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   The xTAG® Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG® GPP:

   - *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only)
   - *Clostridium difficile* (*C. difficile*) toxin A/B
   - *Cryptosporidium* (*C. parvum* and *C. hominis* only)
   - *Escherichia coli* (*E. coli*) O157
   - Enterotoxigenic *Escherichia coli* (ETEC) LT/ST
   - *Giardia* (*G. lamblia* only - also known as *G. intestinalis* and *G. duodenalis*)
   - Norovirus GI/GII
   - Rotavirus A
   - *Salmonella*
   - Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2
   - *Shigella* (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*)

   The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

   xTAG® GPP positive results are presumptive and must be confirmed by FDA-

cleared tests or other acceptable reference methods.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed 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 xTAG Gastrointestinal Pathogen Panel 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.

xTAG® GPP is not intended to monitor or guide treatment for *C. difficile* infections.

The xTAG® GPP is indicated for use with the Luminex® 100/200™ instrument.

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   For prescription use only. Manufacturer must provide device-specific user training to facilities prior to using the device.

4. Special instrument requirements:

   Extraction: Biomerieux NucliSens® EasyMag® instrument

   Analysis: Luminex® 100/200™ instruments with xPONENT® software

I. Device Description:

The Luminex Molecular Diagnostics xTAG GPP consists of kit reagents and software. The reagents in conjunction with a thermal cycler are used to perform nucleic acid amplification (reverse transcription-polymerase chain reaction, or RT-PCR/PCR), and the protocol configuration file is used to generate results while the data analysis software (TDAS GPP (US)) is used to analyze the results from the Luminex Corporation Luminex 100/200 instrument system (which includes the xPONENT core software).

The components of the xTAG GPP kit are contained within 2 boxes (one that is frozen, and one that is refrigerated). The kit is shipped with the xTAG GPP CD which contains the xTAG GPP T-A (LX) protocol configuration file and the TDAS GPP (US) software. The instrument is shipped with the xPONENT software.

The xTAG Gastrointestinal Pathogen Panel (xTAG GPP) incorporates multiplex reverse transcription and polymerase chain reaction (RT-PCR / PCR) with Luminex's proprietary universal tag sorting system on the Luminex platform. The assay also detects an internal control (bacteriophage MS2) that is added to each sample prior to extraction. Each sample is pre-treated prior to extraction and is then put through extraction using the Biomerieux NucliSens EasyMag kit (product code JJH, class I, an IVD-labeled automated system for nucleic acid extraction).

Post-extraction, for each sample, 10 µL of extracted nucleic acid is amplified in a single multiplex RT-PCR/PCR reaction. Each target or internal control in the sample results in PCR amplicons ranging from 58 to 202 bp (not including the 24-mer tag). A five µL aliquot of the RT-PCR product is then added to a hybridization/detection reaction containing bead populations coupled to sequences from the Universal Array ("antitags"), streptavidin, R-phycoerythrin conjugate. Each Luminex bead population detects a specific microbial target or control through a specific tag/anti-tag hybridization reaction. Following the incubation of the RT-PCR products with the xTAG GPP Bead Mix and xTAG Reporter Buffer, the Luminex instrument sorts and reads the hybridization/detection reactions.

A signal, or median fluorescence intensity (MFI), is generated for each bead population. These fluorescence values are analyzed to establish the presence or absence of bacterial, viral or parasitic targets and/or controls in each sample. A single multiplex reaction identifies all targets.

The xTAG Data Analysis Software for the Gastrointestinal Pathogen Panel (TDAS GPP (US)) analyzes the data to provide a report summarizing which pathogens are present. Before data are analyzed, a user has the option to select a subset of the targets from the intended use of the xTAG GPP (for each sample). Consequently the remaining target results are masked and cannot be retrieved.

Target results above or equal to the cutoff are considered positive, while target results below the cutoff are considered negative. For each sample analyzed by TDAS GPP (US), there are individual results for each of the targets and the internal control (bacteriophage MS2).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   None

2. Predicate 510(k) number(s):
   None

3. Comparison with predicate:
   Not applicable

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

Standards Referenced

	Standards No.	Recognition Number (FDA)	Standards Title	Date
1	MM13-A	7-191	Collection, Transport, Preparation and Storage of Specimens	03/18/2009
2	EP15-A2	7-153	User Verification of Performance for Precision and Trueness (2nd edition)	09/09/2008
3	EP05-A2	7-110	Evaluation of Precision Performance of Quantitative measurement Methods (2nd ed.)	10/31/2005
4	EP07-A2	7-127	Interference Testing in Clinical Chemistry (2nd edition)	05/21/2007
5	EP12-A2	7-152	User Protocol for Evaluation f Qualitative Test Performance (2nd edition)	09/09/2008
6	EP17-A	7-194	Protocol for Determination of Limits of Detection and Limits of Quantitation	03/18/2009
7	EP14-A2	7-128 and 7-143	Evaluation of Matrix Effects (2nd edition)	06/01/2004
8	MM03-A2	7-132	Molecular Diagnostic Methods for Infectious Diseases (2nd edition)	09/09/2008
9	CEN 13640	7-84	Stability Testing of In Vitro Diagnostic Reagents	06/01/2004
10	ISO 14971	5-40	Application of Risk Management to Medical Devices	09/12/2007

Guidance Documents Referenced

	Title	Date
1	Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection of *Clostridium difficile*	11/29/10
2	Class II Special Controls Guidance Document: Norovirus Serological Reagents	03/09/12
3	Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems - Guidance for Industry and FDA Staff	03/10/05
4	Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices	5/11/05
5	Guidance document for Format for Traditional and Abbreviated 510(k)s	08/12/05
6	Guidance on the CDRH Premarket Notification Review Program, 510(k) Memorandum #K86-3	06/30/86
7	The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications - Final Guidance	03/20/98
8	The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)]	12/27/11
9	Draft Guidance for Industry and Food and Drug Administration Staff - eCopy Program for Medical Device Submissions	10/17/12
10	Guidance for Industry and FDA Staff - Factors to Consider When	03/28/12

	Making Benefit-Risk Determinations in Medical Device Premarket Approval and De Novo Classifications	
11	Draft Guidance for Industry and Food and Drug Administration Staff - De Novo Classification Process (Evaluation of Automatic Class III Designation)	10/03/11
12	Guidance for Industry and Food and Drug Administration Staff - FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Goals	10/15/12

L. Test Principle:

Human stool samples are pretreated and then subjected to nucleic acid extraction. For each sample, 10 µL of extracted nucleic acid is amplified in a single multiplex RT-PCR/PCR reaction. Each target or internal control in the sample results in PCR amplimers ranging from 58 to 202 bp (not including the 24-mer tag). A five µL aliquot of the RT-PCR product is then added to a hybridization/detection reaction containing bead populations coupled to sequences from the Universal Array ("antitags"), streptavidin, R-phycoerythrin conjugate. Each Luminex bead population detects a specific microbial target or control through a specific tag/anti-tag hybridization reaction. Following the incubation of the RT-PCR products with the xTAG GPP Bead Mix and xTAG Reporter Buffer, the Luminex instrument sorts and reads the hybridization/detection reactions. A signal or median fluorescence intensity (MFI) is generated for each bead population. These fluorescence values are analyzed to establish the presence or absence of bacterial, viral or parasitic targets and/or controls in each sample. A single multiplex reaction identifies all targets.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:
   Site-to-site reproducibility was assessed for each of the indicated microbial targets and for mixed analyte samples (representing co-infected samples). Replicates of simulated samples were tested across 3 sites by 2 operators at each site. All sample replicates tested were prepared through serial dilutions of stock material (pre-treated negative stool spiked with a pathogen or positive stool) containing a microbial target from the intended use. Each sample replicate assayed in the study contained either a single microbial target or 2 microbial targets detected by xTAG GPP in addition to the internal control (bacteriophage MS2). For single analyte samples, dilutions tested fell into 1 of the following 3 categories:

   1. High Negative (HN): microbial target concentrations which generate MFI values not lower than 20-30% below the cut-off MFI for the indicated analyte
   2. Low Positive (LP): microbial target concentrations which generated MFI values that were 1-5X the cut-off MFI for the indicated analyte

3. Moderate Positive (MP): microbial target concentrations which generated MFI values 7- 10X the cut-off MFI for the indicated analyte.

For those samples prepared to simulate co-infections, one microbial target was present at the LP level defined above and the other at a High Positive (HP) level. HP levels were defined as follows:

> High Positive (HP) viral cultures were prepared to a concentration of 10^5 PFU/mL (10^5 $TCID_{50}$/mL) or higher; High Positive (HP) bacterial cultures were prepared to a concentration of 10^6 CFU/mL or higher.

Each sample replicate underwent a single pre-treatment and extraction step. All samples were extracted using the NucliSens EasyMAG extraction method. Extracted material was kept frozen at -70°C until testing. A total of 90 replicates were tested for each sample (3 replicates per run x 5 runs per operator x 2 operators per site x 3 sites = 90 replicates). Reproducibility was assessed both in terms of calls and MFI values.

For single analyte samples prepared at the MP level, depending on the microbial target, 89/90 (99%) to 90/90 (100%) replicates generated a positive result. For LP dilutions, depending on the microbial target, the correct positive call was made in 80/90 (89%) to 90/90 (100%) replicates tested. The only exception in terms of LP detection was *Cryptosporidium*, due to the fact that the initial titer for this particular sample was below the targeted range of 1-5X the cut-off MFI. For HN dilutions, depending on the microbial target, the correct negative call was generated in as few as 54/90 (60%) replicates to as many as 90/90 (100%). Greater variability in the HN dilution, compared to the LP and MP dilution, was expected based on the fact that a microbial target is present in these samples at levels sufficient to generate MFI values 20-30% below the cut-off MFI, and based on the stochastic nature of end-point PCR in the presence of low levels of targeted analytes. Accordingly, percent variability, measured as the coefficient of variation (CV) for MFI values were lowest at the MP dilution and highest at the HN dilution.

For dual analyte samples, all microbial targets generated a positive call when present as a HP dilution. When present at the LP concentration, 3 of the 6 microbial target combinations tested generated a positive call in 90/90 (100%) replicates tested. The following was observed for the remaining 3 targets present at LP concentrations in samples containing a second microbial target at HP concentrations:

- 2/90 replicates of the ETEC (HP) / *Salmonella* (LP) sample generated a negative call for *Salmonella*
- 4/90 replicates of the *Salmonella* (HP) / Rotavirus (LP) sample generated a negative call for rotavirus
- 12/90 replicates of the Rotavirus (HP) / Norovirus GII (LP) sample generated a negative call for norovirus

Reproducibility of Overall Total Raw Median MFI values for All Targets in xTAG GPP

	Panel Member ID	***Campylobacter* Low Positive**	***Campylobacter* Medium Positive**	***Campylobacter* High Negative**	***C. difficile* Toxin A/B Low Positive**		***C. difficile* Toxin A/B Medium Positive**		***C. difficile* Toxin A/B High Negative**		***Cryptosporidium hominis* Low Positive**	***Cryptosporidium hominis* Medium Positive**	***Cryptosporidium hominis* High Negative**
					Probe 1	**Probe 2**	**Probe 1**	**Probe 2**	**Probe 1**	**Probe 2**			
	Concentration	$9.38x10^5$ CFU/mL	$3.75x10^6$ CFU/mL	$1.17x10^5$ CFU/mL	$3.75x10^6$ CFU/mL	$9.38x10^5$ CFU/mL	$1.50x10^7$ CFU/mL	$1.50x10^7$ CFU/mL	$2.34x10^5$ CFU/mL	$2.34x10^5$ CFU/mL	$6.21x10^3$ Copies/mL	$2.05x10^4$ Copies/mL	$6.37x10^2$ Copies/mL
Site 1	Agreement with Expected Result	30/30 100%	30/30 100%	5/30 16.7%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	28/30 93.3%	17/30 56.7%	30/30 100%	30/30 100%
	25th Percentile MFI	1081.0	2022.0	183.5	259.5	256.0	918.0	1821.0	38.5	67.0	165.0	748.0	48.0
	Median MFI Value	1283.3	2269.0	275.5	363.0	297.0	1113.0	1936.3	48.5	80.5	265.0	838.5	54.0
	75th Percentile MFI	1561.0	2520.5	329.5	413.5	383.0	1317.0	2010.0	55.5	105.0	325.0	895.5	66.5
	% CV	23.40	13.06	N/A	32.66	31.19	16.73	8.11	N/A	N/A	35.37	15.89	N/A
Site 2	Agreement with Expected Result	27/30 90%	30/30 100%	19/30 63.3%	29/30 96.7%	30/30 100%	30/30 100%	30/30 100%	29/30 96.7%	17/30 56.7%	27/30 90%	29/30 96.7%	30/30 100%
	25th Percentile MFI	842.5	1924.5	99.0	235.5	315.0	726.0	2529.0	29.0	85.0	284.5	827.0	49.0
	Median MFI Value	1075.3	2086.0	130.3	362.8	412.3	988.5	2723.3	40.8	141.0	327.8	1059.0	56.5
	75th Percentile MFI	1520.0	2515.0	200.0	444.0	509.0	1263.0	2864.0	45.0	166.5	535.5	1140.5	64.0
	% CV	51.66	18.76	N/A	37.17	36.61	33.20	8.95	N/A	N/A	34.46	26.02	N/A
Site 3	Agreement with Expected Result	25/30 83.3%	30/30 100%	30/30 100%	21/30 70%	25/30 83.3%	29/30 96.7%	30/30 100%	30/30 100%	30/30 100%	2/30 6.7%	30/30 100%	30/30 100%
	25th Percentile MFI	160.0	416.0	42.5	145.0	187.0	620.0	1491.0	37.5	55.0	122.0	505.0	51.0
	Median MFI Value	258.5	757.5	55.5	195.3	213.0	790.0	1691.0	44.0	67.0	160.8	559.0	61.8
	75th Percentile MFI	369.0	1086.5	65.0	258.5	250.0	945.0	1851.0	56.0	76.0	191.0	652.0	75.0
	% CV	48.96	49.92	N/A	38.35	28.36	36.07	23.66	N/A	N/A	32.52	19.57	N/A
	Total Agreement with Expected Result	82/90 91.1%	90/90 100%	54/90 60%	80/90 88.9%	85/90 94.4%	89/90 98.9%	90/90 100%	89/90 98.9%	75/90 83.3%	46/90 51.1%	89/90 98.9%	90/90 100%
	95% CI	83.4%-95.4%	95.9%-100.0%	49.7%-69.5%	80.7%-93.9%	87.6%-97.6%	94.0%-99.8%	95.9%-100.0%	94.0%-99.8%	74.3%-89.6%	41.0%-61.2%	94.0%-99.8%	95.9%-100.0%
	Overall 25th Percentile MFI	298.0	1086.5	62.0	218.0	219.0	781.0	1801.5	35.0	61.5	161.0	584.0	48.5
	Overall Median MFI Value	1003.0	1990.3	121.5	281.5	283.0	954.5	1966.3	43.5	79.3	260.0	801.3	58.0
	Overall 75th Percentile MFI	1315.0	2326.0	241.0	382.5	397.5	1193.5	2529.0	52.5	121.0	325.0	944.0	70.0
	Overall % CV	66.01	45.24	N/A	43.21	44.32	32.01	25.52	N/A	N/A	50.13	30.95	N/A

	Panel Member ID	*E. coli O157* Low Positive	*E. coli O157* Medium Positive	*E. coli O157* High Negative	ETEC LT/ST Low Positive		ETEC LT/ST Medium Positive		ETEC LT/ST High Negative		*Giardia* Low Positive	*Giardia* Medium Positive	*Giardia* High Negative
					Probe 1	Probe 2	Probe 1	Probe 2	Probe 1	Probe 2			
	Concentration	$2.34x10^5$ CFU/mL	$3.75x10^6$ CFU/mL	$2.93x10^4$ CFU/mL	$2.93x10^4$ CFU/mL	$9.37x10^5$ CFU/mL	$3.75x10^6$ CFU/mL	$3.75x10^6$ CFU/mL	$7.32x10^3$ CFU/mL	$7.32x10^3$ CFU/mL	$8.79x10^2$ Cells/mL	$3.25x10^3$ Cells/mL	$2.74x10^1$ Cells/mL
Site 1	Agreement with Expected Result	30/30 100%	30/30 100%	24/30 80%	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%
	25th Percentile MFI	546.0	2494.0	74.0	294.0	1387.0	2203.0	2097.0	53.0	25.0	541.0	1145.0	51.0
	Median MFI Value	685.0	2585.0	104.3	344.0	1468.5	2287.8	2149.3	67.5	36.5	657.8	1303.3	59.5
	75th Percentile MFI	840.0	2673.0	140.0	408.5	1545.0	2356.0	2216.5	90.0	42.5	742.0	1392.0	76.5
	% CV	27.64	4.82	N/A	18.49	7.75	6.27	6.32	N/A	N/A	21.87	13.80	N/A
Site 2	Agreement with Expected Result	30/30 100%	30/30 100%	23/30 76.7%	29/30 96.7%	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%
	25th Percentile MFI	506.0	2618.0	69.0	317.0	1617.0	2532.5	2443.0	51.5	36.5	752.0	1506.0	41.0
	Median MFI Value	707.8	3012.3	93.8	419.3	1729.0	2641.0	2537.8	74.0	46.5	901.5	1579.8	57.0
	75th Percentile MFI	947.0	3159.0	120.0	494.5	1867.0	2759.5	2619.0	106.5	52.5	1053.0	1953.0	72.5
	% CV	42.42	12.44	N/A	31.18	18.92	6.26	7.77	N/A	N/A	21.63	25.88	N/A
Site 3	Agreement with Expected Result	27/30 90%	30/30 100%	30/30 100%	29/30 96.7%	30/30 100%	30/30 100%	30/30 100%	25/30 83.3%	30/30 100%	29/30 96.7%	30/30 100%	30/30 100%
	25th Percentile MFI	229.0	1391.0	53.0	425.5	1265.0	2058.5	1723.0	69.0	36.0	278.5	817.0	49.5
	Median MFI Value	314.5	1595.3	67.5	477.0	1384.5	2212.3	1903.3	95.0	43.0	412.3	1065.0	58.5
	75th Percentile MFI	346.0	1773.5	78.0	668.0	1473.0	2326.5	2028.0	146.5	50.0	574.5	1214.0	72.5
	% CV	39.79	34.37	N/A	37.50	22.80	29.95	30.36	N/A	N/A	35.20	24.62	N/A
	Total Agreement with Expected Result	87/90 96.7%	90/90 100%	77/90 85.6%	88/90 97.8%	90/90 100%	90/90 100%	90/90 100%	85/90 94.4%	90/90 100%	89/90 98.9%	89/90 98.9%	90/90 100%
	95% CI	90.7%-98.9%	95.9%-100.0%	76.9%-91.4%	92.3%-99.4%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	87.6%-97.6%	95.9%-100.0%	94.0%-99.8%	94.0%-99.8%	95.9%-100.0%
	Overall 25th Percentile MFI	338.0	1773.5	64.0	328.0	1387.0	2203.0	1978.0	55.5	33.0	472.0	1089.0	45.0
	Overall Median MFI Value	537.0	2548.3	82.8	415.3	1499.5	2327.0	2170.8	76.3	41.3	636.0	1310.0	59.0
	Overall 75th Percentile MFI	764.5	2746.5	116.0	489.0	1650.5	2615.0	2456.5	106.5	49.5	827.5	1539.0	72.5
	Overall % CV	51.27	28.85	N/A	36.90	21.69	18.84	20.99	N/A	N/A	38.43	29.58	N/A

	Panel Member ID	Norovirus GI/GII Low Positive		Norovirus GI/GII Medium Positive		Norovirus GI/GII High Negative		Rotavirus A Low Positive	Rotavirus A Medium Positive	Rotavirus A High Negative	*Salmonella** Low Positive		*Salmonella** Medium Positive		*Salmonella** High Negative	
		Probe 1	**Probe 2**	**Probe 1**	**Probe 2**	**Probe 1**	**Probe 2**				**Probe 1**	**Probe 2**	**Probe 1**	**Probe 2**	**Probe 1**	**Probe 2**
	Concentration	$1.2x10^3$ Copies/mL	$1.74x10^3$ Copies/mL	$4.64x10^3$ Copies/mL	$7.45x10^3$ Copies/mL	$5.84x10^1$ Copies/mL	$5.95x10^1$ Copies/mL	$2.24x10^4$ Copies/mL	$4.47x10^5$ Copies/mL	$1.29x10^3$ Copies/mL	$1.17x10^5$ CFU/mL	$1.17x10^5$ CFU/mL	$9.38x10^5$ CFU/mL	$9.38x10^5$ CFU/mL	$3.66x10^3$ CFU/mL	$3.66x10^3$ CFU/mL
Site 1	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	26/30 86.7%	30/30 100%	29/30 96.7%	30/30 100%	29/30 96.7%	30/30 100%	30/30 100%	24/30 80%	29/30 96.7%
	25th Percentile MFI	477.0	860.0	1405.5	1635.0	50.0	90.0	212.5	546.0	50.0	831.5	469.0	2602.0	2744.0	43.0	38.0
	Median MFI Value	554.5	941.8	1476.5	1797.5	64.3	108.8	315.8	762.0	60.5	938.5	591.0	2681.3	2870.0	75.3	44.5
	75th Percentile MFI	659.5	1177.0	1697.0	2059.0	78.0	132.0	480.0	1101.0	68.5	1189.0	787.0	2843.0	3039.5	146.0	78.0
	% CV	26.14	23.87	12.92	19.34	N/A	N/A	59.70	56.62	N/A	23.48	37.04	6.01	6.07	N/A	N/A
Site 2	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	29/30 96.7%	30/30 100%	26/30 86.7%	30/30 100%	28/30 93.3%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	23/30 76.7%	22/30 73.3%
	25th Percentile MFI	492.0	743.0	1337.0	1643.0	46.0	104.0	212.0	888.0	54.0	867.0	565.5	3098.0	3289.0	42.0	30.0
	Median MFI Value	587.0	1004.0	1620.0	2012.5	58.8	115.5	399.5	1264.0	64.0	992.0	706.3	3272.8	3494.0	96.0	44.3
	75th Percentile MFI	765.0	1288.5	1777.0	2201.0	77.0	131.0	675.0	1548.0	72.5	1143.5	894.0	3393.0	3678.5	191.0	203.0
	% CV	32.86	27.61	17.82	15.20	N/A	N/A	79.96	35.73	N/A	28.89	44.35	15.27	13.89	N/A	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%	28/30 93.3%	30/30 100%	27/30 90%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	28/30 93.3%
	25th Percentile MFI	398.5	614.0	1088.0	1231.0	49.5	92.0	228.0	628.0	58.0	753.5	641.0	2178.0	2383.0	46.0	36.5
	Median MFI Value	491.8	676.5	1218.3	1353.0	62.0	107.0	332.5	748.0	68.0	936.0	691.0	2323.5	2526.3	65.5	56.3
	75th Percentile MFI	603.5	897.0	1324.0	1735.0	83.5	136.0	474.5	978.5	88.0	1154.0	925.0	2564.0	2773.5	99.0	91.5
	% CV	27.79	22.98	19.21	21.50	N/A	N/A	54.09	33.58	N/A	26.03	34.62	20.44	22.38	N/A	N/A
	Total Agreement with Expected Result	90/90 100%	90/90 100%	90/90 100%	90/90 100%	89/90 98.9%	90/90 100%	80/90 88.9%	90/90 100%	84/90 93.3%	90/90 100%	89/90 98.9%	90/90 100%	90/90 100%	77/90 85.6%	79/90 87.8%
	95% CI	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	94.0%-99.8%	95.9%-100.0%	80.4%-93.9%	95.9%-100.0%	86.2%-96.9%	95.9%-100.0%	94.0%-99.8%	95.9%-100.0%	95.9%-100.0%	76.9%-91.4%	79.4%-93.0%
	Overall 25th Percentile MFI	440.0	690.0	1233.0	1484.0	49.0	90.5	221.0	685.0	54.0	807.0	546.0	2401.5	2616.0	43.0	36.0
	Overall Median MFI Value	548.3	887.3	1439.0	1733.8	62.0	112.8	336.5	861.8	63.0	956.0	690.0	2712.8	2986.5	72.8	48.0
	Overall 75th Percentile MFI	693.0	1093.0	1698.0	2059.0	80.0	132.0	510.5	1318.0	76.5	1154.0	874.0	3131.0	3425.0	139.5	108.5
	Overall % CV	30.29	28.23	19.58	21.71	N/A	N/A	70.30	46.14	N/A	26.06	40.15	20.01	19.72	N/A	N/A

*The Salmonella positive (Pos) and negative (Neg) calls presented in this table, represent when the signal from the individual Salmonella probe in question is either above or below the assay threshold for a positive call, it does not represent a true assay positive or negative as information from both probes is required to determine the call for this target.

