510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K220062

B Applicant

QIAGEN GmbH

C Proprietary and Established Names

QIAstat-Dx Gastrointestinal Panel 2

D Regulatory Information

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

II Submission/Device Overview:

A multiplexed nucleic acid-based test intended for use with QIAstat-Dx system for the qualitative *in vitro* detection and identification of multiple bacteria, viruses, and parasites. The QIAstat-Dx Gastrointestinal Panel 2 assay is performed directly from stool samples collected in Para-Pak C&S (Meridian Bioscience) and FecalSwab (COPAN) transport media.

A Purpose for Submission:

To obtain a substantial equivalence determination for the QIAstat-Dx Gastrointestinal Panel 2 microorganism multiplex nucleic acid-based assay.

B Measurand:

Targeted nucleic acid sequences of the following gastrointestinal microorganisms:

- Adenovirus F40/F41
- Astrovirus

- Norovirus GI/GII
- Rotavirus A
- *Campylobacter* (*C. jejuni, C. coli* and *C. upsaliensis*)
- *Shigella*/Enteroinvasive *Escherichia coli* (EIEC)
- Enteropathogenic *Escherichia coli* (EPEC)
- Enterotoxigenic *Escherichia coli* (ETEC) *lt/st*
- Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2** (including specific identification of *E. coli* O157 serogroup within STEC)
- *Salmonella*
- *Plesiomonas shigelloides*
- *Yersinia enterocolitica*
- *Cryptosporidium*
- *Cyclospora cayetanensis*
- *Entamoeba histolytica*
- *Giardia lamblia*

C Type of Test:

A multiplex nucleic acid-based test intended for use with the QIAstat-Dx system for the qualitative *in vitro* detection and identification of multiple bacteria, viruses, and parasites in preserved stool samples collected from individuals suspected of gastrointestinal infection.

III Intended Use/Indications for Use:

A Intended Use(s):

The QIAstat-Dx Gastrointestinal Panel 2 is a multiplexed nucleic acid test intended for use with the QIAstat-Dx Analyzer 1.0. for the simultaneous *in vitro* qualitative detection and identification of nucleic acids from multiple viruses, bacteria, and parasites directly from preserved stool samples (Para-Pak C&S or FecalSwab) obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following viruses, bacteria (including several diarrheagenic *E. coli*/ *Shigella* pathotypes), and parasites are identified with the QIAstat-Dx Gastrointestinal Panel 2:

Pathogen	Para-Pak C&S	FecalSwab
Adenovirus F40/F41	✓	✓
Astrovirus	✓	✓
Norovirus GI/GII	✓	✓
Rotavirus A	✓	✓
Campylobacter (*C. jejuni, C. coli* and *C. upsaliensis*)	✓	✓
Shigella/Enteroinvasive *Escherichia coli* (EIEC)	✓	✓
Enteropathogenic *Escherichia coli* (EPEC)	✓	Not Reported

Enterotoxigenic *Escherichia coli* (ETEC) lt/st	✓	✓
Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1*/*stx2* (including specific identification of *E. coli* O157 serogroup within STEC)	✓	Not Reported
Salmonella	✓	✓
Plesiomonas shigelloides	✓	✓
Yersinia enterocolitica	✓	✓
Cryptosporidium	✓	✓
Cyclospora cayetanensis	✓	✓
Entamoeba histolytica	✓	✓
*Giardia lamblia**	✓	✓

*Also known as *Giardia intestinalis* and *Giardia duodenalis*

Concomitant culture is necessary for organism recovery and further typing of bacterial agents. The QIAstat-Dx Gastrointestinal Panel 2 is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological data. Positive results do not rule-out co-infection with organisms not detected by the QIAstat-Dx Gastrointestinal Panel 2. The organisms detected may not be the sole or definitive cause of the disease.

Negative QIAstat-Dx Gastrointestinal Panel 2 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.

B Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For *in vitro* Diagnostic Use Only

C Special Instrument Requirements:

The QIAstat-Dx Gastrointestinal Panel 2 is intended for use only with the QIAstat-Dx Analyzer 1.0.

IV Device/System Characteristics:

A Device Description:

The QIAstat-Dx Gastrointestinal Panel 2 assay is run on the QIAstat-Dx Analyzer 1.0. The QIAstat-Dx Gastrointestinal Panel 2 cartridge is a single-use cartridge that includes all reagents needed for nucleic acid extraction, nucleic acid amplification, and detection of bacteria, viruses or parasites associated with gastrointestinal infection. Testing requires a 200

µL specimen volume and minimal hands-on time, and the results are available in approximately 78 minutes.