	Panel Member ID	STEC stx1/stx2 Low Positive		STEC stx1/stx2 Medium Positive		STEC stx1/stx2 High Negative		*Shigella* Low Positive	*Shigella* Medium Positive	*Shigella* High Negative
		Probe 1	**Probe 2**	**Probe 1**	**Probe 2**	**Probe 1**	**Probe 2**			
	Concentration	$9.38x10^5$ CFU/mL	$2.34x10^5$ CFU/mL	$3.75x10^6$ CFU/mL	$3.75x10^6$ CFU/mL	$2.93x10^4$ CFU/mL	$2.93x10^4$ CFU/mL	$7.32x10^3$ CFU/mL	$2.93x10^4$ CFU/mL	$2.29x10^2$ CFU/mL
Site 1	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	29/30 96.7%	29/30 96.7%	30/30 100%	27/30 90%
	25th Percentile MFI	715.5	296.5	1573.0	1756.0	42.0	58.5	589.0	1102.0	43.0
	Median MFI Value	813.0	414.8	1632.5	1830.3	57.0	85.3	644.5	1171.0	51.0
	75th Percentile MFI	894.0	509.5	1676.5	1881.0	76.0	105.0	730.0	1240.5	80.0
	% CV	18.73	25.75	8.56	5.86	N/A	N/A	25.21	12.04	N/A
Site 2	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	28/30 93.3%	23/30 76.7%	30/30 100%	30/30 100%	25/30 83.3%
	25th Percentile MFI	875.0	455.5	1974.0	2086.0	45.0	64.0	623.0	1255.0	42.0
	Median MFI Value	995.3	542.0	2051.8	2286.3	58.0	102.0	707.3	1316.3	64.8
	75th Percentile MFI	1140.0	607.0	2198.0	2387.0	100.0	148.0	866.5	1375.0	125.5
	% CV	23.38	22.15	11.04	8.77	N/A	N/A	23.68	10.25	N/A
Site 3	Agreement with Expected Result	29/30 96.7%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	23/30 76.7%	28/30 93.3%	30/30 100%	30/30 100%
	25th Percentile MFI	412.0	412.0	1027.5	1509.5	50.0	68.5	307.5	678.5	45.0
	Median MFI Value	494.5	522.3	1136.5	1641.8	57.0	103.3	432.0	826.3	56.0
	75th Percentile MFI	583.0	597.0	1273.0	1726.0	71.0	148.0	495.0	951.0	71.0
	% CV	29.27	28.07	29.24	30.35	N/A	N/A	41.88	39.24	N/A
	Total Agreement with Expected Result	89/90 98.9%	90/90 100%	90/90 100%	90/90 100%	88/90 97.8%	75/90 83.3%	87/90 96.7%	90/90 100%	82/90 91.1%
	95% CI	94.0%-99.8%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	92.3%-99.4%	74.3%-89.6%	90.7%-98.9%	95.9%-100.0%	83.4%-95.4%
	Overall 25th Percentile MFI	564.5	386.5	1273.0	1663.5	45.0	65.0	438.0	901.0	43.0
	Overall Median MFI Value	769.0	490.3	1622.3	1848.0	57.0	96.5	623.8	1171.0	57.0
	Overall 75th Percentile MFI	958.0	557.5	1984.5	2225.0	77.5	134.0	727.0	1313.0	96.5
	Overall % CV	34.84	27.39	28.49	21.93	N/A	N/A	35.16	26.97	N/A

Reproducibility of Overall Total Raw Median MFI values for Mixed Analytes in xTAG GPP

	Panel Member ID	Rotavirus A Low Positive/ Norovirus GII High Positive		Rotavirus A High Positive/ Norovirus GII Low Positive		Rotavirus A Low Positive/ *Salmonella** High Positive			Rotavirus A High Positive/ *Salmonella** Low Positive		
		Rotavirus A Low Positive	Norovirus GII High Positive Probe 2	Rotavirus A High Positive	Norovirus GII Low Positive Probe 2	Rotavirus A Low Positive	*Salmonella** High Positive		Rotavirus A High Positive	*Salmonella** Low Positive	
							Probe 1	Probe 2		Probe 1	Probe 2
	Concentration	$4.47x10^3$ Copies/mL	$2.94x10^4$ Copies/mL	$1.02x10^5$ Copies/mL	$3.93x10^3$ Copies/mL	$3.78x10^3$ Copies/mL	$3.75 x10^6$ CFU/mL	$3.75 x10^6$ CFU/mL	$1.93x10^4$ Copies/mL	$1.17x10^5$ CFU/mL	$1.17x10^5$ CFU/mL
Site 1	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	29/30 96.7%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	25th Percentile MFI	303.5	2181.0	1995.0	707.0	207.0	2958.0	3220.0	1583.0	1043.0	586.0
	Median MFI Value	416.8	2306.0	2386.0	905.0	302.8	3062.5	3368.3	1944.3	1188.8	664.0
	75th Percentile MFI	665.0	2548.0	2612.0	1053.0	543.0	3200.0	3508.0	2344.0	1303.0	835.0
	% CV	61.05	13.21	25.39	24.85	71.77	9.04	7.37	31.09	17.37	32.68
Site 2	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	28/30 93.3%	29/30 96.7%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	25th Percentile MFI	460.0	1699.5	2424.0	605.0	311.0	3430.0	3549.0	2206.0	1235.5	902.0
	Median MFI Value	625.0	2086.8	3164.5	740.0	432.0	3564.5	3770.8	2430.8	1363.0	1060.0
	75th Percentile MFI	1154.0	2382.0	3660.5	877.0	527.5	3633.5	3924.0	2708.5	1547.0	1398.0
	% CV	59.96	19.54	24.17	31.49	41.02	6.15	8.07	20.78	18.51	37.00
Site 3	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	20/30 66.7%	28/30 93.3%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	25th Percentile MFI	402.5	1277.0	1290.5	331.5	257.0	2501.0	2759.0	1040.5	782.0	603.0
	Median MFI Value	625.5	1486.5	1843.0	388.5	357.8	2750.3	2985.5	1319.8	918.5	774.5
	75th Percentile MFI	796.0	2202.0	2538.0	613.0	601.5	2934.5	3106.5	1765.0	1071.0	899.0
	% CV	71.79	30.68	38.78	39.06	80.66	15.47	13.60	50.31	20.20	33.99
	Total Agreement with Expected Result	90/90 100%	90/90 100%	90/90 100%	78/90 86.7%	86/90 95.6%	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%	78.1%-92.2%	89.1%-98.3%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%
	Overall 25th Percentile MFI	373.0	1675.0	1846.0	432.0	261.0	2782.0	3049.0	1439.0	947.0	608.0
	Overall Median MFI Value	568.0	2181.0	2412.3	700.0	385.3	3079.8	3365.5	1964.5	1146.8	791.3
	Overall 75th Percentile MFI	887.0	2386.0	3015.5	890.5	566.5	3462.0	3673.0	2455.0	1335.0	1065.5
	Overall % CV	67.14	25.14	34.55	41.19	67.20	14.25	13.41	38.01	24.60	41.11

*The Salmonella positive (Pos) and negative (Neg) calls presented in this table, represent when the signal from the individual Salmonella probe in question is either above or below the assay threshold for a positive call, it does not represent a true assay positive or negative as information from both probes is required to determine the call for this target.

	Panel Member ID	ETEC Low Positive / *Salmonella** High Positive				ETEC High Positive / *Salmonella** Low Positive			
		ETEC Low Positive		*Salmonella** High Positive		ETEC High Positive		*Salmonella** Low Positive	
		Probe 1	**Probe 2**	**Probe 1**	**Probe 2**	**Probe 1**	**Probe 2**	**Probe 1**	**Probe 2**
	Concentration	$9.37x10^5$ CFU/mL	$9.37x10^5$ CFU/mL	$3.75 x10^6$ CFU/mL	$3.75 x10^6$ CFU/mL	$7.50x10^6$ CFU/mL	$7.50x10^6$ CFU/mL	$1.17x10^5$ CFU/mL	$1.17x10^5$ CFU/mL
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%	29/30 96.7%
	25th Percentile MFI	1331.0	516.0	3013.0	3234.5	2477.0	2180.0	1111.5	720.0
	Median MFI Value	1526.3	588.0	3083.8	3342.8	2559.0	2361.8	1269.0	919.5
	75th Percentile MFI	1713.5	654.0	3221.0	3488.0	2723.5	2452.0	1501.0	1189.0
	% CV	13.73	18.49	6.22	5.89	9.94	8.91	22.23	32.28
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%
	25th Percentile MFI	2153.0	883.0	3442.0	3683.0	3175.0	2717.5	1315.0	882.0
	Median MFI Value	2508.8	1104.8	3657.0	3956.0	3399.0	2879.5	1378.0	1065.0
	75th Percentile MFI	2788.0	1378.0	3789.0	4155.5	3519.0	3111.0	1641.0	1236.0
	% CV	18.39	28.80	9.15	11.36	8.61	9.31	24.40	31.01
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%	29/30 96.7%
	25th Percentile MFI	1985.0	660.5	2515.0	2763.0	2530.0	1937.0	816.0	680.0
	Median MFI Value	2163.5	741.5	2918.5	3105.3	2736.5	2026.5	1072.5	889.3
	75th Percentile MFI	2323.0	943.5	3056.0	3418.5	2930.5	2252.0	1221.0	1081.0
	% CV	14.76	31.76	12.79	13.93	12.18	15.95	31.08	36.57
	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%	88/90 97.8%
	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%	92.3%-99.4%
	Overall 25th Percentile MFI	1578.0	610.5	2927.5	3124.0	2545.0	2162.0	1048.5	764.0
	Overall Median MFI Value	2039.8	736.5	3083.8	3382.5	2788.8	2390.5	1279.0	961.0
	Overall 75th Percentile MFI	2398.0	983.0	3442.0	3701.0	3175.0	2717.5	1474.0	1216.0
	Overall % CV	24.93	39.13	13.86	14.54	15.51	18.59	28.02	33.74

*The Salmonella positive (Pos) and negative (Neg) calls presented in this table, represent when the signal from the individual Salmonella probe in question is either above or below the assay threshold for a positive call, it does not represent a true assay positive or negative as information from both probes is required to determine the call for this target.

Overall, adequate site-to-site reproducibility has been established for the 11 viral, bacterial and parasitic targets that xTAG GPP has been designed to detect.

Repeatability

Repeatability was assessed for each microbial target by testing 20 replicates of each of two different analyte concentrations: a very low positive sample (at the LoD) and a moderate positive dilution level (5x-10x above the cut-off MFI). All replicates for each dilution level were examined starting from sample extraction with the bioMérieux NucliSENS easyMAG system followed by xTAG GPP in a single run. For each set of 20 replicates, the same operator performed the testing on the same instrument system, using the same lot of extraction kit and xTAG GPP reagents. Due to a limitation in the sample volume available for the *Cryptosporidium* analyte, and for the Rotavirus analyte, the Moderate Positive dilution level was not assessed for these targets. Results of testing were as follows:

Assay Repeatability

Analyte		**Dilution Level**	**Concentration**	**xTAG GPP Calls**	**Mean MFI Value**	**% CV**
Campylobacter		Moderate	$2.34x10^5$ CFU/mL	20 of 20	896	12.91%
		Low	$5.86x10^4$ CFU/mL	20 of 20	383	25.85%
C. difficile Toxin A/B	Probe 1	Moderate	$1.50x10^7$ CFU/mL	20 of 20	1224	25.56%
		Low	$3.75x10^6$ CFU/mL	20 of 20	450	19.68%
	Probe 2	Moderate	$3.75x10^6$ CFU/mL	20 of 20	1126	11.10%
		Low	$9.38x10^5$ CFU/mL	20 of 20	362	24.83%
Cryptosporidium hominis		Moderate Positive	Not Assessed^	Not Assessed	Not Assessed	Not Assessed
		Low Positive/LoD	$3.51x10^4$ Copies/mL (extracted DNA)	19 of 20 POS	810	24.17%
E. coli O157		Moderate	$9.38x10^5$ CFU/mL	20 of 20	1674	13.82%
		Low	$2.34x10^5$ CFU/mL	20 of 20	585	28.45%
ETEC LT/ST	Probe 1	Moderate	$9.38x10^5$ CFU/mL	20 of 20	930	19.89%
		Low	$2.34x10^5$ CFU/mL	20 of 20	321	27.28%
	Probe 2	Moderate	$7.50x10^6$ CFU/mL	20 of 20	1741	7.55%
		Low	$3.75x10^6$ CFU/mL	20 of 20	515	20.89%
Giardia		Moderate	$8.81x10^2$ cells/mL	20 of 20	1913	20.97%
		Low	$2.20x10^2$ cells/mL	20 of 20	1243	18.97%
Norovirus GI/GII	Probe 1	Moderate Positive	$1.95x10^6$ Copies/mL (extracted RNA)	20 of 20 POS	1756	18.37%
		Low Positive/LoD	$6.56x10^5$ Copies/mL (extracted RNA)	20 of 20 POS	991	21.53%
	Probe 2	Moderate Positive	$2.44x10^6$ Copies/mL (extracted RNA)	20 of 20 POS	1025	32.96%
		Low Positive/LoD	$1.15x10^6$ Copies/mL (extracted RNA)	20 of 20 POS	808	28.87%

Rotavirus A		Moderate Positive	Not available§	20 of 20 POS	980	16.37%
		Low Positive/LoD	$6.84x10^4$ Copies/mL (extracted RNA)	19 of 20 POS	486	20.70%
Salmonella	Probe 1	Moderate	$9.38x10^5$ CFU/mL	20 of 20	2100	6.42%
		Low	$2.34x10^5$ CFU/mL	20 of 20	1377	17.87%
	Probe 2	Moderate	$9.38x10^5$ CFU/mL	20 of 20	1916	11.20%
		Low	$2.34x10^5$ CFU/mL	20 of 20	1005	25.29%
Shigella		Moderate	$2.93x10^4$ CFU/mL	20 of 20	1715	6.26%
		Low	$3.67x10^3$ CFU/mL	20 of 20	795	28.19%
STEC stx1/stx2	Probe 1	Moderate	$3.75x10^6$ CFU/mL	20 of 20	1271	10.29%
		Low	$9.38x10^5$ CFU/mL	20 of 20	503	17.11%
	Probe 2	Moderate	$9.38x10^5$ CFU/mL	20 of 20	1002	11.65%
		Low	$2.34x10^5$ CFU/mL	20 of 20	334	31.91%

^Due to limited sample volume (pooled positive clinical material was used)
§A clinical specimen was used for which the concentration was not available

The correct qualitative result was obtained for ≥ 19 of 20 replicates at the low positive level and for 20 of 20 replicates at the moderate positive level for each analyte tested at these concentrations.

b. Linearity/assay reportable range:

Not applicable, qualitative assay.

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

Before using the Luminex system to read samples prepared by the xTAG assay, prepare and calibrate the Luminex instrument system following the procedures in the appropriate system user manual.

Negative Controls - Negative controls are defined as either RNase-free water added to the RT-PCR/PCR step (amplification/detection negative control) or lysis buffer that has undergone the entire assay procedure (pretreatment/extraction/amplification/detection negative control). At least one negative control that underwent extraction process must be included in each batch of specimens run on xTAG GPP. The recommended number of negative controls to be included in a batch is dependent on batch size. For batches of 1-30 samples, one negative control must be included. For batches of 31-61 samples, two negative controls are recommended. For batches of 62-92 samples, three negative controls are recommended. When running multiple negative controls disperse the controls throughout the batch.

NOTE: Users will need to identify all the negative controls (including extraction controls) from the TDAS software before the test data is analyzed. If a negative control has a significant signal detected for an analyte, the TDAS software will generate a 'no

call' for the samples that were positive for the specific analyte and they will need to be retested.

External Positive Controls - Known strains or positive clinical samples with known results for the targeted viruses, bacteria or parasites should be included in routine quality control procedures ("external controls") as positive controls for the assay. At least one of these external controls are analyte positive controls and should be included with each batch of patient specimens and controls positive for different targets should be rotated from batch to batch. External controls should be prepared, extracted and tested in the same manner as patient samples. Results from external controls should be examined before the results from the patient samples. The interpretation of the correct positive control results is performed by the user and not the data analysis software (TDAS). If a given analyte control does not perform as expected, all results for that analyte in the batch of samples should be examined to determine if a re-run is required. If any unexpected calls occur where one or more analytes with signal exceeding the thresholds are detected in any of the positive controls (i.e. non-specific positive signals) for a given run then samples that were positive for the specific analyte(s) that triggered a control failure will need to be re-run. At least one positive control per PCR run must pass, i.e. all expected calls made in order to report any results from the plate.

Internal Control - Bacteriophage MS2 is the internal control for the assay. This internal positive control is added to each patient specimen prior to extraction. This internal control allows the user to ascertain whether the assay is functioning properly. Failure to generate a PRES (present) call for the MS2 control indicates a failure at either the extraction step, and/or the reverse-transcription step, and/or the PCR step, and may be indicative of the presence of amplification inhibitors, which can lead to false negative results.

d. Detection limit:

The LoD was assessed by analyzing serial dilutions of simulated samples made from high-titer stocks of commercial strains or high-titer clinical specimens (when commercial strains were not available). All simulated specimens were prepared in negative clinical matrix (stool). The data from serial dilutions were confirmed in at least 20 replicates of the selected dilution for each analyte target. Results of testing were as follows:

Summary Limit of Detection (LoD) for GPP Analytes

Analyte	**Strain ID**	**Titre (corresponding to the estimated LoD)**	**Average MFI Value**	**%CV**
Campylobacter	*Campylobacter jejuni*, 49943 (Strain LRA 094.06.89)	$5.86x10^4$ CFU/mL	383	25.85%
C. difficile Toxin A/B	*Clostridium difficile*, BAA-1805 (toxinotype III A+B+)	$9.38x10^5$ CFU/mL	362	24.83%
	Clostridium difficile, 43255 (toxinotype 0 A+B+)	$3.75x10^6$ CFU/mL	527	19.63%
Cryptosporidium hominis	Clinical sample	$3.51x10^4$ Copies/mL	810	24.17%

E. coli O157	*E. coli* O157, 0801622 (EDL933; O157:H7; STEC Toxin I+II+)	$2.34x10^5$ CFU/mL	585	28.45%
ETEC LT/ST	*E. coli*, 35401 (O78:H11; ST+LT+)	$2.34x10^5$ CFU/mL	321	27.28%
Giardia	Giardia lamblia, PRA-243	$2.20x10^2$ cells/mL	1243	18.97%
Norovirus GI/GII	Clinical sample – GI	$6.56x10^5$ Copies/mL	991	21.53%
	Clinical sample – GII	$1.15x10^6$ Copies/mL	808	28.87%
Rotavirus A	Clinical sample	$6.84x10^4$ Copies/mL	486	20.70%
Salmonella	*Salmonella enterica*, 13311 (Serotype Typhimurium)	$2.34x10^5$ CFU/mL	Probe 1=1377 Probe 2=1005	Probe 1=17.87% Probe 2=25.29%
STEC stx1/stx2	*E. coli* O157, 0801622 (EDL933; O157:H7; STEC Toxin I+II+)	$2.34x10^5$ CFU/mL	334	31.91%
Shigella	*Shigella sonnei*, 25931 (Subgroup D)	$3.67x10^3$ CFU/mL	795	28.19%

The data summarized above establish a limit of detection for each indicated analyte.

e. Analytical specificity:

Analytical Reactivity

Analytical reactivity was assessed through empirical testing of a wide range of clinically relevant GI pathogen strains, genotypes, serotypes and isolates representing temporal and geographical diversity for each analyte. (Note: Some differences in sensitivity may be expected as a result of sequence diversity within the gene targeted by the GPP assay primers.) Pathogens were diluted two to three times (2x-3x) the claimed Limit of Detection (LoD) in a negative clinical matrix (stool) prior to pre-treatment and extraction. An internal control (MS2) was added to each diluted pathogen sample prior to pre-treatment. Extraction was performed with the Biomerieux Nuclisens EasyMag extraction method. Through testing of 265 unique samples covering all intended use pathogens, reactivity was established at concentrations 2 to 3 times the limit of detection. The following table lists the samples tested:

Reactivity

Pathogen	**ATCC/Other Reference**	**Pathogen**	**ATCC/Other Reference**
Campylobacter jejuni	ATCC 29428	*Salmonella enterica* subsp. *enterica*, Serovar Typhimurium	ATCC 14028
Campylobacter jejuni subsp. *Jejuni*	ATCC 33291	*Salmonella enterica* subsp. *enterica*, Serotype Dublin	ATCC 15480
Campylobacter coli	ATCC 33559	*Salmonella enterica* subsp. *Enterica*, serotype Typhi	ATCC 19430
Campylobacter jejuni subsp. *jejuni*	ATCC 33560	*Salmonella bongori* type strain	ATCC 43975 / NCTC 12419

Campylobacter lari	ATCC 35221	*Salmonella enterica* subsp. *enterica*, Serotype Virchow	ATCC 51955
Campylobacter lari subsp. *lari*	ATCC 35223	*Salmonella enterica* subsp. *enterica*, Serotype Hadar	ATCC 51956
Campylobacter jejuni subsp. *jejuni*	ATCC 35920	*Salmonella enterica* subsp. *enterica*, Serotype Agona	ATCC 51957
Campylobacter coli	ATCC 43473	*Salmonella enterica* subsp. *Enterica*, Serotype Paratyphi B variant Java	ATCC 51962
Campylobacter coli	ATCC 43474	*Salmonella enterica* subsp. *Enterica*, Serotype Derby	ATCC 6960
Campylobacter coli	ATCC 43482	*Salmonella enterica* subsp. *Enterica*, Serotype Newport	ATCC 6962
Campylobacter coli	ATCC 43485	*Salmonella enterica* subsp. *enterica*, Serotype Braenderup	ATCC 700136
Campylobacter lari	ATCC 43675	*Salmonella enterica* subsp. *enterica*, Serotype Choleraesuis	ATCC 7001
Campylobacter lari	ATCC BAA-1060	*Salmonella enterica* subsp. *Enterica*, Serotype Stanley	ATCC 7308
Campylobacter coli	ATCC BAA-372	*Salmonella enterica* subsp. *enterica*, Serotype Panama	ATCC 7378
Campylobacter	Zeptometrix 0801650	Salmonella enterica subsp. Enterica, Serotype Heidelberg	ATCC 8326
Clostridium difficile toxin A/B	ATCC 9689	*Salmonella enterica* subsp. *enterica*, Serotype Montevideo	ATCC 8387
Clostridium difficile toxin A/B	ATCC 17857 (870)	*Salmonella enterica* subsp. *enterica*, Serotype Muenchen	ATCC 8388
Clostridium difficile toxin A/B	ATCC 17858 (1253)	*Salmonella enterica* subsp. *enterica*, Serotype Thompson	ATCC 8391
Clostridium difficile toxin A/B	ATCC 43594	*Salmonella enterica* subsp. *Enterica*, Serotype Paratyphi B	ATCC 8759
Clostridium difficile toxin A/B	ATCC 43596	*Salmonella enterica* subsp. *Enterica*, Serotype Bareilly	ATCC 9115
Clostridium difficile	ATCC 43598	*Salmonella enterica*	ATCC 9239

toxin A/B		subsp. *Enterica*, Serotype Oranienburg	
Clostridium difficile toxin A/B	ATCC 43599	*Salmonella enterica* subsp. *enterica*, Serotype Kentucky	ATCC 9263
Clostridium difficile toxin A/B	ATCC 43600	*Salmonella enterica* subsp. *Enterica*, Serotype Anatum	ATCC 9270
Clostridium difficile toxin A/B	ATCC 51695	*Salmonella enterica* subsp. *Enterica*, Serotype Saintpaul	ATCC 9712
Clostridium difficile toxin A/B	ATCC 700792	*Salmonella enterica* subsp. *enterica*, Serotype Infantis	ATCC BAA-1675
Clostridium difficile toxin A/B	ATCC BAA-1382	*Salmonella enterica* subsp. *enterica*	CDC_Salmonella A
Clostridium difficile toxin A/B	ATCC BAA-1803	*Salmonella enterica* subsp. *Enterica*, Serotype Paratyphi B var. L(+) tartrate+	CDC_Salmonella B
Clostridium difficile toxin A/B	ATCC BAA-1814	*Salmonella enterica* subsp. *enterica*, 4:i:-	07-7741, CNR[#]
Clostridium difficile toxin A/B	ATCC BAA-1870	*Salmonella enterica* subsp. *enterica*, 4:i:-	07-2537, CNR
Clostridium difficile toxin A/B	ATCC BAA-1871	*Salmonella enterica* subsp. *enterica*, Agona	05-960, CNR
Clostridium difficile toxin A/B	ATCC BAA-1872	*Salmonella enterica* subsp. *enterica*, Agona	1137/72, CNR
Clostridium difficile toxin A/B	ATCC BAA-1875	*Salmonella enterica* subsp. *Enterica*, Anatum	84K, CNR
Clostridium difficile toxin A/B	ATCC BAA-2155	*Salmonella enterica* subsp. *Enterica*, Anatum	08-2926, CNR
Clostridium difficile toxin A/B	ATCC BAA-2156	*Salmonella enterica* subsp. *enterica*, Braenderup	49K, CNR
Clostridium difficile toxin A/B	Zeptometrix 0801620	*Salmonella enterica* subsp. *enterica*, Brandenburg	24K, CNR
Cryptosporidium parvum	Waterborne Inc.	*Salmonella enterica* subsp. *enterica*, Choleraesuis var Decatur	2/84, CNR
Cryptosporidium hominis	Waterborne Inc.	*Salmonella enterica* subsp. *Enterica*, Choleraesuis var Kunzendorf	36K, CNR
Cryptosporidium parvum	Zeptometrix 0801700	*Salmonella enterica*	34K, CNR

		subsp. *enterica*, Choleraesuis var sensu stricto	
Cryptosporidium parvum	ATCC PRA-67D	*Salmonella enterica* subsp. *Enterica*, Corvallis	263K, CNR
Cryptosporidium parvum	ATCC 87668	*Salmonella enterica* subsp. *enterica*, Derby	20K, CNR
Cryptosporidium parvum	ATCC 87712	*Salmonella enterica* subsp. *enterica*, Derby	354/67, CNR
Cryptosporidium parvum	ATCC 87763	*Salmonella enterica* subsp. *enterica*, Dublin	05-1078, CNR
Cryptosporidium parvum	ATCC 87765	*Salmonella enterica* subsp. *enterica*, Dublin	65K, CNR
Escherichia coli O157	ATCC 43888	*Salmonella enterica* subsp. *enterica*, Enteritidis	89-323, CNR
Escherichia coli O157	ATCC 43890	*Salmonella enterica* subsp. *enterica*, Enteritidis	02-131, CNR
Escherichia coli O157	ATCC 43894	*Salmonella enterica* subsp. *enterica*, Enteritidis	02-9053, CNR
Escherichia coli O157	ATCC 43895	*Salmonella enterica* subsp. *enterica*, Enteritidis	89-329, CNR
Escherichia coli O91 (Produces shiga-like toxin II)	ATCC 51435	*Salmonella enterica* subsp. *enterica*, Enteritidis	5-56, CNR
Escherichia coli O157	ATCC 700376	*Salmonella enterica* subsp. *enterica*, Enteritidis	03-3527, CNR
Escherichia coli O157	ATCC 700377	*Salmonella enterica* subsp. *enterica*, Enteritidis	02-4884, CNR
Escherichia coli O157	ATCC 700378	*Salmonella enterica* subsp. *enterica*, Hadar	02-2760, CNR
Escherichia coli O113 (Produces shiga toxin 2)	ATCC BAA-176	*Salmonella enterica* subsp. *enterica*, Hadar	2-74, CNR
Escherichia coli O113 (Produces shiga toxin 1 and 2)	ATCC BAA-177	*Salmonella enterica* subsp. *enterica*, Heidelberg	16K, CNR
Escherichia coli O111	ATCC BAA-181	*Salmonella enterica*	08-2380, CNR

(Produces shiga toxin 1 and 2)		subsp. *enterica*, Heidelberg	
Escherichia coli O104 (produces shiga toxin 2)	ATCC BAA-182	*Salmonella enterica* subsp. *enterica*, Infantis	158K, CNR
Escherichia coli O26	ATCC BAA-1653	*Salmonella enterica* subsp. *enterica*, Infantis	05-6334, CNR
Escherichia coli O104 (produces shiga toxin 2)	ATCC BAA-2326	*Salmonella enterica* subsp. *enterica*, Javiana	4-57, CNR
Escherichia coli O78:H11 (produces LT and ST)	ATCC 35401	*Salmonella enterica* subsp. *enterica*, Javiana	214K, CNR
Escherichia coli O25:K98:NM (produces LT)	ATCC 43886	*Salmonella enterica* subsp. *enterica*, Kentucky	98K, CNR
Escherichia coli	Zeptometrix 0801624	*Salmonella enterica* subsp. *enterica*, Kentucky	07-6574, CNR
Escherichia coli O78::K80H12 (produces ST)	ATCC 43896	*Salmonella enterica* subsp. *enterica*, Kentucky	06-5737, CNR
Giardia intestinalis	ATCC 30888	*Salmonella enterica* subsp. *Enterica*, Mississippi	1933/77, CNR
Giardia intestinalis	ATCC 30957	*Salmonella enterica* subsp. *enterica*, Montevideo	126K, CNR
Giardia intestinalis	ATCC 50114	*Salmonella enterica* subsp. *enterica*, Montevideo	06-7410, CNR
Giardia intestinalis	ATCC 50137	*Salmonella enterica* subsp. *enterica*, Montevideo	46K, CNR
Giardia intestinalis	ATCC 50581	*Salmonella enterica* subsp. *enterica*, Montevideo	06-8080, CNR
Giardia intestinalis	ATCC 50584 (JH)	*Salmonella enterica* subsp. *enterica*, Montevideo	06-8107, CNR
Giardia intestinalis	ATCC 50585	*Salmonella enterica* subsp. *enterica*, Montevideo	05-8072, CNR
Giardia lamblia	ATCC PRA-242	*Salmonella enterica* subsp. *enterica*,	54K, CNR

		Muenchen	
Giardia lamblia	ATCC PRA-244	*Salmonella enterica* subsp. *enterica*, Newport	05-815, CNR
Giardia lamblia	ATCC PRA-247	*Salmonella enterica* subsp. *enterica*, Newport	50K, CNR
Giardia lamblia	ATCC PRA-249	*Salmonella enterica* subsp. *enterica*, Newport	04-2487, CNR
Giardia lamblia	Waterborne Inc.	*Salmonella enterica* subsp. *enterica*, Newport	01-2174, CNR
Norovirus GI	CDC – GP-001	*Salmonella enterica* subsp. *enterica*, Newport	02-7891, CNR
Norovirus GI	CDC – GP-003	*Salmonella enterica* subsp. *Enterica*, Oranienburg	42K, CNR
Norovirus GI	CDC – GP-005	*Salmonella enterica* subsp. *enterica*, Panama	73K, CNR
Norovirus GI	CDC – GP-007	*Salmonella enterica* subsp. *enterica*, Paratyphi A	1K, CNR
Norovirus GI	CDC – GP-008	*Salmonella enterica* subsp. *enterica*, Paratyphi A	06-2065, CNR
Norovirus GI	CDC – GP-009	*Salmonella enterica* subsp. *enterica*, Paratyphi B	CIPA214, CNR
Norovirus GI	CDC – GP-010	*Salmonella enterica* subsp. *enterica*, Paratyphi B	05-4862, CNR
Norovirus GI	CDC – GP-012	*Salmonella enterica* subsp. *enterica*, Paratyphi B	02-9348, CNR
Norovirus GI	CDC – GP-013	*Salmonella enterica* subsp. *enterica*, Paratyphi B	5K, CNR
Norovirus GI	CDC – GP-015	*Salmonella enterica* subsp. *enterica*, Paratyphi B	02-2529, CNR
Norovirus GI	CDC – GP-016	*Salmonella enterica* subsp. *enterica*, Paratyphi B	6332/88-1, CNR
Norovirus GI	CDC – GP-018	*Salmonella enterica* subsp. *enterica*, Paratyphi C	32K, CNR

Norovirus GI	CDC – GP-020	*Salmonella enterica* subsp. *enterica*, Saintpaul	108K, CNR
Norovirus GII	CDC – GP-023	*Salmonella enterica* subsp. *enterica*, Saintpaul	05-5166, CNR
Norovirus GII	CDC – GP-024	*Salmonella enterica* subsp. *Enterica*, Stanley	15K, CNR
Norovirus GII	CDC – GP-025	*Salmonella enterica* subsp. *Enterica*, Stanley	397K, CNR
Norovirus GII	CDC – GP-027	*Salmonella enterica* subsp. *Enterica*, Tennessee	142K, CNR
Norovirus GII	CDC – GP-030	*Salmonella enterica* subsp. *enterica*, Thompson	40K, CNR
Norovirus GII	CDC – GP-033	*Salmonella enterica* subsp. *enterica*, Typhimurium	38 (98) MN, CNR
Norovirus GII	CDC – GP-034	*Salmonella enterica* subsp. *enterica*, Typhimurium	49 (98) MN, CNR
Norovirus GII	CDC – GP-035	*Salmonella enterica* subsp. *enterica*, Typhimurium	150 (98) MN, CNR
Norovirus GII	CDC – GP-036	*Salmonella enterica* subsp. *enterica*, Typhimurium	226 (97) MN, CNR
Norovirus GII	CDC – GP-038	*Salmonella enterica* subsp. *enterica*, Typhimurium	31 (98) MN, CNR
Norovirus GII	CDC – GP-039	*Salmonella enterica* subsp. *enterica*, Typhimurium	02-1180, CNR
Norovirus GII	CDC – GP-041	*Salmonella enterica* subsp. *enterica*, Typhimurium	14-58, CNR
Norovirus GII	CDC – GP-042	*Salmonella enterica* subsp. *enterica*, Typhimurium	00-7866, CNR
Norovirus GII	CDC – GP-045	*Salmonella enterica* subsp. *enterica*, Typhimurium	75-2099, CNR

Norovirus GII	CDC – GP-047	*Salmonella enterica* subsp. *enterica*, Typhimurium	75/67, CNR
Norovirus GII	CDC – GP-048	*Salmonella enterica* subsp. *enterica*, Typhimurium	SonLa1/Hoang63, CNR
Norovirus GII	CDC – GP-049	*Salmonella enterica* subsp. *enterica*, Typhimurium	02-3215, CNR
Norovirus GII	CDC – GP-050	*Salmonella enterica* subsp. *enterica*, Typhimurium	02-4577, CNR
Norovirus GII	CDC – GP-053	*Salmonella enterica* subsp. *enterica*, Typhimurium	DK4, CNR
Norovirus GII	CDC – GP-054	*Salmonella enterica* subsp. *enterica*, Typhimurium	LT2, CNR
Norovirus GII	CDC – GP-056	*Salmonella enterica* subsp. *enterica*, Typhimurium	01-1639, CNR
Norovirus GII	CDC – GP-057	Salmonella enterica subsp. *enterica*, Typhimurium	02-4496, CNR
Norovirus GII	CDC – GP-058	*Salmonella enterica* subsp. *enterica*, Virchow	41K, CNR
Norovirus GII	CDC – GP-059	*Salmonella enterica* subsp. *enterica*, Virchow	03-5167, CNR
Norovirus GII	CDC – GP-060	*Salmonella enterica* subsp. *arizonae*, 53:g,z51:-	SO 8/9, CNR
Norovirus GII	CDC – GP-063	*Salmonella enterica* subsp. *Diarizonae*, 17:z10:e,n,z15	1458/74, CNR
Norovirus GII	CDC – GP-064	*Salmonella enterica* subsp. *salamae*, 11:l,z28:enx	1368K, CNR
Norovirus GII	CDC – GP-067	*Salmonella enterica* subsp. *houtenae*, 6,7:z4,z24:-	575K, CNR
Rotavirus Group A	ATCC VR-1546	*Salmonella enterica* subsp. *indica*, 11:b:1,7	437/68, CNR
Rotavirus Group A	ATCC VR-2018	*Salmonella bongori*,	1900/76, CNR

		66:z35:-	
Rotavirus Group A	ATCC VR-2272	*Shigella dysenteriae*, (Subgroup A)	ATCC 11835
Rotavirus Group A	ATCC VR-2273	*Shigella flexneri* (Subgroup B, serotype 3)	ATCC 11836
Rotavirus Group A	ATCC VR-2274	*Shigella dysenteriae* (Subgroup A, serotype 8)	ATCC 12021
Rotavirus Group A	ATCC VR-2275	*Shigella flexneri* (Subgroup B, serotype 4a)	ATCC 12023
Rotavirus Group A	ATCC VR-2417	*Shigella flexneri* (Subgroup B, serotype 6)	ATCC 12025
Rotavirus Group A	ATCC VR-2550	*Shigella boydii* (Subgroup C, serotype 8)	ATCC 12028
Rotavirus Group A	ATCC VR-2551	*Shigella boydii* (Subgroup C, serotype 10)	ATCC 12030
Rotavirus Group A	CDC – GP079	*Shigella boydii* (Subgroup C, serotype 11)	ATCC 12031
Rotavirus Group A	CDC – GP080	*Shigella dysenteriae* (Subgroup A, serotype 9)	ATCC 12037
Salmonella enterica subsp. *enterica*, Serotype Choleraesuis	ATCC 10708	*Shigella dysenteriae* (Type strain, Subgroup A, serotype 1)	ATCC 13313
Salmonella enterica subsp. *Enterica*, Serotype Javiana	ATCC 10721	*Shigella sonnei* , Subgroup D	ATCC 29029
Salmonella enterica subsp. *Enterica*, Serotype Tennessee	ATCC 10722	*Shigella sonnei*, Subgroup D	ATCC 29030
Salmonella enterica subsp. *Enterica*, Serotype Paratyphi A	ATCC 11511	*Shigella dysenteriae* (Subgroup A, serotype 11)	ATCC 49547
Salmonella enterica subsp. *enterica*, Serotype Enteritidis	ATCC 13076	*Shigella dysenteriae*, (Subgroup A, serotype 1)	ATCC 9361
Salmonella enterica subsp. *enterica*, Serotype Typhimurium	ATCC 13311	*Shigella sonnei*	0801627
Salmonella enterica subsp. *Arizonae*	ATCC 13314 / NCTC 8297	*Shigella flexneri*	0801757
Salmonella enterica subsp. *Enterica*, Serotype Paratyphi C	ATCC 13428		

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Analytical Specificity and Potential Interfering Agents

Analytical specificity was assessed with respect to the following parameters:

1. *Propensity for cross - reactivity leading to false positive results*: Potential cross reactivity with pathogens (viruses, bacteria and parasites) associated with gastrointestinal (GI) infections that are not probed by the assay. Potential cross reactivity was also assessed for commensal flora and non-microbial agents.
2. *Propensity for interference leading to false negative results*: Potential interference by pathogens (viruses, bacteria and parasites) associated with gastrointestinal (GI) infections that are not probed by the assay. Potential interference by commensal flora was also assessed.
3. *Propensity for competitive interference leading to false negative results*: Potential interference by GI pathogens that are detected by the assay was evaluated by testing one microbial target prepared at a concentration near the assay cut-off (LP) in the presence of a second microbial target prepared at a very high concentration (HP), and vice-versa. The combinations of analytes tested were selected based on the frequency of co-infections reported in the literature.

This study was mainly conducted at LMD (Toronto) with some runs performed at (1) the National Calicivirus Laboratory, Center for Disease Control (CDC) in Atlanta, (2) Scott & White Hospital, Temple, Texas, and (3) Luminex Headquarters, Austin, Texas. Viral cultures were prepared by growing the virus in the appropriate cell host, to a titer of 10^5 pfu/mL (10^5 $TCID_{50}$/mL) or higher, if available (high positive (HP) sample). Bacterial cultures were prepared at concentrations of 10^6 cfu/mL or higher (high positive (HP) sample). Parasites were tested at a clinically relevant level as supported by literature or clinical trial data (e.g. a high titer clinical sample). Low positive samples (LP) were prepared at a concentration that gave MFI values approximately 1-5 times the assay cutoff (depending on the target). Non-microbial agents were prepared at the concentration noted in the table. Microbial and non-microbial agents were prepared in negative clinical matrix.

Results for the 3 categories of testing outlined above were as follows:

1. There was no cross-reactivity observed in the majority (84) of the 86 relevant pathogen strains, genotypes, serotypes and isolates tested. Note that 9 of the 84 samples that did not cross-react did generate a positive call as they include analytes that are detected by the assay (i.e. they were included to show non-cross reactivity with another analyte). The remaining 2 cross-reacting species are described below and will be addressed in product labeling:

a) *Campylobacter fetus* subsp. *fetus* (NCTC 10842, type strain [ATCC 27374]) at a concentration of 6.00E+08 cfu/mL resulted in a positive call for *Campylobacter* and

b) *Escherichia coli* (Migula) Castellani and Chalmers strain CDC EDL 1284 [929-78] (serotype O124:NM [ATCC 43893]) (enteroinvasive) resulted in a positive call for *Shigella*.

Pathogenic flora evaluated for potential cross reactivity

Pathogenic Flora	ATCC/Other Reference	Titer Tested	Cross-Reactive Yes (Y) / No (N)
Acinetobacter baumannii	ATCC19606	2.4 x 10^9 cfu/mL	N
Adenovirus serotype 1^	ATCC VR-1	1.58 x 10^7 $TCID_{50}$/mL	N
Adenovirus serotype 3	Zeptometrix 0810062CF	5.89 x 10^7 $TCID_{50}$/mL	N
Adenovirus serotype 4	Zeptometrix 0810070CF	7.24 x 10^5 $TCID_{50}$/mL	N
Adenovirus serotype 5	Zeptometrix 0810020CF	1.02 x 10^8 $TCID_{50}$/mL	N
Adenovirus serotype 8	Zeptometrix 0810069CF	3.16 x 10^5 $TCID_{50}$/mL	N
Adenovirus serotype 14	ATCC VR-15	1.58 x 10^9 $TCID_{50}$/mL	N
Adenovirus serotype 18	ATCC VR-1095	3.16 x 10^6 $TCID_{50}$/mL	N
Adenovirus serotype 31	GP-092 (CDC)	Not known	N
Aeromonas hydrophila	ATCC 35654	6 x 10^8 cfu/mL	N
Aichi virus	SO603Dijon (CDC)	1.00 x 10^8 copies/uL	N
Arcobacter butzleri	ATCC 49616	6 x 10^8 cfu/mL	N
Arcobacter cryaerophilus	ATCC 43158	>10^6 cfu/vial	N
Astrovirus Type 1	GP-086 (CDC)	6.00 x 10^7 copies/uL	N
Astrovirus Type 2	GP-087 (CDC)	6.00 x 10^7 copies/uL	N
Bacillus cereus	ATCC 14579	6 x 10^8 cfu/mL	N
Bacillus cereus	ATCC 6464	6 x 10^8 cfu/mL	N
Campylobacter fetus subsp. fetus (NCTC 10842, type strain)	ATCC 27374	6 x 10^8 cfu/mL	**Y** with Campylobacter (C. jejuni, C. coli, and C. lari only)
Campylobacter fetus subsp. fetus	ATCC 33246	4.43 x 10^5 copies/mL	N
Campylobacter fetus subsp. fetus	ATCC 33247	4.25 x 10^4 copies/mL	N
Campylobacter fetus subsp. venerealis	ATCC 19438	4.11 x 10^4 copies/mL	N
Campylobacter fetus subsp. venerealis	ATCC 33561	4.10 x 10^4 copies/mL	N
Campylobacter hyointestinalis	ATCC 35217	6 x 10^8 cfu/mL	N

Campylobacter jejuni subsp. jejuni*	ATCC 33291	6 x 10^8 cfu/mL	**Y (expected)** with Campylobacter (C. jejuni, C. coli, and C. lari only)
Campylobacter upsaliensis	ATCC 43954	2.57 x 10^9 copies/mL	N
Chlamydia trachomatis	ATCC VR-346	2.81 x 10^6 $TCID_{50}$/mL	N
Clostridium perfringens	ATCC 13124	6 x 10^8 cfu/mL	N
Clostridium septicum	ATCC 12464	6 x 10^8 cfu/mL	N
Clostridium sordellii	ATCC 9714	6 x 10^8 cfu/mL	N
Clostridium tertium	ATCC 14573	6 x 10^8 cfu/mL	N
Clostridium tetani	ATCC 19406	6 x 10^8 cfu/mL	N
Coxsackie virus	ATCC VR-28	8.89 x 10^7 $TCID_{50}$/mL	N
Cronobacter sakazakii	Zeptometrix 0801533	2.83 x 10^9 cfu/mL	N
Cryptosporidium meleagridis	Waterborne, Cat # SPECIAL 1867	2.50 x 10^5 oocysts/mL	N
Cryptosporidium muris	Waterborne P-104-1X10-6-L	2.50 x 10^5 cells/mL	N
Cytomegalovirus	ATCC VR-1590	Not known	N
Cytomegalovirus	Zeptometrix 0810003CF	9.55 x 10^6 $TCID_{50}$/mL	N
Echovirus	ATCC VR-41	8.89 x 10^6 $TCID_{50}$/mL	N
Edwardsiella tarda	ATCC 15947	6 x 10^8 cfu/mL	N**
Enterovirus (Human enterovirus D (Enterovirus Type 70)), strain J670/71	ATCC VR-836	8.89 x 10^6 $TCID_{50}$/mL	N
Enterovirus (Sabin 3)	GP-090 (CDC), cell culture	Not known	N
Escherichia blattae	ATCC 29907	6 x 10^8 cfu/mL	N
Escherichia coli (Migula) Castellani and Chalmers strain CDC EDL 1284 [929-78] (serotype O124:NM) (enteroinvasive)	ATCC 43893	6 x 10^8 cfu/mL	**Y** with Shigella
Escherichia coli (Migula) Castellani and Chalmers strain CFT073 (uropathogenic strain)	ATCC 700928	6 x 10^8 cfu/mL	N
Escherichia coli (Migula) Castellani and Chalmers (serotype O16:K1(L):NM)	ATCC 23511	6 x 10^8 cfu/mL	N
Escherichia coli (Migula) Castellani and Chalmers serotype O111:H8 strain CDC 1999-3249) (Produces Shiga toxin 1 and 2)	ATCC BAA-181	1 x 10^7 cfu/mL	**Y (expected)** with STEC stx1 / stx2
Escherichia fergusonii	ATCC 35469	6 x 10^8 cfu/mL	N

Escherichia hermanii	ATCC 33650	6 x 10^8 cfu/mL	N
Escherichia vulneris	ATCC 33821	6 x 10^8 cfu/mL	N
Gardnerella vaginalis	ATCC 14019	6 x 10^8 cfu/mL	N
Helicobacter felis	ATCC 49179	6 x 10^8 cfu/mL	N
Helicobacter pylori	ATCC 43504	No titer available	N
Helicobacter pylori	Zeptometrix 0801486	3.57 x 10^6 cfu/mL	N
Hepatitis A virus	GP-088, strain HM175 (CDC)	2.00 x 10^6 pfu/mL	N
Klebsiella oxytoca	ATCC 13182	6 x 10^8 cfu/mL	N
Klebsiella ozaenae (K. pneumonia subsp. ozaenae)	ATCC 11296	6 x 10^8 cfu/mL	N
Listeria grayi	ATCC 19120	6 x 10^8 cfu/mL	N
Listeria monocytogenes	ATCC BAA-839	6 x 10^8 cfu/mL	N
Norovirus GIV	GP-068, Clinical stool sample collected during an outbreak (CDC)	Not known	N
Plesiomonas shigelloides	ATCC 14029	6 x 10^8 cfu/mL	N
Porphyromonas asaccharolytica	ATCC 25260	6 x 10^8 cfu/mL	N
Providencia alcalifaciens	ATCC 9886	6 x 10^8 cfu/mL	N
Providencia rettgeri	ATCC 9250	6 x 10^8 cfu/mL	N
Providencia stuartii	ATCC 33672	6 x 10^8 cfu/mL	N
Rotavirus A (strain WA)*	ATCC VR-2018	1.58 x 10^8 $TCID_{50}$/mL	**Y (expected)** with Rotavirus A
Rotavirus Group B	CDC, clinical stool sample collected during an outbreak	Not known	N
Rotavirus Group C	CDC, cell culture	Not known	N
Salmonella enterica subsp. enterica serovar Choleraesuis*	ATCC 7001	6 x 10^8 cfu/mL	**Y (expected)** with Salmonella
Salmonella enterica subsp. enterica serovar Typhimurium (formerly Salmonella choleraesuis subsp. Choleraesuis serotype Typhimurium)*	ATCC 51812	6 x 10^8 cfu/mL	**Y (expected)** with Salmonella
Salmonella enterica subsp. enterica serovar Typhimurium (formerly Salmonella choleraesuis subsp. Choleraesuis serotype Typhimurium)*	ATCC 19585	6 x 10^8 cfu/mL	**Y (expected)** with Salmonella
Sapovirus GI	GP-082, clinical isolate (CDC)	1.00 x 10^5 copies/uL	N
Sapovirus GII	GP-083, clinical isolate (CDC)	1.00 x 10^3 copies/uL	N
Sapovirus GIII (porcine)	GP-084 (CDC)	2.00 x 10^5 $TCID_{50}$/mL	N

Sapovirus GIV	GP-085, clinical stool sample (CDC)	1.00 x 10^6 copies/uL	N
Serratia liquefaciens	ATCC 35551	6 x 10^8 cfu/mL	N
Serratia marcescens subsp. marcescens	ATCC 13880	3.8 x 10^9 bacteria/mL	N
Shigella boydii*	ATCC 12028	6 x 10^8 cfu/mL	**Y (expected)** with Shigella
Shigella dysenteriae serotype 1*** strain AMC 43-A-14	ATCC 9361	1.00 x 10^7 cfu/mL	**Y (expected)** with Shigella and STEC stx1 / stx2
Shigella sonnei*	ATCC 25931	6 x 10^8 cfu/mL	**Y (expected)** with Shigella
Stenotrophomonas maltophilia	ATCC 13637	6 x 10^8 cfu/mL	N
Streptococcus dysgalactiae subsp. dysgalactiae	ATCC 43078	6 x 10^8 cfu/mL	N
Streptococcus pyogenes	ATCC 51500	5.85 x 10^7 cells/mL	N
Vibrio parahaemoliticus	ATCC 17802	6 x 10^8 cfu/mL	N
Yersinia bercovieri	ATCC 43970	6 x 10^8 cfu/mL	N
Yersinia pseudotuberculosis	ATCC 29833	6 x 10^8 cfu/mL	N
Yersinia rohdei	ATCC 43380	6 x 10^8 cfu/mL	N

*Although these analytes are probed by xTAG GPP, they have been included in this study as it has been recommended in the FDA Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection of *Clostridium difficile* guidance document to test for cross-reactivity

** Both replicates showed high MFI for *Salmonella* probe 2 (1895, 1779.5). However, this sample is called NEG because in order to make the *Salmonella* call either probe 1 is ≥1400 or both probe 1 and probe 2 must be ≥200.