B Principle of Operation:

The QIAstat-Dx Gastrointestinal Panel 2 is part of the QIAstat-Dx system and works with the QIAstat-Dx Analyzer 1.0.

The QIAstat-Dx Gastrointestinal Panel 2 is intended to be used with stool samples collected in Para-Pak C&S or FecalSwab transport media.

Once the cartridge has been inserted into the instrument, the test starts automatically and runs for approximately 78 minutes. he QIAstat-Dx Analyzer 1.0 automatically interprets test results and displays a summary on the analyzer display screen.

Sample Pre-treatment for PCR Inhibitors removal
Following insertion of the cartridge, the buffer located in Reservoir 1 is added inside the lysis chamber and homogenized with the sample using a rotor in the presence of silica beads. This enables separation of commonly found inhibitory substances in stool from the DNA/RNA by chemical means.

Resuspension of Internal Control (IC) and Proteinase K
Following sample pre-treatment, the IC and Proteinase K is resuspended with the buffer located in Reservoir 2 (resuspension buffer). The buffer from Reservoir 2 is added to the interconnected IC cavity and Proteinase K cavity and transferred repeatedly between the Transfer Chamber and the cavities to ensure resuspension. The resuspended IC and Proteinase K are transferred to the sample cavity.

Cell Lysis
Primary lysis of the cells and analytes present in a stool sample and IC occurs by a combination of chemical and mechanical processes using a rotor inside the lysis chamber in the presence of silica beads and a buffer that acts as a chemical agent in aiding the mechanical process. The fast movement of the rotor in the presence of the silica beads results in sample agitation, which creates turbulence and shear forces that favor the lysis of the cell wall.

After mechanical lysis is completed, the primary lysate is transferred to the purification chamber through a frit with 2-5 µm pore size. The second lysis buffer (from Reservoir 3) is added to the primary lysate to complete chemical lysis.

Purification
Binding reagent (from Reservoir 4) is added to the lysate in the purification chamber, and the mix is passed through the silica membrane. In this process, the DNA/RNA molecules stick to the membrane, and the remaining components of the lysate are delivered to the waste chamber. Then the membrane is washed with a first washing buffer (from Reservoir 5) to wash away proteins. This is followed by a second washing step with a second washing buffer (from Reservoir 6) to remove any remaining substances other than the nucleic acids. A subsequent drying step

eliminates volatile substances from the silica membrane. Prior to the elution step, the Transfer Chamber is rinsed with the rinsing buffer (from Reservoir 7) in order to remove any potential inhibitors from previous processing steps. At the end of the process, the nucleic acids are released from the membrane using an elution buffer (from Reservoir 8). The eluate is collected in the Transfer Chamber.

Rehydration of Master Mix

A defined volume (135 µL) of the eluate is delivered to the reservoir of the Dry Chemistry Container (DCC) to rehydrate the Master Mix. Any remaining eluate is transferred to the Reservoir 7. The eluate/Master Mix solution is mixed by repeated transfer between the Transfer Chamber and the DCC.

Aliquoting and PCR

Defined aliquots (15 µL) of mixed eluate/Master Mix are sequentially transferred from the Transfer Chamber to each of eight Reaction Chambers containing the specified, air dried primers and probes.

Within each Reaction Chamber, a reverse-transcription step followed by real time, multiplex PCR ("rtPCR") is performed. Increase in fluorescence (indicative of detection of each target analyte) is detected directly within each Reaction Chamber.

The rtPCR process is conducted by two submodules of the QIAstat-Dx Analyzer 1.0: the Thermal Cycler and the qPCR Sensor.

Components Description

QIAstat-Dx Gastrointestinal Panel 2 Cartridge:

The QIAstat-Dx Gastrointestinal Panel 2 cartridge is a disposable plastic device that allows performing fully automated molecular assays. The main features of the QIAstat-Dx Gastrointestinal Panel 2 cartridge for the Gastrointestinal assay include the ability to test liquid samples and the capacity to store all necessary reagents within the cartridge needed for such testing. All sample preparation and assay steps will be performed within the cartridge.

All the reagents required for the complete execution of the test are pre-loaded and self-contained in the QIAstat-Dx Gastrointestinal Panel 2. The user does not need to manipulate any reagents. During the test, reagents are handled by pneumatically operated microfluidics without any direct contact with the user or the analyzer actuators. This eliminates any possibility of exposure of the user or the analyzer to chemicals contained in the cartridge during the test and up to the disposal of used cartridges.

Reagents may be found in three different physical forms: liquid, air-dried on surfaces or lyophilized powder cake.

Within the cartridge, multiple steps are automatically performed in sequence by using pneumatic pressure and a multiport valve to transfer sample and fluids via the Transfer Chamber to their intended destinations.

QIAstat-Dx Analyzer 1.0

The QIAstat-Dx Analyzer 1.0 is the unit that hosts a cartridge and, on command from the user, is able to run predefined assay protocols. The software specific to this test is pre-loaded on the QIAstat-Dx Analyzer 1.0.

Other Materials

Each QIAstat-Dx Gastrointestinal Panel 2 cartridge will be used with a transfer pipette (provided with device). The stool sample from the patient will be collected in Cary-Blair medium following the manufacturer's instructions for use (not provided with device).

QIAstat-Dx Analyzer 1.0 – the QIAstat-Dx Gastrointestinal Panel 2 cartridge can only be run on the QIAstat-Dx Analyzer 1.0.

Calibrators and/or Controls

Blank controls are not applicable to the device because it is a single test disposable cartridge. Negative and positive external controls are recommended but not provided with the QIAstat-Dx Gastrointestinal Panel 2.

QIAGEN provides an internal control ("IC") within the QIAstat-Dx Gastrointestinal Panel 2 cartridge which provides a full process control covering lysis, nucleic acid purification, reverse transcription and DNA amplification. The IC is *Schizosaccharomyces pombe*. The IC is located in the IC cavity and is mixed with the sample during sample preparation and the eluate is mixed with the Master Mix, then aliquoted in all Reaction Chambers. The results screen displays a message indicating that the Internal Control "Passed" when the test is run successfully. An IC message of "Failed" indicates that the internal control was not amplified; 'Positive' test results are then reported as POSITIVE* (positive with warning), all 'Negative' results are invalid.

The QIAstat-Dx Analyzer 1.0 is provided factory calibrated and does not require user calibration. The QIAstat-Dx Analyzer 1.0 includes self-check controls to verify the performance of all sensors and actuators and will alert the user in case of failure.

V Substantial Equivalence Information:

A Predicate Device Name(s):

FilmArray Gastrointestinal (GI) Panel

B Predicate 510(k) Number(s):

K140407

C Comparison with Predicate(s):

Device & Predicate Device(s):	K220062	K140407
Device Trade Name	QIAstat-Dx Gastrointestinal Panel 2	FilmArray Gastrointestinal (GI) Panel
General Device Characteristics Similarities		

Device & Predicate Device(s):	K220062	K140407
Pathogens Detected	Adenovirus *F40/F41,* Astrovirus Norovirus GI/GII Rotavirus A *Campylobacter* (*C. jejuni*, *C. coli*, *C. upsaliensis*), *Shigella*/Enteroinvasive *E. coli* (EIEC) Enteropathogenic *E. coli* (EPEC) Enterotoxigenic *E. coli* (ETEC) Shiga-like toxin-producing *E.coli* (STEC) *stx1*/*stx2* (including specific identification of *E. coli* O157 serogroup within STEC) *Salmonella* *Plesiomonas shigelloides* *Yersinia enterocolitica* *Cryptosporidium* *Cyclospora cayetanensis* *Entamoeba histolytica* *Giardia lamblia*	Same plus additional pathogens detected (See below for differences)
Analyte	DNA/RNA	DNA/RNA
Amplification and Detection Technology	PCR	PCR
Technological Principles	Multiplex nucleic acid test	Same (See below for differences)
Intended Use/Indications For Use	The QIAstat-Dx Gastrointestinal Panel 2 is a multiplexed nucleic acid test intended for use with the QIAstat-Dx Analyzer 1.0 for the simultaneous qualitative detection and identification of nucleic acids from multiple viruses, bacteria, and parasites directly from preserved stool samples (Para-Pak C&S and FecalSwab) obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following viruses, bacteria (including	The FilmArray Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based test intended for use with the FilmArray Instrument for the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary Blair medium, obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria (including several diarrheagenic E. coli/Shigella pathotypes),