***Although this analyte is probed by xTAG GPP, it has been included in this study to evaluate potential cross-reactivity of this organism with the STEC stx 1 toxin gene.

There was no cross-reactivity observed with 120 of the 121 commensal flora tested. A false positive call for Shigella was obtained when *Salmonella subterranea* was tested. *Salmonella subterranea* (ATCC BAA-836), a facultatively anaerobic, acid-resistant bacterium, was originally isolated from a low-pH, nitrate- and U(VI)-contaminated subsurface sediment (Shelobolina et al. 2004). However, according to the latest White-Kauffman-Le Minor Scheme maintained by Institut Pasteur, the species called *Salmonella subterranea* (Appl. Environ. Microbiol., 2004, 70, 2959-2965) does not belong in the genus *Salmonella* (Grimont, A.D., Weill, F-X. 2007, Antigenic Formulae of the *Salmonella* Serovars, 9th edition, Pasteur Institute, Paris France, available at http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089). Unfortunately, the only sequence available in GenBank at this time is a partial 16S sequence (AY373829.2) making it difficult to determine the basis of the cross-reactivity with *Shigella*. A dilution study was performed to see at what concentration the cross-reactivity occurred. *Salmonella subterranea* (ATCC BAA-836) cross-reactivity

with *Shigella* was detected at a concentration of 6.0 x 10^8 cfu/mL, but was no longer observed at a concentration of 1.5 x 10^8 cfu/mL or lower. This information will be included in product labeling.

Commensal flora evaluated for potential cross-reactivity

Commensal Flora	ATCC/Other Reference	Titer Tested	Cross-Reactive Yes (Y) / No (N)
Abiotrophia defectiva†	ATCC 49176	6 x 10^8 cfu/mL	N
Acinetobacter haemolyticus	ATCC 17906	1.64 x 10^7 cells/mL	N
Acinetobacter lwoffii	ATCC 15309	6 x 10^8 cfu/mL	N
Actinomyces naeslundii	ATCC 12104	6 x 10^8 cfu/mL	N
Akkermansia muciniphila	ATCC BAA-835	6 x 10^8 cfu/mL	N
Alcaligenes faecalis subsp. Faecalis	ATCC 15554	6 x 10^8 cfu/mL	N
Anaerococcus tetradius	ATCC 35098	6 x 10^8 cfu/mL	N
Atopobium vaginae	ATCC BAA-55	6 x 10^8 cfu/mL	N
Bacillus subtilis subsp. Spizizenii	ATCC 6633	1.9 x 10^7 cfu/mL	N
Bacillus subtilis subsp. Subtilis	ATCC 6051	6 x 10^8 cfu/mL	N
Bacteroides caccae	ATCC 43185	6 x 10^8 cfu/mL	N
Bacteroides fragilis	ATCC 25285	6 x 10^8 cfu/mL	N
Bacteroides stercoris	ATCC 43183	6 x 10^8 cfu/mL	N
Bacteroides thetaiotaomicron	ATCC 29148	6 x 10^8 cfu/mL	N
Bacteroides vulgatus	ATCC 8482	6 x 10^8 cfu/mL	N
Bifidobacterium adolescentis	ATCC 15703	6 x 10^8 cfu/mL	N
Bifidobacterium bifidum	ATCC 29521	6 x 10^8 cfu/mL	N
Bifidobacterium longum subsp. Longum	ATCC 15707	6 x 10^8 cfu/mL	N
Blastocystis hominis	ATCC 50587	≥ 10^6 cells/mL	N
Blastocystis hominis	ATCC 50608	2 x 10^7 cells/mL	N
Campylobacter concisus	ATCC 33237	3.11 x 10^5 copies/mL	N
Campylobacter curvus	ATCC 35224	1.71 x 10^5 copies/mL	N
Campylobacter gracilis	ATCC 33236	1.41 x 10^5 copies/mL	N
Campylobacter helveticus	ATCC 51209	4.64 x 10^7 copies/mL	N
Campylobacter hominis	ATCC BAA-381	6.61 x 10^3 copies/mL	N
Campylobacter rectus	ATCC 33238	1.18 x 10^5 copies/mL	N
Campylobacter showae	ATCC 51146	2.49 x 10^3 copies/mL	N
Campylobacter sputorum biovar Sputorum	ATCC 35980	1.56 x 10^6 copies/mL	N
Candida albicans	ATCC 10231	6 x 10^8 cfu/mL	N
Candida catenulate	ATCC 10565	6 x 10^8 cfu/mL	N
Capnocytophaga gingivalis	ATCC 33624	6 x 10^8 cfu/mL	N

Cedecea davisae	ATCC 33431	6 x 10^8 cfu/mL	N
Chryseobacterium gleum	ATCC 35910	6 x 10^8 cfu/mL	N
Citrobacter amalonaticus	Zeptometrix 0801718	1.35 x 10^10 cfu/mL	N
Citrobacter freundii	ATCC 8090	1.3 x 10^8 bacteria/mL	N
Citrobacter koseri	ATCC 27028	6 x 10^8 cfu/mL	N^
Citrobacter sedlakii	ATCC 51115	6 x 10^8 cfu/mL	N
Clostridium beijerinckii	ATCC 8260	6 x 10^8 cfu/mL	N
Clostridium bifermentans	ATCC 628	6 x 10^8 cfu/mL	N
Clostridium bolteae	ATCC BAA-613	6 x 10^8 cfu/mL	N
Clostridium butyricum	ATCC 19398	6 x 10^8 cfu/mL	N
Clostridium chauvoei	ATCC 11957	6 x 10^8 cfu/mL	N
Clostridium difficile (non-toxigenic)	ATCC 43593	6 x 10^8 cfu/mL	N
Clostridium difficile (non-toxigenic)	ATCC 43601	6 x 10^8 cfu/mL	N
Clostridium difficile (non-toxigenic)	ATCC 700057	6 x 10^8 cfu/mL	N
Clostridium fallax	ATCC 19400	6 x 10^8 cfu/mL	N
Clostridium haemolyticum	ATC 9650	6 x 10^8 cfu/mL	N
Clostridium histolyticum	ATCC 19401	6 x 10^8 cfu/mL	N
Clostridium innocuum	ATCC 14501	6 x 10^8 cfu/mL	N
Clostridium methylpentosum	ATCC 43829	6 x 10^8 cfu/mL	N
Clostridium nexile	ATCC 27757	6 x 10^8 cfu/mL	N
Clostridium novyi	ATCC 3540	6 x 10^8 cfu/mL	N
Clostridium paraputrificum	ATCC 25780	6 x 10^8 cfu/mL	N
Clostridium ramosum	ATCC 25582	6 x 10^8 cfu/mL	N
Clostridium scindens	ATCC 35704	6 x 10^8 cfu/mL	N
Clostridium sphenoides	ATCC 19403	6 x 10^8 cfu/mL	N
Clostridium sporogenes	ATCC 3584	6 x 10^8 cfu/mL	N
Clostridium symbiosum	ATCC 14940	6 x 10^8 cfu/mL	N
Corynebacterium genitalium	ATCC 33030	3.53 x 10^7 cells/mL	N
Corynebacterium glutamicum	ATCC 13032	6 x 10^8 cfu/mL	N
Desulfovibrio piger	ATCC 29098	6 x 10^8 cfu/mL	N
E. coli (strain: (Migula) Castellani and Chalmers) strain Crooks	ATCC 8739	6 x 10^8 cfu/mL	N
E. coli (strain: (Migula) Castellani and Chalmers) serotype O26:K60(B6)	ATCC 12795	6 x 10^8 cfu/mL	N
E. coli (strain: (Migula) Castellani and Chalmers) O Group 26	ATCC 11840	6 x 10^8 cfu/mL	N
E. coli (strain: (Migula) Castellani and Chalmers) serotype O103:K:H8	ATCC 23982	6 x 10^8 cfu/mL	N
E. coli (strain: (Migula) Castellani and Chalmers) serotype O111:NM	Zeptometrix 0801747	1.05 x 10^10 cfu/mL	N

E. coli (strain: (Migula) Castellani and Chalmers) – feces, human (feces from a healthy human), strain HGH21	ATCC BAA-97	6 x 10^8 cfu/mL	N
E. coli (strain: (Migula) Castellani and Chalmers) – adult, human NewYork, strain ECOR2	ATCC 35321	6 x 10^8 cfu/mL	N
E. coli (strain: (Migula) Castellani and Chalmers) – adult, human Sweden, ECOR 9 (reference strain)	ATCC 35328	6 x 10^8 cfu/mL	N
E. coli (strain: (Migula) Castellani and Chalmers) – adult, human Tonga, ECOR 41 (reference strain)	ATCC 35360	6 x 10^8 cfu/mL	N
Eggerthella lenta	ATCC 25559	6 x 10^8 cfu/mL	N
Entamoeba dispar	ATCC PRA-260	6.80 x 10^6 copies/mL	N
Entamoeba moshkovskii	ATCC 50004	Not known	N
Enterobacter aerogenes	ATCC 35028	6 x 10^8 cfu/mL	N
Enterobacter cloacae subsp. cloacae	ATCC 13047	6 x 10^8 cfu/mL	N
Enterococcus casseliflavus	ATCC 25788	6 x 10^8 cfu/mL	N
Enterococcus cecorum	ATCC 43198	6 x 10^8 cfu/mL	N
Enterococcus dispar	ATCC 51266	6 x 10^8 cfu/mL	N
Enterococcus faecalis	ATCC 19433	6 x 10^8 cfu/mL	N
Enterococcus faecalis vanB	ATCC 51299	1.1 x 10^9 bacteria/mL	N
Enterococcus faecium	ATCC 19434	6 x 10^8 cfu/mL	N
Enterococcus faecium vanA	ATCC 700221	6 x 10^8 cfu/mL	N
Enterococcus gallinarum	ATCC 49573	6 x 10^8 cfu/mL	N
Enterococcus hirae	ATCC 8043	5.8 x 10^9 bacteria /mL	N
Enterococcus raffinosus	ATCC 49427	6 x 10^8 cfu/mL	N
Eubacterium rectale	ATCC 33656	6 x 10^8 cfu/mL	N
Faecalibacterium prausnitzii (formerly Fusobacterium prausnitzii)	ATCC 27766	6 x 10^8 cfu/mL	N
Fusobacterium varium	ATCC 8501	6 x 10^8 cfu/mL	N
Gemella morbillorum	ATCC 27824	6 x 10^8 cfu/mL	N
Hafnia alvei	ATCC 13337	6 x 10^8 cfu/mL	N
Helicobacter fennelliae	ATCC 35683	6 x 10^8 cfu/mL	N
Homo sapiens	ATCC MGC-15492	Titer not available; used from stock	N
Klebsiella pneumoniae subsp. pneumoniae	ATCC 13883	6 x 10^8 cfu/mL	N
Lactobacillus acidophilus	ATCC 4356	6 x 10^8 cfu/mL	N
Lactobacillus casei	ATCC 393	6 x 10^8 cfu/mL	N
Lactobacillus reuteri	ATCC 23272	6 x 10^8 cfu/mL	N
Lactococcus lactis subsp. lactis	ATCC 11454	9 x 10^8 cfu/mL	N

Leminorella grimontii	ATCC 33999	6 x 10^8 cfu/mL	N
Listeria innocua	ATCC 33090	6 x 10^8 cfu/mL	N
Mycoplasma fermentans	ATCC 19989	Titer not available; used from stock	N
Peptoniphilus asaccharolyticus	ATCC 14963	6 x 10^8 cfu/mL	N
Peptostreptococcus anaerobius	ATCC 27337	6 x 10^8 cfu/mL	N
Porphyromonas levii	ATCC 29147	6 x 10^8 cfu/mL	N
Prevotella melaninogenica	ATCC 25845	3.2 x 10^7 bacteria/mL	N
Proteus mirabilis	ATCC 4630	6 x 10^8 cfu/mL	N
Proteus penneri	ATCC 35198	6 x 10^8 cfu/mL	N
Proteus vulgaris	ATCC 6380	6 x 10^8 cfu/mL	N
Pseudomonas aeruginosa	ATCC 27853	6 x 10^8 cfu/mL	N
Pseudomonas putida	ATCC 47054	6 x 10^8 cfu/mL	N
Ruminococcus bromii	ATCC 27255	Not known	N
Salmonella subterranea**	ATCC BAA-836	6 x 10^8 cfu/mL	**Y** with Shigella
Staphylococcus aureus subsp. Aureus strain FDA 209	ATCC 6538	6 x 10^8 cfu/mL	N
Staphylococcus aureus subsp. aureus, Cowan's serotype 1 (contains a protein A)	ATCC 12598	6 x 10^8 cfu/mL	N
Staphylococcus epidermidis	ATCC 12228	6 x 10^8 cfu/mL	N
Streptococus intermedius	ATCC 27335	6 x 10^8 cfu/mL	N
Streptococcus salivarius	ATCC 7073	6 x 10^8 cfu/mL	N
Streptococcus sp.	ATCC 12973	6 x 10^8 cfu/mL	N
Streptococcus uberis	ATCC 19436	6 x 10^8 cfu/mL	N
Trabulsiella guamensis	ATCC 49490	1.84 x 10^8 cfu/mL	N
Veillonella atypica	ATCC 12641	6 x 10^8 cfu/mL	N
Veillonella parvula	ATCC 10790	6 x 10^8 cfu/mL	N

Note: *Streptococcus faecalis* is another name for *Enterococcus faecalis.* Therefore, only one of the two (*Enterococcus faecalis)* were tested.
† - Added following release of the *C. difficile* FDA guidance document Nov. 29, 2010.
***Salmonella subterranea* is closely related to *Escherichia hermanii* and does not belong to the genus *Salmonella*.
^ One of eight replicates cross-reacted with *Shigella*.

An additional 20 pathogens were not attainable but were evaluated '*in silico'* to assess the potential for cross-reactivity that could lead to false positive results. While 2 of these 20 could potentially cross-react based on BLAST analysis (*Entamoeba coli* and *Taenia saginata*), positive detection of these pathogens by xTAG GPP is highly unlikely based

on either thermodynamic (Tm) analysis of the pathogen sequence with the kit primers or lack of incorporation of biotin required to produce a signal.

***In silico* evaluation of pathogens for potential cross-reactivity**

Pathogen
Ascaris lumbricoides (roundworm)
Chilomastix mesnili
Cryptosporidium canis
Cryptosporidium felis
Cyclospora cayetanensis
DF-3 – Dysgonomonas capnocytophagoides
Dientamoeba fragilis
Diphyllobothrium species
Endolimax nana
Entamoeba coli
Entamoeba hartmanni
Entamoeba polecki
Enterobius vermicularis (pinworm)
Enteromonas hominis
Hymenolepis nana (the dwarf tapeworm)
Idamoeba buetschlii
Isospora belli
Strongyloides stercoralis
Taenia sp.
Trichuris trichiura

Interference

There was no interference observed for analytes probed by the assay when low positive concentrations of these analytes (Norovirus GI/GII, Rotavirus A and *C. difficile*) were assayed in the presence of high concentrations of the 4 non-panel gastrointestinal pathogens listed below.

Non-panel GI pathogens tested for potential interference

xTAG GPP Analyte (concentration)	Source	Potentially Interfering Organism (concentration)	Source	Interference Yes (Y) /No (N)
Norovirus GI (LP) (6.56 x 10^5 copies/mL)	CDC	None		N
		Aichi virus (HP) (1.00 x 10^8 pfu/mL)	CDC	N
		Astrovirus (HP) (6.00 x 10^10 copies/mL)	CDC	N
		Sapovirus (HP) (5.00E+08 copies/mL)	CDC	N
Norovirus GII (LP) (1.01 x 10^8 copies/mL)	CDC	None		N
		Aichi virus (HP) (1.00 x 10^8 pfu/mL)	CDC	N
		Astrovirus (HP) (6.00 x 10^10 copies/mL)	CDC	N
		Sapovirus (HP) (5.00 x 10^8 copies/mL)	CDC	N
Rotavirus (LP) (4.85 x 10^9 copies/mL)	CDC	None		N
		Aichi virus (HP) (1.00 x 10^8 pfu/mL)	CDC	N
		Astrovirus (HP) (6.00 x 10^10 copies/mL)	CDC	N
		Sapovirus (HP) (5.00 x 10^8 copies/mL)	CDC	N
Clostridium difficile toxin A/B (LP) (3.75 x 10^6 cfu/mL)	ATCC	None		N
		Enterococcus faecium, vancomycin resistant (HP) (6.00 x 10^8 cfu/mL)	ATCC 700221	N

None of the ten common non-panel commensal bacteria, yeast and parasites listed below interfered with the detection of the panel analytes (*Campylobacter, C. difficile, Cryptosporidium, E. coli* 0157, ETEC LT/ST, *Giardia,* Norovirus GI/GII, Rotavirus A, *Salmonella,* STEC stx1/stx2, and *Shigella*).

Common commensal bacteria, yeast and parasites tested for interference

Bacteroides thetaiotaomicron (ATCC 29148)
Citrobacter koseri (ATCC 27028)
Clostridium sporogenes (ATCC 3584)
E. coli strain ECOR2 (ATCC 35321)

Enterobacter cloacae (ATCC 13047)
Klebsiella pneumoniae subsp. *pneumoniae* (ATCC 13883)
Pseudomonas putida (ATCC 47054)
Proteus penneri (ATCC 35198)
Candida albicans (ATCC 10231)
Blastocystis hominis (ATCC 50587 or 50608)

There was no interference observed with the 18 non-microbial agents tested. In addition, none of the non-microbial agents tested in the presence of *C. difficile* inhibited the detection of the *C. difficile* Toxin A and B analytes.

Non-microbial agents evaluated for interference

Non-microbial agents	Brand	Lot Number	Cross-Reactive Yes (Y) / No (N)
Whole blood (40% v/v)	Bioreclamation	BRH288023	N
Mucin (3.5% w/v)	Sigma-Aldrich	039k7003v	N
Fecal fat - triglcerides (4.8% w/v)	Supleco	LB81189	N
Fecal fat - cholesterol (4.8% w/v)	Sigma-Aldrich	061m53001v	N
Hemoglobin (tarry stool) (12.5% w/v)	Sigma-Aldrich	051m7004v	N
Pepto-Bismol (5% w/v) (Bismuth subsalicylate)	Pepto-Bismol	1151171951	N
Kaopectate (5 mg/mL) (Attapulgite)	Kaopectate	L0705	N
Imodium (5% w/v) (Loperamide hydrochloride)	Imodium	CNER	N
Nystatin† (50% w/w) (antifungal)	Ratio-nystatin	655900	N
Hydrocortisone† (50% w/v)	Rexall Hydrocortisone cream USP	F1022	N
Calcium Carbonate† (5% w/v) (antacids)	Tums	1C21	N
Magnesium Hydroxide, Aluminum Hydroxide† (5% v/v) (antacids)	Maalox	10114204	N
Mineral Oil† (50% v/v)	Rexall Mineral Oil heavy ISP	150-1	N
Sennosides† (5% w/v) (laxative)	Sennokot	F328	N
Naproxen Sodium† (2170 µmol/L) (non-steroid anti-inflammatory)	Rexall Naproxen	p6172	N

Benzalkonium Chloride, Ethanol† (50% v/v) (moist towellets)	Sigma-Aldrich, Commercial alcohols	szba3280, 9163	N
Ampicillin sodium salt† (152 µmol/L) (antibiotic)	Sigma-Aldrich	bcbf5293v	N
Polymyxin B sulfate, bacitracin zinc†, (50% w/v) (antibiotic, topical)	Polysporin	1410	N

† - Added following release of the C. difficile FDA guidance document Nov. 29, 2010

Competitive Interference

There was no competitive interference observed between pathogens probed by xTAG GPP when testing was carried out with the mixed analyte samples described below.

Mixed analyte samples tested for competitive interference

xTAG GPP Analyte #1	xTAG GPP Analyte #2
Campylobacter jejuni (HP) (6.00E+08 cfu/mL)	No Analyte #2
	Shigella sonnei (LP) (1.01E+04 cfu/mL)
Campylobacter jejuni (LP) (2.93E+05 cfu/mL)	No Analyte #2
	Shigella sonnei (HP) (6.00E+08 cfu/mL)
Cryptosporidium parvum (HP) (2.50E+05 oocysts/mL)	No Analyte #2
	Giardia lamblia (LP) (1.10E+03 cells/mL)
Cryptosporidium parvum (LP) (6.25E+04 oocysts/mL)	No Analyte #2
	Giardia lamblia (HP) (9.00E+06 cells/mL)
E. coli (enterotoxic) (HP) (6.00E+08 cfu/mL)	No Analyte #2
	Shigella sonnei (LP)* (1.01E+04 cfu/mL)
	Campylobacter jejuni (LP) (2.93E+05 cfu/mL)
E. coli (enterotoxic) (LP) (3.51E+05 cfu/mL)	No Analyte #2
	Shigella sonnei (HP) (6.00E+08 cfu/mL)
	Campylobacter jejuni (HP) (6.00E+08 cfu/mL)
Norovirus (HP) (stock)	No Analyte #2
	Clostridium difficile (LP) (3.75E+06 cfu/mL)**
Norovirus (LP) (dil 3 = 160x dilution of stock concentration)	No Analyte #2
	Clostridium difficile (HP) (6.00E+08 cfu/mL)
Rotavirus (HP)	No Analyte #2

xTAG GPP Analyte #1	xTAG GPP Analyte #2
(1.58E+06 $TCID_{50}$/mL)	*Shigella sonnei* (LP) (1.01E+04 cfu/mL)
	Campylobacter jejuni (LP) (2.93E+05 cfu/mL)
	Giardia lamblia (HP) (1.10E+03 cells/mL)
	Cryptosporidium hominis (LP) (2.15E+04 copies/mL)
	E. coli (enterotoxic) (LP) (3.51E+05 cfu/mL)
	Clostridium difficile (LP) (3.75E+06 cells/mL)
Rotavirus (LP) (5.27E+05 $TCID_{50}$/mL)	No Analyte #2
	Shigella sonnei (HP) (6.00E+08 cfu/mL)
	Campylobacter jejuni (HP) (6.00E+08 cfu/mL)
	Giardia lamblia (HP) (9.00E+06 cells/mL)
	Cryptosporidium parvum (HP) (2.50E+05 oocysts/mL)
	E. coli (enterotoxic) (HP) (6.00E+08 cfu/mL)
	Clostridium difficile (HP) (6.00E+08 cells/mL)

*In addition, no interference with *Shigella* at a concentration of 5.04 x 10^3 cfu/mL

***Clostridium difficile* was also tested at 8.33 x 10^6 cfu/mL

Carry-over Contamination

The likelihood of carry-over contamination events was assessed by testing 2 representative pathogens: bacterial (*C. difficile*), and parasitic (*Giardia*). These analytes were examined in the form of simulated samples prepared at concentrations just below the assay cut-off (High Negative, HN) and well above the assay cut-off (High Positive, HP). Each target was examined in a set of 2 identical runs (including the pre-treatment and extraction steps) arranged in a checkerboard manner on a 96-well plate.

Results showed that all 96 high negative samples remained negative when run on the Luminex 100/200 instrument for both targets (100% HN). In addition, results show that all 96 high positive samples remained positive when run on the Luminex 100/200 instrument for both targets (100% HP). Therefore a lack of carryover contamination has been demonstrated.

f. Assay cut-off:

Clinical specimens, cultured isolates spiked in a synthetic stool matrix sample and extraction controls (negative matrix spiked with MS2) were used to establish cut-offs.

These cutoff values are hard-coded into the TDAS software (US IVD) and can not be modified.

Assay cut-off determination (threshold-setting) consists of three steps for each analyte:

1) Setting an initial cut-off range based on the 95th percentile of signals for the NEG samples and 5th percentile of signals for the POS samples.
2) Recommending optimized cut-offs within this range based on Receiver Operating Characteristic (ROC) analysis of empirical data, and
3) Establishing an MFI cut-off value through a Design Review Committee (DRC) assessment of ROC curves.

Distinct sample sets were used for setting initial cut-offs (step 1 above) and for finding the optimized cut-offs (step 2 above) for GPP. Samples were assigned a "positive" or "negative" call for the analyte in question based on the known sample types or results obtained at the clinical sites. These results were based on the routine diagnostic algorithm at the collection sites (e.g. bacterial culture, EIA/DFA, microscopic examination, real-time PCR, nucleic acid amplification tests followed by bi-directional sequencing). For some samples, comparator results were not available for all 15 targets in the xTAG GPP assay, but rather than drop or lose these samples, these data points were highlighted. When the comparator result was not available for a particular target, the target for that sample was excluded from the threshold-setting data sets.

The sample set used in these two cut-off determination steps also included cultured isolates with confirmed viral, bacterial or parasitic identity which were serially diluted into negative matrix. Finally, the sample set was supplemented with extraction controls (negative matrix spiked with MS2) that were coded as negative for all targets. All samples were extracted using the Biomerieux EasyMag® method prior to being tested with xTAG GPP.

The table below details the final cutoff values selected for each of the targets probed by the xTAG GPP assay. For most targets that have a single probe, sample results above or equal to the cutoff value are considered positive, while sample results below the cutoff value are considered negative. Please note that for multi-probe targets, like *C. Difficile*, Norovirus, Enterotoxigenic *E. coli* (ETEC) LT/ST and Shiga-like toxin producing *E. Coli* (STEC), a single qualitative POS (positive) call is made if either one of their probes is above or equal to the cutoff value, otherwise a single qualitative NEG (negative) call is made. For *Salmonella*, a single qualitative POS (positive) call is made when Probe-1 signal is above or equal to 1400, and a single qualitative NEG (negative) call is made when its signal is less than 200. Probe-2 signal will only be used to determine the final call when the Probe-1 signal falls within the equivocal zone, i.e. signals greater than or equal to 200 but less than 1400.

xTAG GPP Analyte Cutoff Values
for all targets probed by the assay

Analyte	Final Cut-off (MFI) for LX 100/200
Campylobacter	≥ 150 (POS)
C. difficile Probe-1	≥ 150 (POS)
C. difficile Probe-2	≥ 150 (POS)
Cryptosporidium	≥ 250 (POS)
E. coli O157	≥ 150 (POS)
ETEC Probe-1	≥ 200 (POS)
ETEC Probe-2	≥ 200 (POS)
Giardia	≥ 250 (POS)
Norovirus Probe-1	≥ 200 (POS)
Norovirus Probe-2	≥ 350 (POS)
Rotavirus A	≥ 150 (POS)
Salmonella Probe-1	≥200 (NEG), ≥1400 (POS)
Salmonella Probe-2	≥ 200 (POS)
STEC Probe-1	≥ 150 (POS)
STEC Probe-2	≥ 150 (POS)
Shigella	≥ 150 (POS)

Fresh vs. Frozen

The purpose of this evaluation was to generate data to support the hypothesis that no significant difference in the performance of xTAG GPP would be observed between specimens tested from the "fresh" state (i.e., unfrozen) and specimens that were tested after being stored frozen at -70°C to -80°C. Each analyte target probed by the assay was assessed in a set of simulated specimens prepared in negative clinical matrix at a concentration close to the assay cut-off MFI (Low Positive), 5-10x the assay cut-off MFI (Moderate Positive) and, where possible, more than 10x the assay cut-off MFI (High Positive), where MFI is median fluorescent intensity value. Stability of un-extracted specimens, as well as pre-treated specimens, and finally, pre-treated and extracted nucleic acids were evaluated.

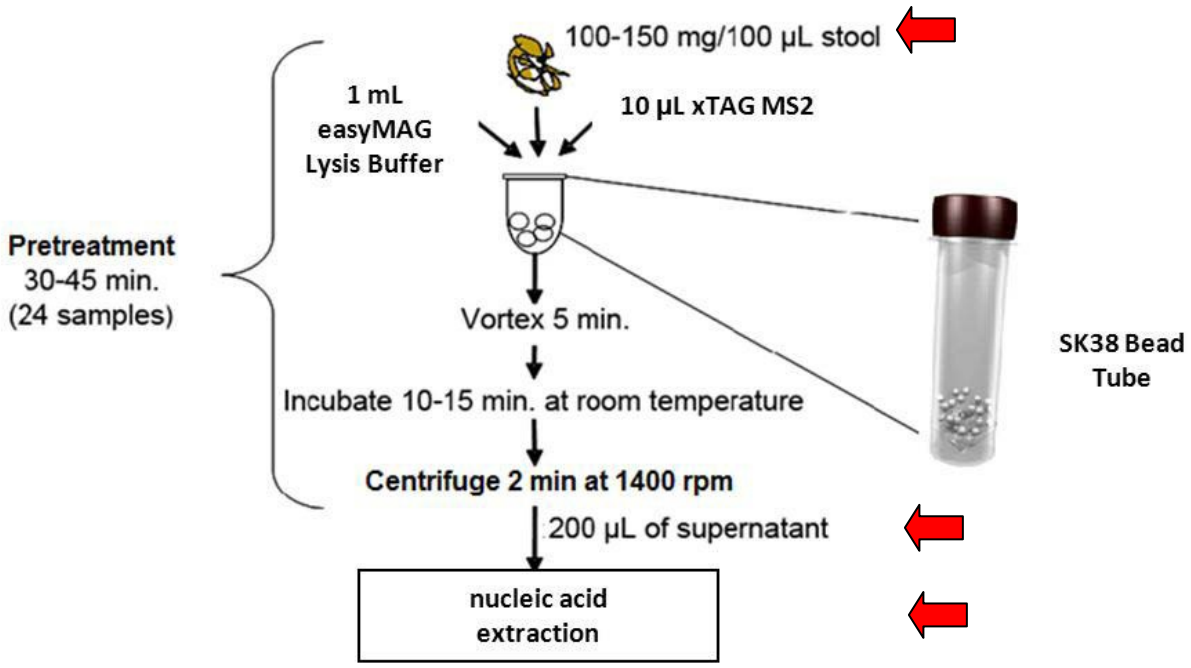
The results of this study will be used to support (or reject) the inclusion of frozen clinical specimens in the multi-site method comparison clinical evaluation of xTAG GPP and will support sample storage claims in the instructions for use.

Following the selection of the appropriate dilution to represent the three different levels (Low Positive, Moderate Positive and High Positive), identical sets of the simulated specimens were prepared for each analyte target so they could be examined at the following intervals: baseline (fresh), 1 month, 3 months (un-extracted specimen and

nucleic acid extracts only) and 6 months after freezing at -70°C to -80°C.

This study examined the stability of un-extracted specimens as well as pre-treated material and nucleic acid extracts (see three horizontal red block arrows to the right side of the Figure) after being stored frozen at -70°C to -80°C for up to 3 months. The first block arrow shows the 'un-extracted stool' material. The second block arrow shows the 'pre-treated' material (prior to extraction). The third block arrow (after nucleic acid extraction) shows the 'extracted' nucleic acid material. Un-extracted, pre-treated and extracted specimen stability will also be examined after storage at -70°C to -80°C for 6 months.

Instructions for Use and Samples Tested (Red Block Arrows) in this Study



For each analyte, HP, MP and LP un-extracted stocks were prepared in negative stool matrix and split into 5 aliquots. Two (2) aliquots, sufficient volume for 36 pre-treatments and extractions of HP, 44 of MP, and 40 of LP, were immediately extracted (no freeze-thaw). When pooled in pairs, the volume for each of these aliquots was enough for 18 pre-treatments and extractions of HP, 22 of MP and 20 of LP. The remaining 3 aliquots were stored at -70 to -80°C for later stability testing (see Figure below).

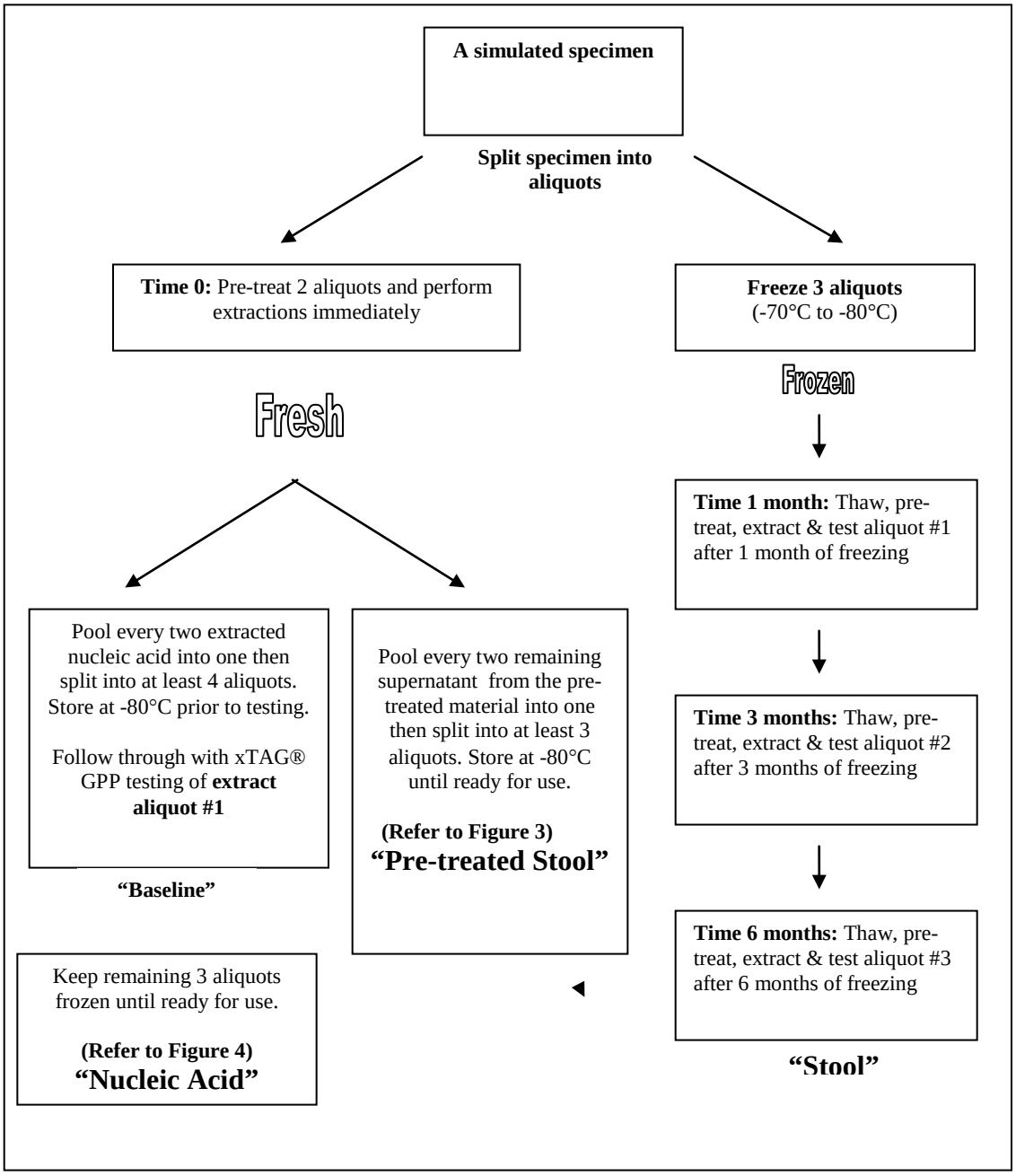
For each dilution, two aliquots of extracted nucleic acid were pooled and pooled material was split into four aliquots. One aliquot was immediately tested by xTAG GPP (no freeze-thaw) to generate **"Baseline"** values for all sample types (i.e. un-extracted, pre-treated and extracted stool). The remaining three aliquots (**"Nucleic Acids"**) were stored at -80°C for later stability testing at 1-month, 3-month and 6-month stability time points.

In the same manner, two Pre-treated samples (**"Pre-treated Stool"**) were also pooled,

split into aliquots and stored -70 to -80°C for stability testing at 1-month and 6-month stability time points.

Frozen un-treated specimens were pre-treated, extracted and tested by xTAG GPP at each designated stability time point (see Figure below).

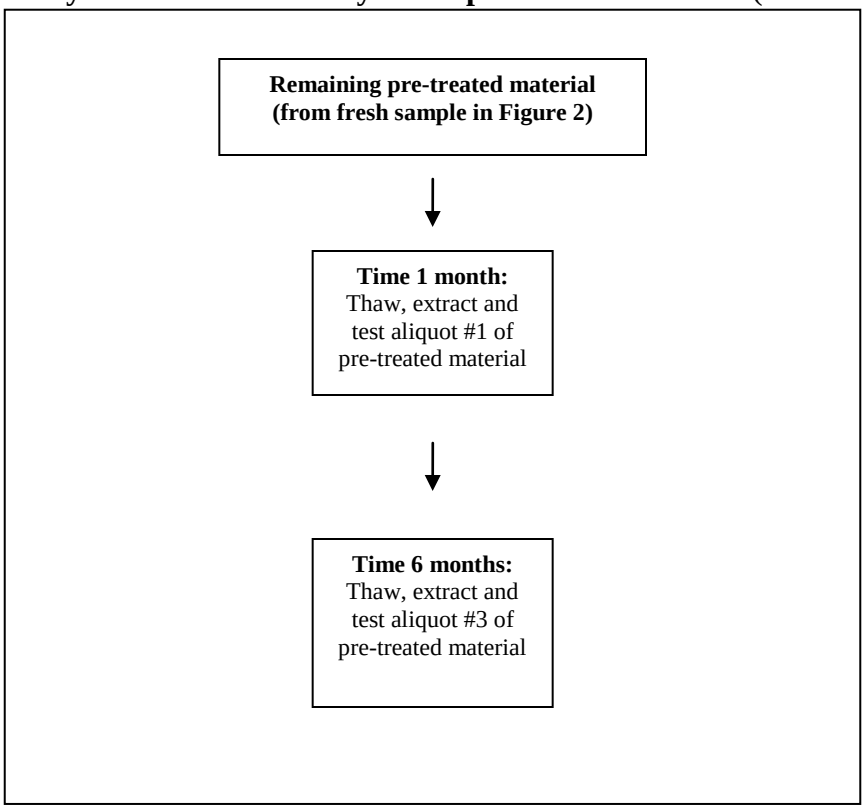
Study workflow of the stability of fresh ("Baseline") and frozen ("un-extracted Stool") specimens.



In order to assess pre-treated sample and extracted nucleic acid stability for each analyte target, the remaining three aliquots of pre-treated material and extracted nucleic acid from the "Fresh" arm in the Figure above were tested by xTAG GPP at the following time points post freezing: 1 month (both) and 3 months (extracted nucleic acid only). Pre-treated and extracted sample stability will also be examined after storage at -70°C to -80°C for 6 months.

Data generated at each time point (1 month and 3 months) on frozen un-extracted specimens, nucleic acid extracts and pre-treated material were compared to the data generated at baseline (time 0 or Fresh). The 6- month time-point is not yet available. It is not expected that clinical specimens will be stored for longer than 30 days (1 month) in clinical practice.

Study workflow of stability of the pre-treated material (**"Pre-treated Stool"**).



Acceptance Criteria

In order to demonstrate no significant difference in assay performance between fresh and frozen un-extracted specimens and the stability of frozen pre-treated material and nucleic acid at each time point, the positive agreement (i.e. the agreement between positive results generated in fresh aliquots compared to positive results generated in frozen aliquots) should be $\geq$ 95% with a lower bound of the 95% (two- sided) confidence interval exceeding 85% for each claimed analyte.

1-Month Stability Results

1-month stability acceptance criteria were met for all of the targets except the following:

- Campylobacter (un-extracted and extracted specimens only)
- Giardia (un-extracted and pre-treated specimens only)
- Norovirus GII (un-extracted specimen only)

For those targets that met the 1-month stability acceptance criteria, MFIs generated on HP, MP and LP replicates of frozen un-extracted, extracted and extracted specimens were generally close to those generated at baseline.

For *Campylobacter*, MFIs generated on HP, MP and LP replicates of frozen un-extracted and extracted specimens were well below those generated at baseline. The same observation was made for HP, MP and LP replicates of *Giardia* (un-extracted and pre-treated specimens only). In addition, internal control (MS2) values generated on frozen replicates of un-extracted and pre-treated samples were generally lower for these 3 analytes compared to baseline MS2 values suggesting that sub-optimal extraction may be the cause for these results.

For Norovirus GII, although the 1-month stability acceptance criteria was not met for un-extracted specimens, MFI generated from HP and MP replicates of both fresh and frozen specimens were similar. The mean MFI value generated on frozen LP replicates was 360 (1x assay cut-off) compared to 868 for fresh specimens (2.5x assay cut-off).

Luminex was unable to source suitable stock material of cryptosporidium to generate enough replicates of HP, MP and LP concentrations for the study. Therefore, only LP dilutions were generated for this target. xTAG GPP only generated 40/60 positive results at baseline and MFIs ranged from 0.7x to 2.6x the assay cut-off. Although 1-month stability criteria were not met for this target, MFIs generated on frozen un-extracted, pre-treated and extracted specimens ranged from 0.35x - 1.66x, 0.33x – 0.36x and 0.6x – 1.74x the assay cut-off respectively suggesting that these results are most likely due to low starting titer rather than specimen stability.

3-Month Stability Results

To date, 3-month stability results for un-extracted and extracted specimens are available for *Campylobacter*, *C. difficile*, *E. coli* O157, ETEC, *Giardia*, Norovirus GII, Rotavirus A, *Salmonella*, STEC and *Shigella*.

3-month stability acceptance criteria for frozen <u>un-extracted</u> specimens were met for all targets tested to date with the exception of Norovirus GII. For this target, MFI values generated for LP replicates bracket the assay cut-off.

3-month stability acceptance criteria for frozen <u>extracted</u> specimens were met for all targets tested to date with the exception of Norovirus GII and *Giardia*.

3-month stability results of *Campylobacter*, Norovirus GII and *Giardia* are of particular interest as they do not reflect the 1-month stability results. That is, study acceptance criteria were met for *Campylobacter* un-extracted and extracted specimens at the 3-month stability time point but not at the 1-month time point. Similarly, study acceptance criteria were met for *Giardia* and Norovirus GII un-extracted specimens at the 3-month stability time point but not at the 1-month time point. One possible explanation for the discrepant results generated on frozen replicates of un-extracted samples at 1-month and 3-month time points is sub-optimal extraction. This hypothesis is supported by the fact that the internal control (MS2) values generated on frozen replicates of un-extracted samples, in particular *Giardia*, were generally lower at Month 1 compared to baseline and Month 3. LMD is unable to explain the discrepant results observed at Month 1 and Month 3 for *Campylobacter* nucleic acid extracts.

Supplemental Stability Results - *Cryptosporidium* (pre-treated and extracted)
In order to verify that the results obtained at the Month-1 time point for *Cryptosporidium* un-extracted, pre-treated and extracted specimens were attributed to sample titer rather than to stability, LP and MP results generated as part of the multi-site reproducibility study at site 1 (Luminex) were re-analyzed. All LP and MP un-extracted, pre-treated and extracted specimen remnants were also re-tested by xTAG GPP at a later date.

Results generated on LP specimens in terms of calls and MFIs are consistent with those generated as part of the Fresh vs Frozen study. However, mean MFI values generated on MP dilutions of un-extracted, pre- treated and extracted left-over specimens at different time points were similar and ranged from 2.2x to 4.4x the assay cut-off MFI. These results suggest that un-extracted, pre-treated and extracted *Cryptosporidium* specimens prepared at a concentration 1-5x the assay cut-off MFI are stable for at least 1-month when stored frozen at -70°C to -80°C.

Conclusion for Fresh vs. Frozen Study
Stability results generated to date support the inclusion of frozen clinical specimens positive for all targets in the multi-site clinical evaluation of the xTAG GPP. Results generated to date also indicate that pre-treated material and nucleic acid extracts of all targets evaluated to date are stable for at least 1 month post freezing (with the exception of *Giardia* pre-treated material).

Summary of Stability Results

Analyte Target	Un-extracted 1 month	Un-extracted 3 months	Pre-Treated 1 month	Extracted 1 month	Extracted 3 months
Campylobacter	X	√	√	X	√
C. difficile Toxin A/B	√	√	√	√	√
Cryptosporidium	√^	Pending	√^	√^	Pending
E. coli O157	√	√	√	√	√

ETEC (LT/ST)	√	√	√	√	√
Giardia	X	√	√^	√	Pending
Norovirus GI	√	Pending	√	√	Pending
Norovirus GII	X	√	√	√	X
Rotavirus A	√	√	√	√	√
Salmonella	√	√	√	√	√
STEC (stx1/stx2)	√	√	√	√	√
Shigella	√	√	√	√	√

^Based on supplemental testing results, possible titer or extraction issue with sample rather than stability failure

Comparator Assays Analytical Validation Studies

PCR followed by bi-directional sequencing assays (PCR/sequencing) are used as a comparator method and to resolve discordant results to establish analyte identity during the clinical evaluation of xTAG assays. They are validated to evaluate certain performance characteristics including analytical sensitivity (limit of detection), analytical reactivity and specificity (cross-reactivity).

The primers were chosen to perform sequencing as a comparator method for Campylobacter, Enterotoxigenic Escherichia Coli (ETEC) LT and ST, and Rotavirus A targets of the xTAG Gastrointestinal Pathogen Panel (xTAG GPP). Two different primer sets were designed and validated for ETEC LT and ST and one primer set was designed and validated for Campylobacter, and Rotavirus A.

To the extent possible, the sequencing primers were designed to amplify regions of the genomic sequence that are not covered by the xTAG GPP kit primers. The second set of sequencing primers designed for ETEC LT and ETEC ST targets were designed to flank the GPP kit amplicon. Bi-directional (both forward and reverse sequences of the produced amplicon) Sanger dideoxy - sequencing method and BLAST analysis were used to confirm sequence identity.

Sequencing primers were validated using samples from the following sources:

1. **Representative Clinical Sample:** Wherever possible, known positive clinical samples were tested with the sequencing primers to evaluate detection from an extracted clinical stool sample.
2. **Limit of Detection (LoD)**: Serial dilutions of the target analytes were tested to establish the lower limit of primer sensitivity. Samples tested for "Evaluation of the Limit of Detection and Repeatability of xTAG Gastrointestinal Pathogen Panel (FDA)," study were used here.
3. **Cross-reactivity:** For the xTAG GPP panel targets, samples representing all the targtes in the xTAG GPP panel, were tested at the highest available titres. For the xTAG GPP non-panel cross-reactivity targets, BLAST analysis was preformed with each sequencing primer. If both the forward and reverse sets contained an 11 base pair match up to the 3' end (Kwok S, 1994) of the primer with any of the non-panel cross-reactivity species, then a

representative sample for that strain was tested to evaluate cross-reactivity.

4. **Reactivity:** Various strains for each target were analyzed to evaluate the strain coverage of the sequencing primers. Samples tested for "Evaluation of Analytical Reactivity of the xTAG Gastrointestinal Pathogen Panel (FDA)" study were used here**.**

Detailed descriptions of the types of samples tested are listed below:

- **Clinical Sample:** Pre-characterized target-specific clinical samples for Rotavirus and Campylobacter were tested with the sequencing primers. For ETEC, since there is no validated comparator other than the sequencing method, clinical samples positive for either ETEC LT or ST by the xTAG GPP assay were used.