Device & Predicate Device(s):	K220062	K140407
	several diarrheagenic *E.coli*/*Shigella* pathotypes), and parasites are identified with the QIAstat-Dx Gastrointestinal Panel 2: • Adenovirus F40/F41 • Astrovirus • Norovirus GI/GII • Rotavirus A • *Campylobacter* (*C. jejuni, C. coli* and *C. upsaliensis*) • *Shigella*/Enteroinvasive *Escherichia coli* (EIEC) • Enteropathogenic *Escherichia coli* (EPEC) (only with Para-Pak C&S, not reported for FecalSwab) • Enterotoxigenic *Escherichia coli* (ETEC) *lt/st* • Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2** (including specific identification of *E. coli* O157 serogroup within STEC) (only with Para-Pak C&S, not reported for FecalSwab) • *Salmonella* • *Plesiomonas shigelloides* • *Yersinia enterocolitica* • *Cryptosporidium* • *Cyclospora cayetanensis* • *Entamoeba histolytica* • *Giardia lamblia (Also known as G. intestinalis* and *G. duodenalis*) Concomitant culture is necessary for organism recovery and further typing of bacterial agents. The QIAstat-Dx Gastrointestinal Panel 2 is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness in	parasites, and viruses are identified using the FilmArray GI Panel*:* • *Campylobacter (C. jejuni/C. coli/C. upsaliensis* • *Clostridium difficile (C. difficile) toxin A/B* • *Plesiomonas shigelloides* • *Salmonella* • *Vibrio (V> parahaemolyticus/V. vulnificus/ V. cholerae) including specific identification of Vibrio cholerae* • *Yersinia enterocolitica* • Enteroaggregative *Escherichia coli* (EAEC) • Enteropathogenic *Escherichia coli* (EPEC) • Enterotoxigenic *Escherichia coli* (ETEC) *lt/st* • Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli* O157 serogroup within STEC) • *Shigella*/Enteroinvasive *Escherichia coli* (EIEC) • *Cryptosporidium* • *Cyclospora cayetanensis* • *Entamoeba histolytica* • *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*) • Adenovirus F 40/41 • Astrovirus • Norovirus GI/GII • Rotavirus A • Sapovirus (Genogroups I, II, IV and V) The FilmArray Gastrointestinal Panel is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical,

Device & Predicate Device(s):	K220062	K140407
	conjunction with other clinical, laboratory, and epidemiological data. Positive results do not rule-out co-infection with organisms not detected by the QIAstat-Dx Gastrointestinal Panel 2. The organisms detected may not be the sole or definitive cause of the disease. Negative QIAstat-Dx Gastrointestinal Panel 2 results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this assay test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.	laboratory, and epidemiological data. Positive results do not rule out co-infection with organisms not included in the FilmArray GI Panel. The agent detected may not be the definite cause of the disease. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. This device is not intended to monitor or guide treatment for *C. difficile* infection. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *E. coli* O157*, Plesiomonas shigelloides, Yersinia enterocolitica,* Astrovirus, and Rotavirus A were established primarily with retrospective clinical specimens. Performance characteristics for *Entamoeba histolytica, and Vibrio (V parahaemolyticus, V. vulnificus, and Vibrio cholerae)* were established primarily using contrived clinical specimens. Negative FilmArray GI 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. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.
Assay Controls	One internal control in each cartridge to control for sample	Two controls are included in each reagent pouch to control for

Device & Predicate Device(s):	K220062	K140407
	processing that is subjected to all nucleic acid extraction and amplification steps similar to patient samples. External controls are not provided with the QIAstat-Dx Gastrointestinal Panel 2. Labeling recommends use of negative and positive external controls regularly. Use transport media as the external Negative Control, and previously characterized positive samples or negative sample spiked with well characterized target organisms as external positive controls.	sample processing and both stages of PCR and melt analysis. Labeling recommends the use of external positive and negative controls regularly. Enteric transport media can be used as an external negative control, and previously characterized positive samples or negative samples spiked with well characterized organisms as external positive controls.
General Device Characteristic Differences		
Assay Targets	No additional targets to those listed above which are in common with the predicate device.	The FilmArray Gastrointestinal Panel has four additional targets: *Clostridium difficile (C. difficile)* toxin A/B, *Enteroaggregative Escherichia coli (EAEC),* Sapovirus and a generic *Vibrio* target which differentiates *Vibrio cholerae*.
Specimen Types	Human stool sample collected in Para-Pak C&S and FecalSwab transport media.	Human stool sample collected in Cary Blair transport media
Nucleic Acid Extraction	Extraction of nucleic acids using silica membrane	Extraction of nucleic acids using magnetic beads
Technology	QIAstat-Dx Gastrointestinal Panel 2 detection of amplified targets uses an increase in fluorescence due to specific probe binding to generate the assay results.	The FilmArray Gastrointestinal Panel uses melting curve analysis to confirm the identity of amplified targets to generate assay results.
Amplification and Detection Instrument System	QIAstat-Dx Analyzer 1.0	FilmArray Instrument