- **Limit of Detection Study:** The same sample sets prepared for the Evaluation of the Limit of Detection and Repeatability of xTAG GPP study, were used for this Sequencing Primer Validation study. Briefly, stock solutions were diluted to a starting concentration and dilution series were prepared by making sequential 4-fold dilutions to about 10 dilution levels. Sample dilutions were prepared and tested in triplicates.

- **Cross-reactivity:** To test for cross-reactivity of the sequencing primers the following studies were conducted.
    - For the xTAG GPP panel targets, samples representing all the targets in the xTAG GPP panel, were tested at the highest available titers.

    - For the xTAG GPP non-panel cross-reactivity targets, BLAST analysis was preformed with each sequencing primer. If both the forward and reverse sets contained an 11 base pair match up to the 3' end (Kwok S, 1994) of the primer with any of the non-panel cross-reactivity species, then a representative sample for that strain was tested to evaluate cross-reactivity.

- **Reactivity:** A variety of strains, genotypes and serotypes for ETEC, Rotavirus, and Campylobacter used in the Analytical Reactivity study were tested with each sequencing primer set.

<u>Categorizing Sequencing Results</u>
Positive - Samples were considered positive by sequencing if the following criteria were met:

- The generated sequences, from bidirectional sequencing, should be at least 200 bases of an acceptable quality, defined as a minimum of 90% of the total bases (20 bases per 200bp read) with PHRED quality score of 20 or higher (accuracy of base call is $\geq$ 99%)
- For sequences containing ambiguous base calls such as "N"s, the total number of ambiguous bases in the acceptable quality sequences generated using bidirectional sequencing should not exceed 5% of total bases (or 10

bases per 200 bp read).

- Blast analysis of the acceptable quality sequences generated by bidirectional sequencing should have at least 95% query coverage compared to reference and at least 95% identity to reference.
- Sequence matches the reference or sequence generates an Expected Value (E-Value) < 10^{-30} for the specific target following a BLAST search in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/).

Negative – Samples were considered negative by sequencing if any one of the above criteria were not met.

Acceptance Criteria

- **Clinical Sample:** The clinical sample of known identity, if available, must be positive by sequencing for the expected target.
- **Limit of Detection Study:** At least, 2 of the 3 extraction replicates must be positive by sequencing at the equivalent or lower titer than the established limit of detection recorded for the xTAG GPP analyte.
- **Cross-reactivity Study:** All samples tested should generate no sequencing reactions of acceptable quality.
- **Reactivity:** Strains, genotypes and serotypes should generate positives results with their respective sequencing primers.

Conclusion

All sequencing primers met the acceptance criteria for all studies.

Summary of Sequencing Primers Validation Studies

	Rotavirus	ETEC LT		ETEC ST		Campylobacter
	Partially Flanking 2F/3R	**Outside 101**	**Flanking 2F/2BR**	**Partially Flanking 102F/1BR**	**Flanking 7AF/12.1R**	**Outside 101**
Limit of Detection	Equal to kit	More Sensitive than kit	Equal to kit	More Sensitive than kit	More Sensitive than kit	More Sensitive than kit
Cross-Reactivity	None	None	None	None	None	None
Reactivity	8of9 strains reacted[1]	6of7 LT positive strains reacted[2]	6of7 LT positive strains reacted[2]	5of6 ST positive strains reacted[2]	5of6 ST positive strains reacted[2]	14of14 strains reacted

[1] The 1 strain of Rotavirus that did not react was ATCC 2275

[2] The 1 strain that did not react (ATCC 43896) with the ETEC primers also did not react with the ETEC LT and ST targets of the GPP kit

Summary of negative control failures and sample re-run rates for analytical performance studies

There were a total of 217 xTAG GPP runs performed over the course of analytical performance studies. Each xTAG run has at least one no template negative control depending on batch size. Of the 217 runs, 11 (5.07%) had one or more negative control (NC) failures. These are summarized in the table below.

Summary of Negative Control Failures for Analytical Performance Studies

Study	Total # of runs (including allowable re-runs)	Total # of runs with at least one NC failure	% total runs with at least one NC failure	Total No. of NCs included in runs and allowable re-runs	Total No. of NC failures	% total NC s included which failed in xTAG runs / allowable re-runs
Multi-site reproducibility	64	6	9.38%	188	7	3.72%
Matrix equivalence	3	0	0	9	0	0
Limit of detection	29	0	0	108	0	0
Carry-over contamination	6	0	0	0	0	0
Analytical specificity and interference	23	1	4.34%	91	1	1.10%
Analytical reactivity	31	2	6.45%	204	3	1.47%
Evaluation of fresh vs. frozen stool	61	2	3.28%	188	2	1.06%
Overall	**217**	**11**	**5.07%**	**788**	**13**	**1.65%**

Included in the 217 xTAG runs summarized above were 12473 specimens. Of these, 99.79% (12447/12473) yielded valid results on the first attempt. The remaining 26 specimens generated valid results following allowable re-runs. Sample re-run rates are summarized in the table below.

Summary of Sample Re-Run Rates for Analytical Performance Studies

Studies	Total # of specimens tested	Total # of invalid results prior to re-run	% invalid results prior to re-run	Invalid results after re-run	% invalid results after re-run
Multi-site reproducibility	4230	22	0.52%	0	0.00%
Matrix equivalence	180	0	0.00%	0	0.00%
Limit of detection	740	1	0.14%	0	0.00%
Carry-over contamination	576	0	0.00%	0	0.00%
Analytical specificity and interference	1319	0	0.00%	0	0.00%
Analytical reactivity	1866	1	0.05%	0	0.00%
Evaluation of fresh vs. frozen stool	3562	2	0.06%	0	0.00%
Overall	**12473**	**26**	**0.21%**	**0**	**0.00%**

2. Comparison studies:

a. Method comparison with predicate device:

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

b. Matrix comparison:

Matrix Equivalency

The purpose of this study was to determine if the performance of the xTAG Gastrointestinal Panel (xTAG GPP) in stool re-suspended in pre-treatment buffer (designated as PT buffer) prior to spiking known concentrations of analytes is equivalent to that of native (raw and untreated) stool (designated as NS) spiked with known concentrations of analytes before the pre-treatment step. The performance of xTAG GPP in these two matrices (raw untreated stool and stool re-suspended in pre- treatment buffer) was assessed by comparing serial dilution curves of analyte targets generated using a single lot of xTAG GPP.

Based on comparative analysis of dilution curves, this study suggests that xTAG GPP performance is equivalent between samples prepared in native stool and stool re-suspended in pre-treatment buffer when extracted with the Biomerieux NucliSens® EasyMag® system. Thus, negative stool re-suspended in pre-treatment buffer as a base matrix can be used for all analytical studies of xTAG GPP.

3. Clinical studies:

a. Clinical Sensitivity:

Microbial Detection in Asymptomatic Volunteers

In order to determine baseline levels for each analyte included in xTAG GPP for individuals who are not exhibiting signs and symptoms of infectious gastroenteritis, 200 clinical stool samples were collected from healthy, asymptomatic donors. Asymptomatic donors from various age groups were included in this study.

Demographic information for the asymptomatic donors is shown in the table below.

Demographic Information for Asymptomatic Donors

Gender	Number of Subjects
Male	92 (46%)
Female	108 (54%)
Total	200
Age	
0 - 1	5 (2.5%)

2 - 5	7 (3.5%)
6 - 21	43 (21.5%)
22 - 60	111 (55.5%)
≥61	34 (17.0%)

PCR inhibition, as determined by results for the internal control used with xTAG GPP (bacteriophage MS2), was observed in 23 of the 200 samples tested (11.5%). After re-running these specimens in accordance with the instructions for use, PCR inhibition was still observed in eight samples (4%). The absence of a detectable internal control signal in these samples meant that negative results for the indicated microbial targets could not be reported. Therefore, the final data analysis was conducted on 192 of the 200 samples collected for this study.

A total of 13 samples that were positive by xTAG GPP were sequenced. Two (2) out of 13 samples were positive by sequencing (*C. Difficile* Toxin A/B), while 11 of 13 samples were not positive by sequencing.

These results are summarized in the table below:

Asymptomatic Donor Results for xTAG GPP

Target	**Percent Negative Results by xTAG GPP for all samples**
Campylobacter	100.0% (192/192)
C. difficile toxin A/B	98.4% (189/192) [1]
Cryptosporidium	100.0% (192/192)
E. coli O157	100.0% (192/192)
ETEC LT/ST	100.0% (192/192)
Giardia	99.0% (190/192) [2]
Norovirus GI/GII	98.4% (189/192) [3]
Rotavirus A	100.0% (192/192)
Salmonella	97.4% (187/192) [4]
STEC stx1/stx2	100.0% (192/192)
Shigella	100.0% (192/192)

NOTE: Sample 216 was positive by xTAG GPP for both Norovirus GII and *C. Difficile*

[1] Two (2) out of 3 xTAG GPP *C. Difficile* positive samples were confirmed as positive by sequencing analysis.

[2] None of the 2 xTAG GPP *Giardia* positive samples was confirmed as positive by sequencing analysis.

[3] None of the 3 xTAG GPP Noroviris GI/GII positive samples was confirmed as positive by sequencing analysis.

[4] None of the 5 xTAG GPP *Salmonella* positive samples was confirmed as positive by sequencing analysis.

Samples (at the specimen level) that were positive by xTAG GPP but negative by sequencing were considered false positives (11/192, 5.3%). These samples had MFI values that were relatively close to the cut-offs. Two samples at the specimen level that were called positive by xTAG GPP were also positive by sequencing analysis for *C. difficile*. These two samples positive for *C. difficile* by both xTAG GPP and sequencing may represent asymptomatic infections.

Prospective Clinical Study

The clinical performance of the xTAG GPP was evaluated during prospective studies at six clinical laboratories in North America (four sites in the U.S. and two sites in Canada). Stool specimens were collected and tested at the six clinical laboratories (Sites 1, 2, 3, 4, 5, and 6) during June 2011 thru February 2012. Clinical study sites were selected based on the types of patients usually referred (e.g. pediatrics, adults), conditions often treated (e.g. *C. difficile* colitis), as well as the geographical prevalence of particular targeted pathogens.

Six geographically separated clinical study sites participated in the clinical evaluation of the xTAG GPP. The study sites were located in East-Central Canada (Toronto, Ontario and Hamilton, Ontario), and Southeast (Nashville, TN), Southwest (Temple, TX and Tucson, AZ), and Midwest (St Louis, MN) of the U.S. Each study location was representative of the intended use setting (clinical laboratories) and testing was performed by trained clinical laboratory personnel.

The table below summarized the total number of patients recruited at each site:

Number of Patients Per Site

Site #	# Patients Recruited
1	461
2	449
3	188
4	295
5	97
6	44
	1534

Patient specimens (one specimen from each of the recruited patients) that met all of the following characteristics were eligible for the study.

1. An exemption from the requirement for Informed Consent had been granted by the site IRB to include the left-over stool specimen in the study.
2. The specimen was from a pediatric or adult, male or female subject who was either hospitalized, admitted to a hospital emergency department, visiting an outpatient clinic or resident of a long-term care facility.
3. The specimen was from a patient for whom a requisition had been made for testing of microbial pathogens suspected of gastrointestinal tract infections.
4. The specimen was from a patient exhibiting clinical signs and symptoms of infectious colitis (including *C. difficile* colitis) or gastroenteritis (including traveler's diarrhea), such as diarrhea, nausea and vomiting, loss of appetite, fever, abdominal pain and tenderness, cramping, bloating, flatulence, bloody stools, fainting and weakness.
5. The volume of the specimen was ≥ 8.5 ML or ≥ 6 g.

Patient specimens with any one of the following characteristics was not eligible for study entry:

1. The specimen was collected at a site which was not covered under the study IRB.
2. The specimen was a preserved stool, stool in Cary-Blair media or rectal swab.
3. The specimen was from an individual who did not exhibit clinical signs and symptoms of infectious colitis or gastroenteritis.
4. Based on available clinical information, the specimen was from an individual with known and documented non-infectious conditions such as ulcerative colitis, irritable bowel syndrome and/or Crohn's disease.
5. The specimen was not properly collected, transported, processed or stored according to the instructions provided by the sponsor.
6. The specimen could not be tested by the relevant comparator assays within 72 hours of collection.

Of the 1534 stool specimens, 127 were excluded from the study. The reasons for exclusion are summarized in the table below.

Summary of Excluded Specimens (N=127)

Reason for Specimen Exclusion	Exclusion Criteria	# Excluded Specimens
The specimen was collected from a site not covered under the study IRB	1	5 (0.3%)
The specimen was from an individual with known and documented non-infectious conditions such as ulcerative colitis, irritable bowel syndrome and/or Crohn's disease	4	67 (4.3%)
The specimen was not properly collected, transported, processed or stored according to the instructions provided by the sponsor	5	50 (3.2%)
The specimen could not be tested by the relevant comparator assays within 72 hours of collection	6	4 (0.2%)
Other: multiple extraction failures	N/A	1 (0.05%)
	Total	**127**

The following table provides a summary of demographic information for the 1407 subjects whose stool specimens were included in the prospective study.

General Demographic Details for the Prospective Data Set (N=1407)

Sex	Number of Subjects
Male	632 (44.9%)
Female	775 (55.1%)
Total	1407
Age (yrs)	
0 – 1	6 (0.4%)
>1 – 5	20 (1.4%)
>5 – 12	25 (1.8%)
>12 – 21	51 (3.6%)
>21 – 65	879 (62.5%)
>65	426 (30.3%)
Total	1407
Subject Status	

Outpatients	421 (29.9%)
Hospitalized	804 (57.1%)
Emergency Department	118 (8.4%)
Long Term Care Facility	18 (1.3%)
Not Determined	46 (3.3%)
Total	1407
Immune Status	
Immuno-compromised	493 (35.0%)
Immuno-competent	758 (53.9%)
Not Determined	156 (11.1%)
Total	1407

In addition to patients' demographic details, every effort was made to ensure that information on clinical signs and symptoms of infectious colitis or gastroenteritis was available on all subjects enrolled in the prospective study. This information was collected by way of chart reviews. Chart reviews were conducted by an individual at the sites who was not directly involved in the study (e.g. research nurse) so that information was collected in a manner that did not make the specimen source identifiable to the investigator or any other individual involved in the investigation including the Sponsor. Local IRB approval for the study was obtained prior to study start. If available, the following information was also collected:

- Stool consistency (based on Bristol Stool Scale)
- Clinical signs and symptoms of infectious colitis or gastroenteritis such as diarrhea, nausea and vomiting, loss of appetite, fever, abdominal pain and tenderness, cramping, bloating, flatulence, bloody stools, fainting and weakness
- Duration and severity of symptoms prior to enrolment
- Method of transmission (e.g. food-borne outbreak or close contact method)
- Prior and concomitant medications including dose, type, frequency and duration.
- Other orally ingested substances (e.g. fiber, stool bulking agents), including dose, type, frequency and duration
- Other laboratory results (e.g. viral/bacterial culture, gram positive/negative infection, hematology and serum chemistry etc.)

Wherever available in the medical charts, the duration and severity of each specific sign or symptom was also recorded.

Stool consistency (based on the Bristol Stool Form Scale) was recorded for each clinical specimen included in the prospective clinical study. A summary of this information is provided in the table below.

Stool consistency (N=1407)

Stool Consistency	# Specimens (%)
Type 1 Separate hard lumps	8 (0.5%)
Type 2 Sausage-shaped but lumpy	24 (1.7%)
Type 3 Like a sausage but with cracks	26 (1.8%)
Type 4 Like sausage/snake, smooth, soft	77 (5.5%)

Type 5 Soft blobs with clear-cut edges	160 (11.4%)
Type 6 Fluffy pieces with ragged edges	354 (25.2%)
Type 7 Watery, no solid pieces	758 (53.9%)

Information on clinical signs and symptoms of infectious colitis or gastroenteritis were available on 918 patients (65.2%). A summary of the findings from the patient medical charts is provided in the table below.

Summary of Clinical Signs and Symptoms (N=918)

Clinical Signs and Symptoms	# Events Reported (%)	Duration Reported
Diarrhea	807 (87.9%)	1 day to 6 months
Nausea	327 (35.6%)	1 day to 6 months
Vomiting	228 (24.8%)	1 to 30 days
Loss of appetite	179 (19.4%)	1 day to 2 months
Fever	170 (18.5%)	1 day to 2 weeks
Abdominal pain	284 (30.9%)	1 day to 6 months
Tenderness	118 (12.8%)	1 day to 4 months
Cramping	101 (11.0%)	1 day to 4 months
Bloating	62 (6.7%)	1 day to 6 months
Flatulence	50 (5.4%)	1 day to 3 months
Bloody stool	89 (9.7%)	1 day to 4 months
Weakness	159 (17.3%)	1 day to 4 months
Other (e.g. Constipation)	87 (9.5%)	1 to 25 days

All prospective clinical specimens were submitted fresh to the sites and were processed according to their routine algorithm and as ordered by the referring physician. Upon receipt at the laboratory, any left-over stool specimen that met the study inclusion / exclusion criteria was placed into the following six containers.

1. Meridian sterile, leak-proof, wide-mouthed empty container (unpreserved stools)
2. Meridian container containing Cary-Blair holding medium (Para-Pak® C&S)
3. Meridian container containing PVA fixative (Para-Pak® LV-PVA Fixative)
4. Meridian container containing formalin (Para-Pak® 10% Buffered Neutral Formalin)
5. Container containing ACTD medium (swab)
6. Sterile container for xTAG GPP testing (unpreserved stools)

The time from collection to processing into the appropriate containers was kept to a minimum (<24 hours). Prior to study initiation, processing instructions as well as shipping details were provided to each clinical site by the central laboratories carrying out reference and comparator method testing. Specimens were shipped to the central laboratories within 24 hours of processing. Prospective clinical specimens were then processed for both comparator testing and xTAG GPP testing as described below.

For all prospective specimens, reference and comparator method testing was performed at central laboratories independent of xTAG GPP testing sites. Reference/comparator testing was performed for all analytes on all prospectively collected specimens. In the event that comparator results were not available for all targets on a given specimen, then

the specimen in question was excluded from performance calculations of xTAG GPP.

Reference and comparator methods for each analyte target are listed in the table below.

Reference/Comparator Methods and Shipping Requirements

xTAG GPP Analytes	Reference/Comparator Method	Shipping Requirements
Rotavirus A	Composite comparator consisting of Premier Rotaclone EIA (Meridian BioScience k852969) directly on the stool specimen and one PCR/ sequencing assay directly from clinical specimen[1]	Unpreserved stool in sterile tubes
Norovirus	Composite comparator consisting of CDC real-time PCR and conventional PCR followed by bi-directional sequencing assays directly from clinical specimen[1]	Unpreserved stool in sterile tubes
Clostridium difficile Toxin A/B	Bartels Cytotoxicity Assay for *Clostridium difficile* Toxin (Bartels k833447) using diluted stool filtrate processed directly from clinical specimen	Unpreserved stool in sterile tubes
Salmonella	Bacterial culture	Stool in Cary-Blair holding medium
Shigella	Bacterial culture	Stool in Cary-Blair holding medium
Campylobacter	Bacterial culture (A PCR/Sequencing assay was also performed directly on clinical specimens that were tested positive by culture for species identification only)	Stool in Cary- Blair holding medium
E. coli O157	Bacterial culture	Stool in Cary-Blair holding medium
Shiga-Like Toxin Producing *E. coli* (STEC)	Broth enrichment followed by ImmunoCard STAT EHEC (Meridian BioScience, k062546)	Unpreserved stool in sterile tube
Enterotoxigenic *E. coli* (ETEC) LT/ST	Composite comparator consisting of PCR/sequencing directly from clinical specimen using four PCR/sequencing assays, two assays each for the LT and the ST gene[1]	Unpreserved stool in sterile tube
Cryptosporidium	Microscopy	Preserved stool in 10% Formalin

Giardia	Microscopy	Preserved stool in PVA fixative

[1] Refer to more detailed descriptions below.

Performance of the xTAG GPP detecting ETEC-LT and ETEC-ST was compared to a composite comparator method consisted of four separate analytically validated PCR followed by bi-directional sequencing assays (two for ETEC-LT and two for ETEC-ST). "True" ETEC positives were considered as any sample that was tested positive for LT or ST by any of the four PCR/sequencing assays. "True" ETEC negatives were considered as any sample that was tested negative for LT and ST by all four PCR/sequencing assays. PCR/sequencing assays were performed on nucleic acid extracted directly from clinical specimens using primers that targeted different genomic regions from the ones probed by xTAG GPP. Generated sequence results were analyzed as follows:

- For a given base from the consensus sequence generated from bi-directional sequencing, the PHRED score was calculated by averaging the PHRED quality score from the forward and reverse sequencing.
- The generated sequence should be at least 200 bases of an acceptable quality, defined as a minimum of 90% of the total bases with PHRED quality score of 20 or higher.
- Blast analysis of the consensus sequence generated by bi-directional sequencing should have at least 95% query coverage compare to reference, at least 95% identity to reference and an Expected Value (E-Value) [1]of at least 10^{-30}.
- For sequences containing "N"s, the consensus generated using bi-directional sequencing should correspond to the strand including the high quality base instead of the strand including the "N" called base. In addition, the total number of N's should not exceed 5% of total bases (or 10 bases per 200 bp read).

Performance of the xTAG GPP detecting rotavirus was compared to a composite comparator method consisted of an FDA cleared EIA test and one analytically validated PCR followed by bi-directional sequencing assay. "True" rotavirus positives were considered as any sample that was tested positive for rotavirus by the EIA and/or the PCR/sequencing assay. "True" rotavirus negatives were considered as any sample that was tested negative for rotavirus by both the EIA and the PCR/sequencing assay. PCR/sequencing was performed on nucleic acid extracted directly from clinical specimens using primers that targeted different genomic regions from the ones probed by xTAG GPP. Generated sequence results were analyzed described above.

Performance of the xTAG GPP for norovirus was assessed by comparing test results to the "patient norovirus infected status" of each specimen. The "patient norovirus infected

[1] 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. 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).

status" was determined using a composite comparator method consisting of the CDC norovirus real-time Taqman RT-PCR assay and the CDC Conventional RT-PCR (Region-C and D primers) followed by bi-directional sequencing assays. The following interpretation algorithm was used to determine the "patient norovirus infected status":

Composite Comparator Algorithm for Norovirus

Clinical runs and re-runs (per the instructions provided in the product package insert)

CDC Norovirus Real- Time Taqman RT-PCR Result	CDC Conventional RT-PCR Result (Region C) Followed by Bi-Directional Sequencing	CDC Conventional RT-PCR Result (Region D) Followed by Bi-Directional Sequencing	Final Composite Comparator Result
Positive	Positive	N/A	Positive
Negative	Positive	N/A	Positive
Positive	Negative	Positive	Positive
Positive	Negative	Negative	Negative
Negative	Negative	N/A	Negative

using xTAG GPP were carried out on left-over clinical specimens that had been extracted from the fresh or frozen state using the NucliSENS EasyMAG method (BioMérieux, Inc., Durham, NC) according to the manufacturer's instructions. Total extracted nucleic acid material was stored at -70^{0}C prior to testing with xTAG GPP.

PCR negative (water blanks, NTC) control and external rotating positive controls (RC) representing analytes probed by the assay were also included with each xTAG GPP run. The external positive controls used in the study are listed in the table below and, for the most part (except for *Cryptosporidium*), consisted of chemically-inactivated bacteria, viruses and parasites from ZeptoMetrix. These controls were used to control the entire assay process including nucleic acid extraction, amplification, and detection. The external positive controls contained low organism copy numbers and were designed to mimic patient specimens. These were run as separate samples, concurrently with patient specimens. External positive controls were included in each assay plate in a rotating manner.

External Positive Controls

External Positive Control	Source	Dilution Factor
Campylobacter	Natrol (ZeptoMetrix)	Stock*
C difficile Toxin A/B	Natrol (ZeptoMetrix)	1/100
Cryptosporidium	Pooled clinical specimens	Stock**
E. coli 0157 / STEC	Natrol (ZeptoMetrix)	1/100
ETEC	Natrol (ZeptoMetrix)	1/10
Giardia	PRA-243 (ATCC)	Stock
Norovirus GI	Natrol (ZeptoMetrix)	1/100
Norovirus GII	Natrol (ZeptoMetrix)	1/1000
Rotavirus	Natrol (ZeptoMetrix)	1/10
Salmonella	Natrol (ZeptoMetrix)	1/10
Shigella	Natrol (ZeptoMetrix)	1/1000

* Stock material was used as MFI signals generated for *campylobacter* in the initial clinical runs using1/10

dilution of the stock were too close to the assay cut-off.
** Pooled clinical specimens positive for *Crytopsoridium hominis* were used as positive control for this target. MFI values generated were however close to the assay cut-off and, in a number of clinical runs were below the threshold for a positive call.