VI Standards/Guidance Documents Referenced:

None

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Reproducibility/Repeatability:

Reproducibility Study

Reproducibility testing evaluating contrived samples was performed at three test sites, including one internal site (Site A) and two external sites (Site B and Site C). The study incorporated a range of factors that may introduce variability in assay results, including sites, days, replicates, cartridge lots, operators, and QIAstat-Dx analyzers. For each site, testing was performed across five non-consecutive days with two replicates per day (total of 30 replicates per target, concentration, and site), four QIAstat-Dx Analyzers (two analyzers per operator and per site), and at least two operators on each testing day. A total of five sample mixes (including evaluation of analyte levels corresponding to 1x LoD and 3x LoD as well as negative samples containing no analyte) were prepared in a matrix consisting of pooled stool in Para-Pak C&S transport medium. For each mix, six replicates were tested and evaluated, and data obtained at all three sites were compiled to calculate the two-sided 95% Confidence Interval by target and concentration.

The percent agreement with expected results for all analytes was ≥95% for samples tested at 1x and 3x LoD, meeting the acceptance criteria for the study. All analytes evaluated were detected within performance expectations for qualitative agreement across all concentrations tested. The Reproducibility Study site-to-site qualitative results (agreements with expected results) are presented in Table 1 below.

Table 1: Reproducibility Study, Qualitative Results

Pathogen Tested	**Concentration Tested**	**Expected Result**	**% Agreement with Expected Result**			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Adenovirus F41 ZeptoMetrix 0810085CF	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	None	Not Detected	29/30 100%	29/30 100%	29/30 100%	87/90[1] 96.7% (90.98 – 98.9%)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Campylobacter ZeptoMetrix 801650	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
Escherichia coli EPEC[4] ZeptoMetrix 801747	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	29/30 96.67%	30/30 100%	89/90 98.89% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
Entamoeba histolytica ATCC 30459	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	30/30 100%	29/30 96.67%	89/90 98.89% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
Giardia lamblia[2] ATCC 30888	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Norovirus GII ZeptoMetrix 0810087CF	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	29/30 96.67%	30/30 100%	30/30 100%	89/90 98.89% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
Rotavirus A[3] ZeptoMetrix 0810280CF	3x LoD	Detected	29/30 96.67%	29/30 96.67%	30/30 100%	88/90 97.8% (92.20 – 99.73%)
	1x LoD	Detected	23/30 76.67%	26/30 86.67%	12/12 100%	61/72 84.7% (74.31 – 92.12%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
Escherichia coli (STEC) O157:H7[4] ZeptoMetrix 0801622	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	30/30 100%	29/30 96.67%	89/90 98.89% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
Escherichia coli (STEC) [4] *stx1/stx2 ZeptoMetrix 0801622	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Salmonella enterica ZeptoMetrix 0801437	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	29/30 96.67%	29/30 96.67%	88/90 97.78% (92.20 - 99.73%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 – 100%)
Yersinia enterocolitica Zeptometrix 0801734	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 – 100%)

[1] Three (3) Adenovirus F40/41 false positives were observed when testing negative sample. Retesting of the three samples resulted in the expected negative results.

[2] One (1) *Giardia lamblia* false positive was observed when testing a positive sample not containing the pathogen. Repeat testing of this sample resulted in the expected negative result.

[3] The Reproducibility study was fully re-tested for Rotavirus A with a new sample set due to an unexpected number of false negatives for Rotavirus A at the 1x LoD concentration. This was observed with during an interim data evaluation (61/72, 84.7%) and that was attributed to the sample manufacture and while unrelated to the study workflow variables (operator, lot, day, instrument and site). Test runs derived from Rotavirus A new sample set resulted in 90/90 (100%; 95.98-100% CI) for the 3x LoD and 89/90 (98.89%; 93.96-99.97% CI) for the 1x LoD. During this testing, one (1) *Campylobacter* false positive was observed Retesting of this sample resulted in the expected negative result.