Clinical specimens were tested in accordance with the package insert for xTAG GPP assay and were tested by a single operator at each of the clinical sites.

The xTAG GPP assay includes an internal control (MS2 bacteriophage) that is added to each sample prior to extraction. In the event that none of the pathogen targets probed by xTAG GPP were detected in a clinical specimen and the MS2 call in that specimen was "Absent", a 1/10 dilution of the nucleic acid remnant (from the original extraction) was prepared and tested by xTAG GPP. Two outcomes of running a 1/10 dilution were addressed in the following manner:

- If the MS2 call was "Present" following a 1/10 dilution of the original extract, it is likely that the original result was due to PCR inhibition. All additional positive results generated in this scenario were reported as "Positive" in the calculation of sensitivity and specificity (or positive and negative agreement). Negative results generated in this scenario were reported as "inhibited" and excluded from the calculation of sensitivity and specificity (or positive and negative agreement) for the targets in question. However, inhibited results are presented in the performance tables as "invalid" for each microbial target.
- If the MS2 signal was "Absent" following a 1/10 dilution of the original extract and none of the pathogen targets were detected, then the sample was re-tested with xTAG GPP, starting from the extraction step. If MS2 signal was "Present" after re-testing from the extraction step, it is likely that the original result was due to sub-optimal extraction. Negative and positive results generated in this allowable re-run were included in the calculation of sensitivity and specificity (or positive / negative agreement) for each individual target. If MS2 signal was still "Absent" after re-testing from the extraction step and none of the pathogen targets were detected, then the sample was coded as "inhibited" and was excluded from the calculation of sensitivity and specificity (or positive and negative agreement) for the targets in question. However, inhibited results are presented in the performance tables as "invalid" for each microbial target.

In the event that an unexpected positive call was made in any of the assay controls included in the xTAG GPP run (negative or external positive control), then all clinical specimens that tested positive for the analyte(s) in question were re-tested by xTAG GPP. Negative and positive results generated in this allowable re-run were included in the calculation of sensitivity and specificity (or positive and negative percent agreements) for each individual target.

Discrepant results between the xTAG GPP and the reference methods were also evaluated using analytically validated PCR/sequencing assays or FDA cleared molecular assays (i.e., for *C. difficile* Toxin), and results are footnoted in the performance tables below.

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

Campylobacter

xTAG GPP	Reference			
	Positive	Negative	Invalid	TOTAL
Positive	3	21[2]	0	24
Negative	0	1155	0	1155
Invalid	0	228	0	228
TOTAL	3[1]	1404	0	1407
		95% CI		
Sensitivity	100%	43.8% - 100%		
Specificity	98.2%	97.3% - 98.8%		
Invalid Rate due to PCR Inhibition	16.2%			

[1]Sequencing results from these specimens revealed that all three were *campylobacter jejuni*.
[2]A total of six *Campylobacter* xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

***Clostridium difficile* Toxin A/B**

xTAG GPP	Comparator			
	Positive	Negative	Invalid	TOTAL
Positive	107	105[1]	8	220[3]
Negative	7	922	62	991
Invalid	1	170	25	196
TOTAL	115	1197	95[2]	1407
		95% CI		
Positive Percent Agreement	93.9%	87.9% - 97.0%		
Negative Percent Agreement	89.8%	87.8% - 91.5%		
Invalid Rate due to PCR Inhibition	13.9%			

[1]A total of 48 *C. difficile* Toxin A/B xTAG GPP positive specimens that were negative by the comparator method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP, or FDA cleared *C. difficile* Toxin molecular assays.
[2]A total of 95 specimens generated a "Nonspecific reaction, not characteristic of *Clostridium difficile* toxin". A titration test was performed on all 95 specimens and it was determined that in each case, the cytotoxicity reaction was not typical of *C. difficile* toxin. This finding is consistent with the expected values for invalid results noted in the package insert of the Bartels Cytotoxicity Assay for *Clostridium difficile* Toxin.
[3]A total of 151 (151/220, 68.7%) *C. difficile* Toxin A/B xTAG GPP positive specimens were positive for both the Toxin A and B gene targets by the xTAG GPP Test. A total of 57 (57/220, 25.9%) *C. difficile* Toxin A/B xTAG GPP positive specimens were positive for the Toxin B target and 12 (12/220, 5.4%) were positive for the Toxin A target.

Cryptosporidium

xTAG GPP	Reference			
	Positive	Negative	Invalid	TOTAL
Positive	12	53[2]	0	65
Negative	1	1131	0	1132
Invalid	0	210	0	210
TOTAL	13[1]	1394	0	1407
		95% CI		

Sensitivity	92.3%	66.7% - 98.6%		
Specificity	95.5%	94.2% - 96.6%		
Invalid Rate due to PCR Inhibition	14.9%			

[1]All 13 *Cryptosporidium* reference positive specimens were collected during a single outbreak which occurred at Site 2 and were typed as *Cryptosporidium hominis.*

[2]A total of eight *Crytosporidium* xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

E. coli O157

xTAG GPP	**Reference**			
	Positive	Negative	Invalid	TOTAL
Positive	2	9[1]	0	11
Negative	0	1158	0	1158
Invalid	0	238	0	238
TOTAL	2[2]	1405	0	1407
		95% CI		
Sensitivity	100%	34.2% - 100%		
Specificity	99.2%	98.5% - 99.6%		
Invalid Rate due to PCR Inhibition	16.9%			

[1]A total of four *E. coli* O157 xTAG GPP positive specimens that were negative by the comparator method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

[2] Both reference positive *E. coli* 0157 specimens were also positive for STEC by xTAG GPP. Only one was positive for STEC by the reference culture and EIA.

ETEC

xTAG GPP	**Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	2[1]	4	0	6
Negative	6[2]	1156	0	1162
Invalid	1	238	0	239
TOTAL	9	1398	0	1407
		95% CI		
Positive Percent Agreement	25.0%	7.1% - 59.1%		
Negative Percent Agreement	99.7%	99.1% - 99.9%		
Invalid Rate due to PCR Inhibition	17.0%			

[1] One sample was positive for LT by both ETEC-LT PCR/sequencing assays. The other sample was positive for ST by both ETEC-ST PCR/sequencing assays.

[2] ETEC performance were calculated against a composite comparator consisting of four well-characterized PCR/bi-directional sequencing assays, two ETEC-LT PCR/sequencing assays and two ETEC-ST PCR/sequencing assays. All six specimens were positive by only one of the four PCR/sequencing assays.

Giardia

xTAG GPP	**Reference**			
	Positive	Negative	Invalid	TOTAL
Positive	4	39	0	43
Negative	0	1132	0	1132
Invalid	0	232	0	232

TOTAL	4	1403	0	1407
		95% CI		
Sensitivity	100%	51.0% - 100%		
Specificity	96.7%	95.5% - 97.6%		
Invalid Rate due to PCR Inhibition	16.5%			

Norovirus GI/GII

xTAG GPP	**Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	74	96	0	170
Negative	4[1]	1023	0	1027
Invalid	0	210	0	210
TOTAL	78[2]	1329	0	1407
		95% CI		
Positive Percent Agreement	94.9%	87.5% - 98.0%		
Negative Percent Agreement	91.4%	89.6% - 92.9%		
Invalid Rate due to PCR Inhibition	14.9%			

[1] All four xTAG GPP false negative Norovirus specimens were Norovirus GII.
[2] Five of the 78 Norovirus comparator positive specimens were typed as GI at the CDC by sequencing, and 73 of the 78 Norovirus comparator positive specimens were typed as GII at the CDC by sequencing.

Rotavirus A

xTAG GPP	**Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	2	2	0	4
Negative	0	1162	0	1162
Invalid	0	241	0	241
TOTAL	2	1405	0	1407
		95% CI		
Positive Percent Agreement	100%	34.2% - 100%		
Negative Percent Agreement	99.8%	99.4% - 100%		
Invalid Rate due to PCR Inhibition	17.1%			

Salmonella

xTAG GPP	**Reference**			
	Positive	Negative	Invalid	TOTAL
Positive	10	18[2]	0	28
Negative	0	1143	0	1143
Invalid	0	236	0	236
TOTAL	10[1]	1397	0	1407
		95% CI		
Sensitivity	100%	72.2% - 100%		
Specificity	98.4%	97.6% - 99.0%		
Invalid Rate due to PCR Inhibition	16.8%			

[1] Cultured isolates from all 10 *salmonella* reference positive clinical specimens were typed at the Ontario Public Health Laboratory in Toronto. Three specimens were typed as *Salmonella enterica* subsp. *enterica*,

Typhimurium; one specimen as *Salmonella enterica* subsp. *enterica*, Typhi; one specimen as *Salmonella enterica* subsp. *enterica*, Salamae; one specimen as *Salmonella enterica* subsp. *enterica*, Javiana; one specimen as *Salmonella enterica* subsp. *enterica*, Bredeney; one specimen as *Salmonella enterica* subsp. *enterica*, Mississippi; one specimen as *Salmonella enterica* subsp. *enterica*, Heidelberg; one specimen as *Salmonella enterica* subsp. *enterica*, Muenchen.

[2]A total of two *salmonella* xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

Shiga-Like Toxin Producing *E. coli* (STEC) *stx*1/*stx*2

xTAG GPP	Reference			
	Positive	Negative	Invalid	TOTAL
Positive	1	16[2]	0	17
Negative	0	1153	0	1153
Invalid	0	237	0	237
TOTAL	1[1]	1406	0	1407
		95% CI		
Sensitivity	100%	20.7% - 100%		
Specificity	98.6%	97.8% - 99.2%		
Invalid Rate due to PCR Inhibition	16.9%			

[1] This STEC reference positive specimen was typed a Shiga-like toxin 2 using the ImmunoCard STAT EHEC.

[2]A total of one STEC xTAG GPP positive specimen that was negative by the reference method was confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

Shigella

xTAG GPP	Reference			
	Positive	Negative	Invalid	TOTAL
Positive	2	17[2]	0	19
Negative	0	1154	0	1154
Invalid	0	234	0	234
TOTAL	2[1]	1405	0	1407
		95% CI		
Sensitivity	100%	34.2% - 100%		
Specificity	98.5%	97.7% - 99.1%		
Invalid Rate due to PCR Inhibition	16.6%			

[1] Two clinical specimens tested positive for *shigella* by bacterial culture; one was reported as *Shigella flexneri* while the other one was reported as *Shigella sonnei*.

[2]A total of two *shigella* xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

The prospective performance data (all sites combined) are presented in the following table by organism:

Organism	Sensitivity		95% CI	Specificity		95% CI
Campylobacter	3/3	100%	43.8% - 100%	1155/1176[1]	98.2%	97.3% - 98.8%
Cryptosporidium	12/13	92.3%	66.7% - 98.6%	1131/1184[2]	95.5%	94.2% - 96.6%
E. coli O157	2/2	100%	34.2% - 100%	1158/1167[3]	99.2%	98.5% - 99.6%
Giardia	4/4	100%	51.0% - 100%	1132/1171	96.7%	95.5% - 97.6%
Salmonella	10/10	100%	72.2% - 100%	1143/1161[4]	98.4%	97.6% - 99.0%

STEC	1/1	100%	20.7% - 100%	1153/1169[5]	98.6%	97.8% - 99.2%
Shigella	2/2	100%	34.2% - 100%	1154/1171[6]	98.5%	97.7% - 99.1%
Organism	**Positive Percent Agreement**		**95% CI**	**Negative Percent Agreement**		**95% CI**
C. difficile Toxin A/B	107/114	93.9%	87.9% - 97.0%	922/1027[7]	89.8%	87.8% - 91.5%
ETEC	2/8	25.0%	7.1% - 59.1%	1156/1160	99.7%	99.1% - 99.9%
Norovirus GI/GII	74/78	94.9%	87.5% - 98.0%	1023/1119	91.4%	89.6% - 92.9%
Rotavirus A	2/2	100%	34.2% - 100%	1162/1164	99.8%	99.4% - 100%

[1] A total of six *Campylobacter* xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

[2] A total of eight *Crytosporidium* xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

[3] A total of four *E. coli* O157 xTAG GPP positive specimens that were negative by the comparator method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

[4] A total of two *Salmonella* xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

[5] A total of one STEC xTAG GPP positive specimen that was negative by the reference method was confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

[6] A total of two *Shigella* xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

[7] A total of 48 *C. difficile* Toxin A/B xTAG GPP positive specimens that were negative by the comparator method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP, or FDA cleared *C. difficile* Toxin molecular assay.

Prospective Clinical Study Mixed Infection Analysis

xTAG GPP detected a total of 91 mixed infections in the prospective clinical evaluation. This represents 18.7% of the total number of xTAG GPP positive specimens (91/486). (62/91; 68.1%) were double infections, 21 (21/91; 23.1%) were triple infections, four (4/91; 4.4%) were quadruple infections, two (2/91; 2.2%) were quintuple infections, one (1/91; 1.1%) was sextuple infection and one was septuple infection (1/91; 1.1%). The single most common co-infections (24/91; 26.4%) was Norovirus GI/GII with *C. difficile* Toxin A/B. Out of the 91 co-infections, 86 contained one or more analytes that had not been detected with the reference/comparator methods, i.e. discrepant co-infections. Distinct co-infection combinations detected by xTAG GPP in the prospective clinical study are summarized in the table below.

Distinct Co-infection Combinations Detected by the xTAG GPP in the Prospective Clinical Trial

Distinct Co-infection Combinations Detected by xTAG GPP							**Total Co-infections**	**Number of Discrepant Co-infections [a]**	**Discrepant Analyte(s) [a]**
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**			
Campyl.	*Crypto.*						1	1	All
Campyl.	*Giardia*						2	2	*Campyl.* (x2); *Giardia* (x1);
C. diff.	*Crypto.*						3	3	All
C. diff.	*E. coli* O157	STEC					1	1	All

C. diff.	*Giardia*						2	2	All
C. diff.	STEC						1	1	STEC (x1);
C. diff.	STEC	*Crypto.*					2	2	All
C. diff.	ETEC						1	1	*C. diff.* (x1);
E. coli O157	STEC						2	1	*E coli* O157 (x1); STEC (x1);
Giardia	*Crypto.*						1	1	All
Norovirus	*Campyl.*	*C. diff.*					1	1	*Campyl.* (x1); *C diff.* (x1);
Norovirus	*Campyl.*	*C. diff.*	*Crypto.*				2	2	All
Norovirus	*Campyl.*	*C. diff.*	*Crypto.*	STEC			2	2	All
Norovirus	*Campyl.*	*Crypto.*					4	4	All
Norovirus	*Campyl.*	*Giardia*					1	1	Norovirus (x1); *Giardia* (x1);
Norovirus	*C. diff.*						24	20	Norovirus (x15); *C diff.* (x12);
Norovirus	*C. diff.*	*E. coli* O157	*Giardia*				1	1	All
Norovirus	*Crypto.*						9	9	Norovirus (x9); *Crypt.* (x6);
Norovirus	*Giardia*	ETEC					1	1	ETEC (x1); *Giardia* (x1);
Norovirus	*Giardia*						6	6	Norovirus (x3); *Giardia* (x6);
Norovirus	*E. coli* O157	STEC					1	1	STEC (x1);
Norovirus	*Giardia*	*Crypto.*					2	2	All
Norovirus	*Crypto.*	STEC					1	1	All
Norovirus	*Giardia*	STEC					1	1	STEC (x1); *Giardia* (x1);
Norovirus	*Salmonella*	*Shigella*					1	1	Norovirus (x1); *Shigella* (x1);
Norovirus	*Shigella*	*C. diff.*					1	1	Norovirus (x1); *Shigella* (x1);
Norovirus	*Shigella*	*C. diff.*	*Campyl.*	STEC	*Crypto.*	ETEC	1	1	Norovirus (x1); *Shigella* (x1); *Campyl.* (x1); ETEC (x1); STEC (x1); *Crypto.* (x1)
Norovirus	*Shigella*	*Campyl.*	*E. coli* O157	*Crypto.*	ETEC		1	1	*Shigella* (x1); *Campyl.* (x1); ETEC (x1); *E coli* O157 (x1); *Crypto.* (x1);
Rotavirus	*C. diff.*						1	1	All
Rotavirus	Norovirus	*Giardia*					1	1	All
STEC	*Crypto.*						1	1	All
Salmonella	*C. diff.*						2	2	*Salmonella* (x2); *C diff.* (x1);
Salmonella	*C. diff.*	*E. coli* O157					1	1	*C diff.* (x1); *E coli* O157 (x1);
Salmonella	*C. diff.*	STEC	*Crypto.*				1	1	All
Salmonella	*Crypto.*						2	2	All
Salmonella	*C. diff.*	*Crypto.*					1	1	All
Salmonella	*Shigella*						1	1	All
Salmonella	*Giardia*						1	1	All
Salmonella	STEC						1	1	STEC (x1);
Salmonella	*Shigella*	*Giardia*					1	1	All
Shigella	*Giardia*						1	1	All

Total Co-infections	91	86	
Total Double Infections	62	57	
Total Triple Infections	21	21	
Total Quadruple infections	4	4	
Total Quintuple infections	2	2	
Total Number of sextuple infections	1	1	
Total Number of septuplet infections	1	1	

[a] A discrepant co-infection or discrepant analyte was defined as one that was detected by the xTAG GPP but not detected by the reference/comparator methods.

[b] One Norovirus /*C. difficile* Tox A/B, one Norovirus /*E. coli* 0157/STEC and one *Salmonella/C.difficile* Tox A/B xTAG GPP reported co-infected specimens that were negative by the reference method were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the xTAG GPP

Additional Distinct Co-infection Combinations Detected by the Reference/Comparator Methods, But Not Detected by the xTAG GPP in the Prospective Clinical Trial

Distinct Co-infection Combinations [a]		**Total Co-infections**	**Number of Discrepant Co-infections**	**Discrepant Analyte(s)** [b]
Analyte 1	**Analyte 2**			
Norovirus	*C. diff.*	1	1	*C. diff.*
Norovirus	ETEC	2	2	ETEC (x2)

[a] This table includes only distinct co-infections that were detected by the reference/comparator method but not by the xTAG GPP; the remaining co-infections detected by the reference methods are already represented in the table above.

[b] Discrepant analyte is defined as one that is detected by the reference/comparator but not detected by the xTAG GPP.

Of the 1407 clinical specimens included in the data analysis, 91 (6.5%) were identified as positive for more than one target by xTAG GPP. In most cases, bacteria presented with viruses (N=29, 31.9%), followed by bacteria + parasites (N=18, 19.8%), viruses + parasites (N=18, 19.8%), bacteria + viruses + parasites (N=15, 16.5%), bacteria + bacteria (N=10, 11.0%), and parasite + parasite (N=1, 1.1%). All enteric pathogens probed by xTAG GPP were implicated in co-infections. Results for co- infections are summarized in the table below.

Prevalence of Individual Analytes in Mixed Infections Detected by the xTAG GPP during the Prospective Clinical Study

Target	**Number Implicated in Co-Infections**	**Percent of Total Co-Infected Specimens (N=91)**
Campylobacter	15	16.5%
C. difficile	48	52.7%
Cryptosporidium	34	37.4%
E. coli 0157	7	7.7%
ETEC	4	4.4%
Giardia	21	23.1%
Norovirus GI/GII	61	67.0%

Rotavirus	2	2.2%
Salmonella	12	13.2%
Shigella	7	7.7%
STEC	15	16.3%

Prospective Clinical Study Per Specimen/Patient Summary Results

Prospective study results were also analyzed on a per sample/patient basis. Results of this analysis are summarized in the table below both without taking into consideration the discrepant analysis by PCR/bi-directional sequencing or FDA cleared molecular assays (Primary Reference/Comparator) and taking into consideration this discrepant analysis (After Discrepant Investigation).

Per Sample/Patient Summary Results – Prospective Sample Set (N=1407)

Analyses	Primary Reference/Comparator	After Discrepant Investigation
# Specimens with at least one pathogen positive by xTAG GPP	486	486
# Specimens with at least one pathogen positive by xTAG GPP and confirmed by reference/comparator	217	286
# Specimens with at least one pathogen positive by xTAG GPP but none confirmed by reference/comparator	269	200
# Specimens with at least one pathogen positive by reference/comparator but none was positive by xTAG GPP	17	17

Prospective Clinical Study Contaminated Runs

Unexpected positive call(s) in negative (NTC) or external rotating positive control(s) (RC) were reported in 10 out of 49 xTAG GPP runs (10/49, 20.4%) during the prospective clinical study. A total of 49 clinical specimens included in these contaminated runs tested positive for analytes that were unexpectedly present in assay controls (49/1407; 3.8%).

Retrospective Clinical Study 1 - Pre-Selected Clinical Specimens

Due to low prevalence observed for most of the xTAG GPP analytes in the prospective clinical study (see above), xTAG GPP performance detecting the following microbial targets was further evaluated in a retrospective clinical study testing pre-selected clinical specimens.

Campylobacter (*C. jejuni*, *C. coli* and *C. lari* only)
Cryptosporidium (*C. parvum* and *C. hominis* only)
E. coli O157
Enterotoxigenic *E. coli* (ETEC) LT/ST
Giardia
Rotavirus A

Salmonella
Shiga-like toxin producing *E. coli* (STEC) *stx1/stx2*
Shigella

Pre-selected stool specimens were collected at multiple sites in North America and Europe. Demographic information (age and gender) was collected on all pre-selected specimens for which these data were available and is summarized in the table below.

General Demographic Details for the Pre-Selected Data Set (N=203)

Sex	**Number of Subjects**
Male	106 (52.2%)
Female	83 (40.9%)
Not known	14 (6.9%)
Total	203
Age (yrs)	
0 - 1	36 (17.7%)
>1 - 5	25 (12.3%)
>5 – 12	13 (6.4%)
>12 - 21	11 (5.4%)
>21 - 65	90 (44.3%)
>65	14 (6.0%)
Not known	14 (6.9%)
Total	203

The table below outlines the number of pre-selected positive specimens included in the retrospective clinical study for each analyte target as well as the characterization method used.

Pre-selected Specimen Information (N=203)

Pre-selected Target	**# Specimens Included**	**Characterization Method (Comparator)**
Campylobacter	41	Bacterial culture
Cryptosporidium	13 (9 *Cryptosporidium parvum and* 4 *Cryptosporidium hominis)*	FDA cleared DFA or microscopy
E. coli O157	8[1]	Bacterial culture
ETEC	39	PCR/sequencing directly from clinical specimen using four PCR/sequencing assays (two for LT and two for ST)
Giardia	17	FDA cleared DFA or microscopy
Rotavirus A	28	FDA cleared EIA or PCR followed by bi-directional sequencing using the same analytically validated primers as those used in the Prospective Clinical Study
Salmonella	27	Bacterial culture
STEC	10[2]	FDA cleared EIA

Shigella	20	Bacterial culture

[1] All eight *E. coli* 0157 clinical specimens were also assessed by PCR followed by bi-directional sequencing for STEC.
[2] All 10 STEC clinical specimens were also assessed by PCR followed by bi-directional sequencing for *E. coli* 0157.

These pre-selected positive specimens were tested with xTAG GPP at three clinical sites along with 277 "negative" clinical specimens in a randomized, blinded fashion. The "negative" designation for these 277 specimens was based on the routine algorithms used at the clinical site (e.g. bacterial culture, EIA, microscopy, in-house real time PCR). These algorithms did not test for all pathogen targets probed by xTAG GPP.

The table below summarizes the positive percent agreement between comparator and xTAG GPP for all pre-selected targets evaluated.

Positive Percent Agreement of xTAG GPP in the Pre-Selected Data Set

Analyte	Positive Percent Agreement		95%CI for Positive Percent Agreement	Number of "Invalid" xTAG GPP Results
	TP / (TP+FN)	percent		
Campylobacter	40/41	97.6%	87.4% - 99.6%	0
Cryptosporidium	12/12	100%	75.7% - 100%	1
E. coli O157 [1]	14/14	100%	78.5% - 100%	0
ETEC	38/39	97.4%	86.8% - 99.5%	0
Giardia	15/16	93.7%	71.7% - 98.9%	1
Rotavirus A	28/28	100%	87.9% - 100%	0
Salmonella	24/27	88.9%	71.9% - 96.1%	0
STEC [2]	18/18	100%	82.4% - 100%	0
Shigella	20/20	100%	83.9% - 100%	0

[1] Eight (8)/8 *E. coli* 0157 were also positive for STEC by xTAG GPP. Sample remnants of all 8 *E. coli* 0157 specimens were tested for the presence of *stx1* and *stx 2* genes by bi-directional sequencing and the results added to those obtained for STEC.
[2] Six (6)/10 STEC were also positive for *E. coli* 0157 by xTAG GPP. Sample remnants of all 10 STEC specimens were assessed by bi-directional sequencing for *E.* coli 0157 and the results added to those obtained for *E. coli* 0157.

Nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was also performed on all available pre-selected clinical specimens that were positive by xTAG GPP for other analytes. More specifically, confirmatory testing was performed for those analytes that were positive by xTAG GPP but not pre-selected at the banking site in order to determine whether these additional positive calls represented True Positive (TP) or False Positive (FP) clinical results. To the extent possible, sequencing primers targeted genomic regions distinct from those of the kit primers. xTAG GPP generated 98 additional positive calls (after allowable re-runs) for analytes that were not pre-selected at the banking site. A summary of these additional calls and confirmatory testing results are provided in the tables below.

Campylobacter

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	3	1	0	4
Negative	NA	NA	369	369
Invalid	NA	NA	66	66
TOTAL	3	1	435	439*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	75.0%			
Invalid Rate due to PCR Inhibition (N=480)	13.7%			

*41 specimens were pre-selected for *Campylobacter*. Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

C. Difficile Toxin A/B

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	16	9	0	25[1]
Negative	NA	NA	394	394
Invalid	NA	NA	61	61
TOTAL	16	9	455	480
Confirmed xTAG GPP Positives/All xTAG GPP Positives	64.0%			
Invalid Rate due to PCR Inhibition (N=480)	12.7%			

[1]A total of 17 (17/25, 68.0%) *C. difficile* Toxin A/B xTAG GPP positive specimens were positive for both the Toxin A and B gene targets by the xTAG GPP Test. A total of 7 (7/25, 28.0%) *C. difficile* Toxin A/B xTAG GPP positive specimens were positive for the Toxin B target and 1 (1/25, 4.0%) were positive for the Toxin A target.

Cryptosporidium

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	1	0	0	1
Negative	NA	NA	401	401
Invalid	NA	NA	65	65
TOTAL	1	0	466	467*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	100%			
Invalid Rate due to PCR Inhibition (N=480)	13.5%			

*13 specimens were pre-selected for *Cryptosporidium.* Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

E. coli o157

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL

Positive	1	0	1	2
Negative	NA	NA	397	397
Invalid	NA	NA	67	67
TOTAL	1	0	465	466*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	50%			
Invalid Rate due to PCR Inhibition (N=480)	13.9%			

*14 specimens were pre-selected for *E. coli* O157. Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

ETEC

xTAG GPP	**PCR/Bi-directional Sequencing**			
	Positive	Negative	Not Done	TOTAL
Positive	4	4	0	8
Negative	NA	NA	369	369
Invalid	NA	NA	64	64
TOTAL	4	4	433	441*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	50%			
Invalid Rate due to PCR Inhibition (N=480)	13.3%			

*39 specimens were pre-selected for ETEC. Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

Giardia

xTAG GPP	**PCR/Bi-directional Sequencing**			
	Positive	Negative	Not Done	TOTAL
Positive	0	5	0	5
Negative	NA	NA	395	395
Invalid	NA	NA	63	63
TOTAL	0	5	458	463*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	0%			
Invalid Rate due to PCR Inhibition (N=480)	13.1%			

*17 specimens were pre-selected for *Giardia*. Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

Norovirus

xTAG GPP	**PCR/Bi-directional Sequencing**			
	Positive	Negative	Not Done	TOTAL
Positive	2	7	8	17
Negative	NA	NA	396	396
Invalid	NA	NA	67	67

TOTAL	2	7	471	480
Confirmed xTAG GPP Positives/All xTAG GPP Positives	11.8%			
Invalid Rate due to PCR Inhibition (N=480)	13.9%			

Rotavirus

xTAG GPP	**PCR/Bi-directional Sequencing**			
	Positive	Negative	Not Done	TOTAL
Positive	6	0	0	6
Negative	NA	NA	379	379
Invalid	NA	NA	67	67
TOTAL	6	0	446	452*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	100%			
Invalid Rate due to PCR Inhibition (N=480)	13.9%			

*28 specimens were pre-selected for Rotavirus. Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

Salmonella

xTAG GPP	**PCR/Bi-directional Sequencing**			
	Positive	Negative	Not Done	TOTAL
Positive	4	6	0	10
Negative	NA	NA	382	382
Invalid	NA	NA	61	61
TOTAL	4	6	443	453*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	40.0%			
Invalid Rate due to PCR Inhibition (N=480)	12.7%			

*27 specimens were pre-selected for *Salmonella*. Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

STEC

xTAG GPP	**PCR/Bi-directional Sequencing**			
	Positive	Negative	Not Done	TOTAL
Positive	3	3	0	6
Negative	NA	NA	390	390
Invalid	NA	NA	66	66
TOTAL	3	3	456	462*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	50.0%			

Invalid Rate due to PCR Inhibition (N=480)	13.7%			

*18 specimens were pre-selected for STEC. Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

Shigella

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	11	2	1	14
Negative	NA	NA	379	379
Invalid	NA	NA	67	67
TOTAL	11	2	447	460*
Confirmed xTAG GPP Positives/All xTAG GPP Positives	78.6%			
Invalid Rate due to PCR Inhibition (N=480)	13.9%			

*20 specimens were pre-selected for *Shigella*. Results are presented in the "Positive Percent Agreement of xTAG GPP in the Pre−Selected Data Set" table.

Retrospective Clinical Study 1 (Pre-Selected Clinical Specimens) Contaminated Runs

Unexpected positive call(s) in negative (NTC) or external rotating positive control(s) (RC) were reported in three out of 15 pre-selected xTAG GPP runs (3/15, 20.0%). A total of 21 clinical specimens included in these runs tested positive by xTAG GPP for analytes that were unexpectedly present in assay controls (21/480; 4.4%).

Supplemental Clinical Study – Botswana Pediatric Stool Specimens

The clinical performance of xTAG GPP for Rotavirus, ETEC, *Cryptosporidium* and *Gardia* was also evaluated in a set of pediatric stool specimens (N=313) prospectively collected between February 2011 and January 2012 from symptomatic pediatric patients admitted to two referral hospitals in Botswana, Africa. All pediatric patients included in this evaluation presented with diarrhea and/or vomiting. General demographic details for these patients are summarized in the table below.

General demographic details of Botswana Sample Set (N=313)

Sex	Number of Subjects
Male	186 (59.4%)
Female	127(40.6%)
Total	313
Age (yrs)	
< 1	231 (73.8%)
1	62 (19.8%)
2	11 (3.5%)
3	3 (0.9%)
4	3 (0.9%)

> 4	3 (0.9%)
Total	313

All specimens were shipped frozen to one of the study sites in Ontario, Canada for xTAG GPP testing. Stools were extracted by the Biomerieux NucliSENS EasyMag and tested using the xTAG GPP per the instructions provided in the product package insert.

Comparator testing by nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was performed on samples positive for Rotavirus, ETEC, *Cryptosporidium* and *Giardia* by xTAG GPP. In order to minimize bias, a random subset of the 313 Botswana specimens that tested negative by xTAG GPP was also assessed by the same nucleic acid amplification followed by bi-directional sequencing method for Rotavirus, ETEC, *Cryptosporidium* and *Giardia*. In the case of *Cryptosporidium* and *Giardia*, the number of xTAG GPP negative specimens assessed was equal to or greater than the number of specimens identified as positive by xTAG GPP. In the case of ETEC, the number of xTAG GPP negative specimens assessed was slightly less than the number of specimens identified as positive by xTAG GPP. Since 178 of 313 specimens tested positive by xTAG GPP for Rotavirus, the number of negative Rotavirus specimens tested by nucleic acid amplification followed by sequencing was less than the number of positive Rotavirus specimens tested by this comparator method. Comparator testing by nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was performed on a total of 308, 56, 24, and 20 specimens for Rotavirus, ETEC, *Cryptosporidium*, and *Giardia*, respectively.

The Botswana Study performance data are presented in the following tables by analyte:

Rotavirus A

xTAG GPP	**Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	175	3	0	178
Negative	18	108	0	126
Invalid	0	4	0	4
TOTAL	193	115	0	308
		95% CI		
Positive Percent Agreement	90.7%	85.7% - 94.0%		
Negative Percent Agreement	97.3%	92.4% - 99.1%		
Invalid Rate due to PCR Inhibition [1]	1.3%			

[1] Four out of a total of 313 samples tested by the xTAG GPP generated an "invalid" result for Rotavirus A.

ETEC

xTAG GPP	**Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	26	3	0	29
Negative	1	26	0	27
Invalid	0	0	0	0
TOTAL	27	29	0	56
		95% CI		
Positive Percent Agreement	96.3%	81.7% – 99.3%		

Negative Percent Agreement	89.7%	73.6% – 96.4%		
Invalid Rate due to PCR Inhibition [1]	1.6%			

[1] Five out of a total of 313 samples tested by the xTAG GPP generated an "invalid" result for ETEC

Cryptosporidium

xTAG GPP	**Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	11	0	0	11
Negative	1	12	0	13
Invalid	0	0	0	0
TOTAL	12	12	0	24
		95% CI		
Positive Percent Agreement	91.7%	64.6% – 98.5%		
Negative Percent Agreement	100%	75.7% – 100%		
Invalid Rate due to PCR Inhibition [1]	1.6%			

[1] Five out of a total of 313 samples tested by the xTAG GPP generated an "invalid" result for *Cryptosporidium*.

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Giardia

xTAG GPP	**Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	9	1	0	10
Negative	0	10	0	10
Invalid	0	0	0	0
TOTAL	9	11	0	20
		95% CI		
Positive Percent Agreement	100%	70.1% - 100%		
Negative Percent Agreement	90.9%	62.3% – 98.4%		
Invalid Rate due to PCR Inhibition [1]	1.6%			

[1] Five out of a total of 313 samples tested by the xTAG GPP generated an "invalid" result for *Giardia.*

The table below summarizes the positive and negative agreement (PPA and NPA) between PCR/bi-directional sequencing results and xTAG GPP for Rotavirus, *Cryptosporidium* and *Giardia*.

Organism	**PPA**		**95% CI**	**NPA**		**95% CI**
Rotavirus A	175/193	90.7%	85.7% - 94.0%	108/111	97.3%	92.4% - 99.1%
ETEC	26/27	96.3%	81.7% – 99.3%	26/29	89.7%	73.6% – 96.4%
Cryptosporidium	11/12	91.7%	64.6% – 98.5%	12/12	100%	75.7% – 100%
Giardia	9/9	100%	70.1% - 100%	10/11	90.9%	62.3% – 98.4%

Nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was also performed on all available clinical specimens that were positive by xTAG GPP for other analytes (i.e., *Campylobacter*, *C. difficile* Toxin A/B, *E. coli* O157, Norovirus, *Salmonella*, *Shigella*, and STEC) in order to determine whether these additional positive calls represented True Positive (TP) or False Positive (FP) clinical results. The tables below summarize the confirmed xTAG GPP positive rate (i.e., confirmed xTAG GPP positives/all xTAG GPP positives) by PCR/bi-directional

sequencing for *Campylobacter*, *C. difficile* Toxin A/B, *E. coli* O157, Norovirus, *Salmonella*, *Shigella*, and STEC.

Campylobacter

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	47	1	1	49
Negative	NA	NA	258	258
Invalid	NA	NA	6	6
TOTAL	47	1	265	313
Confirmed xTAG GPP Positives/All xTAG GPP Positives	95.9%			
Invalid Rate due to PCR Inhibition	1.9%			

***C. Difficile* Toxin A/B**

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	9	3	3	15[1]
Negative	NA	NA	292	292
Invalid	NA	NA	6	6
TOTAL	9	3	301	313
Confirmed xTAG GPP Positives/All xTAG GPP Positives	60.0%			
Invalid Rate due to PCR Inhibition	1.9%			

[1]A total of 9 (9/15, 60.0%) *C. difficile* Toxin A/B xTAG GPP positive specimens were positive for both the Toxin A and B gene targets by the xTAG GPP Test. A total of 3 (3/15, 20.0%) *C. difficile* Toxin A/B xTAG GPP positive specimens were positive for the Toxin B target and 3 (3/15, 20.0%) were positive for the Toxin A target.

***E. coli* O157**

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	4	0	1	5
Negative	NA	NA	303	303
Invalid	NA	NA	5	5
TOTAL	4	0	309	313
Confirmed xTAG GPP Positives/All xTAG GPP Positives	80.0%			
Invalid Rate due to PCR Inhibition	1.6%			

Norovirus

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	29	9	6	44
Negative	NA	NA	263	263
Invalid	NA	NA	6	6
TOTAL	29	9	275	313
Confirmed xTAG GPP Positives/All xTAG GPP Positives	65.9%			
Invalid Rate due to PCR Inhibition	1.9%			

Salmonella

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	6	7	4	17
Negative	NA	NA	290	290
Invalid	NA	NA	6	6
TOTAL	6	7	300	313
Confirmed xTAG GPP Positives/All xTAG GPP Positives	35.3%			
Invalid Rate due to PCR Inhibition	1.9%			

Shigella

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	32	2	2	36
Negative	NA	NA	271	271
Invalid	NA	NA	6	6
TOTAL	32	2	279	313
Confirmed xTAG GPP Positives/All xTAG GPP Positives	88.9%			
Invalid Rate due to PCR Inhibition	1.9%			

STEC

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	3	1	1	5
Negative	NA	NA	302	302
Invalid	NA	NA	6	6
TOTAL	3	1	309	313

Confirmed xTAG GPP Positives/All xTAG GPP Positives	60.0%			
Invalid Rate due to PCR Inhibition	1.9%			

Supplemental Clinical Study (Botswana Pediatric Stool Specimens) Contaminated Runs

Unexpected positive call(s) in negative (NTC) or external rotating positive control(s) (RC) were reported in 2 out of 5 Botswana xTAG GPP runs (40%). A total of 5 clinical specimens included in these runs tested positive by xTAG GPP for analytes that were unexpectedly present in assay controls (5/313; 1.6%).

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

Expected Value (As Determined by the xTAG GPP) Summary by Site for the xTAG GPP Prospective Clinical Evaluation (June 2011 – February 2012)

	Overall (n=1407)		Site 1 (n=434)		Site 2 (n=428)		Site 3 (n=155)		Site 4 (n=260)		Site 5 (n=88)		Site 6 (n=42)	
	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value
Campylobacter	24	1.7%	5	1.2%	15	3.5%	2	1.3%	2	0.8%	0	0.0%	0	0.0%
Cryptosporidium	65	4.6%	11	2.5%	48	11.2%	0	0.0%	6	2.3%	0	0.0%	0	0.0%
E. coli O157	11	0.8%	2	0.5%	2	0.5%	3	1.9%	2	0.8%	2	2.3%	0	0.0%
ETEC LT/ST	6	0.4%	2	0.5%	3	0.7%	0	0.0%	0	0.0%	1	1.1%	0	0.0%
Giardia lamblia	43	3.1%	13	3.0%	17	4.0%	3	1.9%	8	3.1%	2	2.3%	0	0.0%
Salmonella	28	2.0%	11	2.5%	11	2.6%	2	1.3%	4	1.5%	0	0.0%	0	0.0%
STEC (*stx*1/*stx* 2)	17	1.2%	9	2.1%	5	1.2%	1	0.6%	1	0.4%	1	1.1%	0	0.0%
Shigella	19	1.4%	3	0.7%	12	2.8%	4	2.6%	0	0.0%	0	0.0%	0	0.0%
C. difficile Toxin A/B	220	15.6%	57	13.1%	63	14.7%	28	18.1%	42	16.2%	21	23.9%	9	21.4%
Norovirus GI/GII	170	12.1%	24	5.5%	76	17.8%	12	7.7%	41	15.8%	14	15.9%	3	7.1%
Rotavirus A	4	0.3%	2	0.5%	1	0.2%	1	0.6%	0	0.0%	0	0.0%	0	0.0%

Expected Value (As Determined by the xTAG GPP) Summary by Age Group for the xTAG GPP Prospective Clinical Evaluation (June 2011 – February 2012)

	Overall (n=1407)		0-1 year (n=6)		>1-5 years (n=20)		>5-21 years (n=76)		>21-65 years (n=879)		>65 years (n=426)	
	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value
Campylobacter	24	1.7%	0	0.0%	2	10.0%	0	0.0%	15	1.7%	7	1.6%
Cryptosporidium	65	4.6%	0	0.0%	4	20.0%	2	2.6%	46	5.2%	13	3.1%
E. coli O157	11	0.8%	1	16.7%	0	0.0%	2	2.6%	6	0.7%	2	0.5%
ETEC LT/ST	6	0.4%	0	0.0%	0	0.0%	1	1.3%	3	0.3%	2	0.5%
Giardia lamblia	43	3.1%	0	0.0%	0	0.0%	2	2.6%	26	3.0%	15	3.5%
Salmonella	28	2.0%	0	0.0%	1	5.0%	3	3.9%	18	2.0%	6	1.4%
STEC (*stx*1/*stx* 2)	17	1.2%	0	0.0%	0	0.0%	3	3.9%	8	0.9%	6	1.4%
Shigella	19	1.4%	0	0.0%	0	0.0%	0	0.0%	12	1.4%	7	1.6%
C. difficile Toxin A/B	220	15.6%	2	33.3%	2	10.0%	13	17.1%	120	13.7%	83	19.5%
Norovirus GI/GII	170	12.1%	1	16.7%	6	30.0%	11	14.5%	101	11.5%	51	12.0%
Rotavirus A	4	0.3%	0	0.0%	0	0.0%	2	2.6%	1	0.1%	1	0.2%

N. Instrument Name:

Luminex 100/200

O. System Descriptions:

1. Modes of Operation:

   Batch

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:

   Users must fill in Batch Information by providing a unique batch Name, Description and Creator. Users have to enter appropriate patient information, i.e. number of samples, and sample IDs.

4. Specimen Sampling and Handling:

   DNA is extracted using the Biomerieux NucliSens EasyMag system. Samples are manually prepared for amplification according to assay package insert and, once amplified, are transferred to a 96-well microtiter plate for analysis on the Luminex system.

5. Calibration:

   xMAP Calibrator Microspheres, Classification (CAL1) and Reporter (CAL2) serve as system calibrators for Luminex xMAP technology based detectors and are intended to normalize the settings for both the classification channel (CL1, CL2), the doublet discriminator channel (DD), and the reporter channel (RP1). They are not intended to be used as calibrators for a given assay.

6. Quality Control:

   xMAP Control Microspheres, Classification (CON1) and Reporter (CON2) are intended to verify the calibration and optical integrity for the Luminex 100/200 System. Classification Control Microspheres verify both classification channels and the doublet discriminator channel (DD). Reporter Control Microspheres verify the reporter channel. They are not intended to be used as controls for a given assay which are described in the specific assay package insert.

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

Instrument Performance Assurance:

Due to the open system design of the platform there is a potential for contamination, the Intended Use of this device states that all positive results are presumptive and need to be confirmed by another FDA-cleared or approved assay or acceptable reference method. The benefit of this test lies in its ability to rule out infection of a patient with the 11 pathogens on the panel. The following mitigations were instituted for the xTAG GPP:

1. **Proficiency Panel and Training**-A formal training and certification program will be provided by Luminex with mandatory proficiency testing for end users that they would need to complete before running the xTAG GPP.
2. **Trending and Reporting Positivity Rates**-As part of the formal training program, Luminex will include training that specifically focuses on maintaining and monitoring data related to positivity rates for the xTAG GPP. Labs running the xTAG GPP would establish a procedure to monitor unusual spikes in positivity rates and would use this procedure in determining how to report these spikes to Luminex through their existing complaint handling system.
3. **Environmental Monitoring and Cleaning Process**-As part of the formal training progam, Luminex will instruct laboratories to create a procedure that specifically describes an xTAG GPP environmental monitoring program. This procedure would instruct the user to include the appropriate controls on the plate, to swab surfaces in the processing areas and run them with the xTAG GPP at least once per month, to monitor results of this swabbing, and initiate a cleaning protocol in the event of a positive finding. Increased frequency of swabbing would be recommended until the contamination has been adequately addressed.

Q. Proposed Labeling:

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

R. Risks to Health:

FDA has identified the risks that require special controls to be the following: failure of the device to detect and identify a targeted organism when such organism is present in the specimen (i.e., false negative test result for presence of organism) and detection of the targeted microorganism when such organism is not present in the specimen (i.e., false positive test result for presence of organism), both of which can lead to individual and/or public health consequences, and failure to correctly interpret test results

Failure of the device to detect and identify a targeted organism when such organism is present in the specimen (false negative result) may lead to a delay in finding the true cause of the gastrointestinal infection, additional diagnostic tests, and unnecessary treatment or to

inappropriate antibiotic use. For certain microorganisms detected by the device, failure of detection may contribute to incorrect patient management to prevent transmission of infection, or delay recognition of an outbreak. An incorrect positive test result (false positive result) also may lead to unnecessary or ineffective antibiotic therapy and delay in determining the true cause of the patient's illness, which for some microorganisms may lead to a more serious infection. Additionally, in the context of public health, a false positive teste result may lead to misallocation of resources used for disease surveillance and prevention.

Failure to correctly interpret test results in the context of other clinical and laboratory findings may lead to inappropriate or delayed treatment. For example, a microorganism present as a colonizer may be correctly detected, but not be the true cause of illness. Although this identical risk would be present from use of any microbiological assay in this setting, simultaneous testing of multiple analytes in a multiplex assay may be more likely to detect an unanticipated colonizer that might not be tested for individually.

The special controls necessary to address the risks posed by this device are identified in the special controls guideline entitled "Class II Special Controls Guideline: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assays for Detection and Identification of Microorganisms and Toxin Genes from Human Stool Specimens," which includes mitigation measures relating to device characteristics, device specific performance characteristics, and device specific labeling.

S. Benefit/Risks Analysis

We considered the following factors in our analysis of benefit: the ability to more rapidly rule out potentially significant gastrointestinal pathogens in the setting of acute gastroenteritis, the ability of laboratorians to alter workflow, e.g., obviating the need for setting up multiple assays to detect the pathogens included in this panel, more sensitive detection of certain pathogens relative to existing FDA cleared assays (e.g., Norovirus), and potential improved tracking or sentinel detection of acute gastroenteritis outbreaks.

As noted earlier, the risks from this device include failure of the device to detect and identify a targeted organism when such organism is present in the specimen (i.e., false negative test result for presence of organism) and detection of the targeted microorganism when such organism is not present in the specimen (i.e., false positive test result for presence of organism), both of which can lead to individual and/or public health consequences, and failure to correctly interpret test results.

There is a concern with relatively low specificity of two of the analytes tested in the panel (*C. difficile* and Norovirus), but this is addressed by the labelling requirement that all positive test results tests be confirmed by other cleared or reference assays and by consideration of the risks from inaccurate results for each pathogen. The low incidence of many of the pathogens in the panel, despite high specificity, yields a low positive predictive value in most clinical settings; however, this is also addressed by the need for confirmatory testing, clinician evaluation, and the results additional diagnostic testing. The sponsor has also mandated operator training prior to device use to mitigate risks of device contamination and

false positive results, a risk present in open nucleic acid amplification platforms.

There is the additional impact of false positive and/or false negative results as regards infection control within an institution or unrecognized spread of disease, or (in the extreme case where confirmation is not performed), false outbreak identification (i.e., a pseudo-outbreak).

It should be recognized that the device is intended for use as an 'aid in the diagnosis' of gastroenteritis in conjunction with clinical presentation and the results of other laboratory tests. Both clinical presentation and other results would likely substantially mitigate concerns with both false positive and false negative test results; for example, a significantly ill patient with frank dysentery is unlikely to have Norovirus infection, or if so, there is likely to be a co-pathogen or a second concomitant illness. Similarly, an ill patient with a negative GPP result is likely to undergo additional conventional testing since all potential GI pathogens are not tested by this panel, and for sufficiently ill patients, empiric antibiotic use is likely.

It is also important to recognize the potential value of Norovirus testing in this panel; this panel is the first FDA-cleared device for nucleic acid-based testing of Norovirus. Norovirus is a major cause of outbreaks of gastrointestinal disease in closed populations such as nursing homes or cruise ships, and this panel may serve an important role in increasing the confirmed diagnosis of the entity, allowing more rapid intervention.

The benefits of the GPP assay outweigh the risks. The ability to more rapidly rule out potentially significant gastrointestinal pathogens in the setting of acute gastroenteritis is particularly beneficial. The identified risks posed by the device are adequately mitigated.

T. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.3990 with special controls. The special control guidance document "Class II Special Controls Guidance Document: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assays for Detection and Identification of Microorganisms and Toxin Genes from Human Stool Specimens." will be available shortly. The device is classified under the following:

Product Code: PCH, Gastrointestinal microorganism multiplex nucleic acid-based assay
Device Type: Gastrointestinal microorganism multiplex nucleic acid-based assay
Class: II
Regulation: 21 CFR 866.3990