[4] Only applicable for Para-Pak C&S samples

During the study, there were three false positive results for Adenovirus F40/41 in negative (non-spiked) samples as well as one false positive result for *Campylobacter* and one false positive result for *Giardia lamblia* in samples containing other analytes. Repeat testing of the same five samples yielded the expected negative result for each applicable analyte. The investigation into the unexpected false positive results showed that these events were observed in five different analyzers. For the sample that was false positive for *Campylobacter*, the sample tested immediately prior was also positive for *Campylobacter*. For the other four false positive samples, the samples run immediately prior were not positive for the applicable pathogen suggesting that these unexpected results were likely not due to inter-run carryover.

A total of six cartridge failures (6/645, 0.93%) were observed during the study. Failures included cartridge errors (5) and an instrument error (1). No invalid results due to internal control failure were observed.

An additional analysis of variation was conducted across specific potential sources of variability such as sites, days, replicates, cartridge lots, operators, and QIAstat-Dx analyzers. Results from the analysis showed no significant contribution to variability (Standard Deviation and Coefficient of Variation values below 1 and 5%, respectively) caused by any of the assessed variables. Overall, Ct variability was low, and the study demonstrates assay variability within an acceptable range. Results are presented in Table 2 below.

Table 2: Reproducibility Study, Numerical Analysis of Variability

					Variance component (SD, %CV*)						
Pathogen	**Concentration tested**	**Positive (n)**	**Negative (n)**	**Mean**	**Between Site**	**Between Lot**	**Between Day**	**Between Operator**	**Between Instrument**	**Between Replicate**	**Total****
Adenovirus	**1x LoD**	**90**	**0**	**34.15**	(0.2979, 0.87%)	(0.1630, 0.48%)	(0.1135, 0.33%)	(0.0000, 0.00%)	(0.1883, 0.55%)	(0.6153, 1.80%)	(0.7067, 2.07%)
	3x LoD	**90**	**0**	**32.14**	(0.0000, 0.00%)	(0.0741, 0.23%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.3716, 1.16%)	(0.3766, 1.17%)
Campylobacter	**1x LoD**	**90**	**0**	**32.64**	(0.1289, 0.39%)	(0.0000, 0.00%)	(0.0200, 0.06%)	(0.0000, 0.00%)	(0.0661, 0.20%)	(0.4232, 1.30%)	(0.4411, 1.35%)
	3x LoD	**90**	**0**	**31.00**	(0.0397, 0.13%)	(0.1446, 0.47%)	(0.0806, 0.26%)	(0.0000, 0.00%)	(0.1488, 0.48%)	(0.3420, 1.10%)	(0.3855, 1.24%)
EPEC	**1x LoD**	**89**	**1**	**34.24**	(0.4422, 1.29%)	(0.2000, 0.58%)	(0.0000, 0.00%)	(0.1599, 0.47%)	(0.1044, 0.30%)	(0.6699, 1.96%)	(0.7927, 2.31%)
	3x LoD	**90**	**0**	**32.49**	(0.1053, 0.32%)	(0.1429, 0.44%)	(0.0000, 0.00%)	(0.2081, 0.64%)	(0.0000, 0.00%)	(0.5238, 1.61%)	(0.5710, 1.76%)
Entamoeba	**1x LoD**	**89**	**1**	**33.01**	(0.0000, 0.00%)	(0.1349, 0.41%)	(0.0000, 0.00%)	(0.1175, 0.36%)	(0.0000, 0.00%)	(0.3182, 0.96%)	(0.3456, 1.05%)
	3x LoD	**90**	**0**	**31.34**	(0.1826, 0.58%)	(0.1265, 0.40%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0906, 0.29%)	(0.4000, 1.28%)	(0.4449, 1.42%)
Giardia	**1x LoD**	**90**	**0**	**28.65**	(0.2341, 0.82%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.1737, 0.61%)	(0.5386, 1.88%)	(0.5953, 2.08%)
	3x LoD	**90**	**0**	**26.68**	(0.0840, 0.31%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.1025, 0.38%)	(0.0857, 0.32%)	(0.3531, 1.32%)	(0.3798, 1.42%)
Norovirus GII	**1x LoD**	**89**	**1**	**33.53**	(0.1346, 0.40%)	(0.3090, 0.92%)	(0.1111, 0.33%)	(0.0000, 0.00%)	(0.0431, 0.13%)	(0.4102, 1.22%)	(0.5083, 1.52%)
	3x LoD	**90**	**0**	**32.42**	(0.1742, 0.54%)	(0.3405, 1.05%)	(0.2895, 0.89%)	(0.0632, 0.19%)	(0.1972, 0.61%)	(0.4966, 1.53%)	(0.6721, 2.07%)
Rotavirus* **	**1x LoD**	**89**	**1**	**34.86**	(0.1706, 0.49%)	(0.0000, 0.00%)	(0.3458, 0.99%)	(0.1207, 0.35%)	(0.3224, 0.92%)	(0.9814, 2.82%)	(1.0891, 3.12%)
	3x LoD	**90**	**0**	**33.85**	(0.8986, 2.65%)	(0.2050, 0.61%)	(0.5187, 1.53%)	(0.0000, 0.00%)	(0.2847, 0.84%)	(1.3126, 3.88%)	(1.6070, 4.75%)
STEC_O157	**1x LoD**	**89**	**1**	**33.83**	(0.2218, 0.66%)	(0.3083, 0.91%)	(0.0000, 0.00%)	(0.0204, 0.06%)	(0.0000, 0.00%)	(0.5455, 1.61%)	(0.6295, 1.86%)
	3x LoD	**90**	**0**	**32.22**	(0.2431, 0.75%)	(0.2146, 0.67%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.2051, 0.64%)	(0.4625, 1.44%)	(0.5633, 1.75%)
STEC_stx1	**1x LoD**	**90**	**0**	**34.07**	(0.2297, 0.67%)	(0.1214, 0.36%)	(0.1281, 0.38%)	(0.0000, 0.00%)	(0.1170, 0.34%)	(0.4206, 1.23%)	(0.4991, 1.46%)
	3x LoD	**90**	**0**	**32.54**	(0.2380, 0.73%)	(0.2222, 0.68%)	(0.0802, 0.25%)	(0.0000, 0.00%)	(0.2000, 0.61%)	(0.4919, 1.51%)	(0.5887, 1.81%)
STEC_stx2	**1x LoD**	**90**	**0**	**34.11**	(0.0620, 0.18%)	(0.0979, 0.29%)	(0.0310, 0.09%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.3987, 1.17%)	(0.4109, 1.20%)
	3x LoD	**90**	**0**	**32.69**	(0.1173, 0.36%)	(0.2599, 0.80%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.1316, 0.40%)	(0.4131, 1.26%)	(0.4821, 1.47%)
Salmonella	**1x LoD**	**88**	**2**	**34.53**	(0.2888, 0.84%)	(0.2631, 0.76%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.4880, 1.41%)	(0.5857, 1.70%)
	3x LoD	**90**	**0**	**33.44**	(0.0279, 0.08%)	(0.2100, 0.63%)	(0.0000, 0.00%)	(0.1814, 0.54%)	(0.1177, 0.35%)	(0.4088, 1.22%)	(0.4715, 1.41%)
Yersinia	**1x LoD**	**90**	**0**	**34.53**	(0.1616, 0.47%)	(0.1688, 0.49%)	(0.0564, 0.16%)	(0.0000, 0.00%)	(0.1036, 0.30%)	(0.5172, 1.50%)	(0.5637, 1.63%)
	3x LoD	**90**	**0**	**33.02**	(0.0503, 0.15%)	(0.2129, 0.64%)	(0.0370, 0.11%)	(0.0000, 0.00%)	(0.0801, 0.24%)	(0.3434, 1.04%)	(0.3951, 1.20%)

* SD is first figure presented, subsequent figure is CV (Coefficient of variation presented as a percentage (%)
** TOTAL means total variance observed across the study
*** Results in table correspond to the original sample set analyzed for Rotavirus. Results from the new dataset were:

					Variance component (SD, %CV*)						
Pathogen	**Concentration tested**	**Positive (n)**	**Negative (n)**	**Mean**	**Between Site**	**Between Lot**	**Between Day**	**Between Operator**	**Between Instrument**	**Between Replicate**	**Total****
Rotavirus	**1x LoD**	**89**	**1**	**34.86**	(0.1706, 0.49%)	(0.0000, 0.00%)	(0.3458, 0.99%)	(0.1207, 0.35%)	(0.3224, 0.92%)	(0.9814, 2.82%)	(1.0891, 3.12%)
	3x LoD	**90**	**0**	**32.42**	(0.1742, 0.54%)	(0.3405, 1.05%)	(0.2895, 0.89%)	(0.0632, 0.19%)	(0.1972, 0.61%)	(0.4966, 1.53%)	(0.6721, 2.07%)

Repeatability Study:

To further assess the precision of the QIAstat-Dx Gastrointestinal Panel 2, an in-house repeatability study was conducted using a set of samples containing analyte concentrations of 3X LoD and 1X LoD as well as negative samples prepared in stool matrix in Para-Pak C&S transport medium. Specifically, pathogens included in the positive samples were Adenovirus, *Campylobacter*, *Entamoeba histolytica*, *Giardia lamblia*, Norovirus GII, Rotavirus, *Salmonella enterica*, and *Yersinia enterocolitica,* in addition to Enteropathogenic *E. coli* (EPEC), STEC *stx1*/*stx2*, and *E. coli* O157 which are only applicable for Para-Pak C&S samples. Each panel member was tested with the same instrument over 12 days. In total, 60 replicates at 1x LoD and 60 replicates at 3x LoD per each of the tested targets and 60 negative samples were run in the study. Overall results showed a 93.33-100% and 95.00-100% detection rate for 1x LoD and 3x LoD samples, respectively. Negative samples showed 100% of negative calls for all panel analytes. One failed run due to a cartridge error occurred during the study (1/426, 0.23%). Testing results met the study acceptance criteria demonstrating acceptable repeatability of the QIAstat-Dx Gastrointestinal Panel 2.

2. Linearity:

   Not applicable.

3. Analytical Specificity/Interference:

   Analytical Specificity/Cross-Reactivity

   The Analytical Specificity study was performed to demonstrate the absence of cross-reactivity between the QIAstat-Dx Gastrointestinal Panel 2 assay on-panel microorganisms and viruses (intra-panel cross-reactivity) and between on-panel and off-panel microorganisms/viruses (i.e., organisms not covered by the panel and therefore not intended to be detected by the QIAstat-Dx Gastrointestinal Panel 2).

   Samples were prepared by single spiking microorganisms/viruses at high concentrations into negative stool resuspended in Para-Pak C&S transport medium. Sample concentrations tested were 1.0E+06 CFU/mL for bacteria, 1.0E+05 TCID50/mL for viruses and 1.0E+05 cells/mL for parasites, or the highest available concentration based on the pathogen stock. Testing was conducted in triplicate.

Study results indicated no false positive results for on-panel or off-panel analytes with the exception of two non-targeted *Campylobacter* species (*C. helveticus* and *C. lari*) that cross-reacted with the *Campylobacter* assay oligonucleotides included in the QIAstat-Dx Gastrointestinal Panel 2. The on-panel and off-panel microorganisms/viruses evaluated in the study are presented in Tables 3 and 4 respectively.

Table 3: List of Analytical Specificity On-Panel Pathogens Tested

Type	Pathogen	Concentration Tested
Bacteria	*Campylobacter coli*	1.0E+06 CFU/mL
	Campylobacter jejuni	1.0E+06 CFU/mL
	Campylobacter upsaliensis	1.0E+06 CFU/mL
	Escherichia coli (EPEC)	1.0E+06 CFU/mL
	Escherichia coli (ETEC lt/st)	1.0E+06 CFU/mL
	Escherichia coli (STEC)	1.0E+06 CFU/mL
	Plesiomonas shigelloides	1.0E+06 CFU/mL
	Salmonella enterica	1.0E+06 CFU/mL
	Shigella sonnei	1.0E+06 CFU/mL
	Yersinia enterocolitica	1.0E+06 CFU/mL
Parasites	*Cryptosporidium parvum*	1.0E+05 oocysts/mL
	Cyclospora cayetanensis	1.0E+05 genome copies/mL
	Entamoeba histolytica	1.0E+05 cells/mL
	Giardia lamblia	1.0E+05 cells/mL
Viruses	Adenovirus F41	1.0E+05 $TCID_{50}$/mL
	Astrovirus	1.0E+05 copies/mL
	Norovirus GI	1.0E+05 $TCID_{50}$/mL
	Norovirus GII	1.0E+05 $TCID_{50}$/mL
	Rotavirus	1.0E+05 U/mL

Table 4: List of Analytical Specificity Off-Panel Pathogens Tested

Type	Pathogen (Potential Cross-Reactant)	Concentration Tested
Bacteria	*Abiotrophia defectiva*	1.0E+06 cells/mL
	Acinetobacter baumannii	1.0E+06 CFU/mL
	Aeromonas hydrophila	1.0E+06 CFU/mL
	Arcobacter cryaerophilus	1.0E+06 cells/mL
	Bacillus subtilis	1.0E+06 CFU/mL
	Bifidobacterium bifidum	1.0E+06 CFU/mL
	Campylobacter fetus	1.0E+06 cells/mL
	Campylobacter gracilis	1.0E+06 CFU/mL
	Campylobacter helveticus*	1.0E+06 CFU/mL
	Campylobacter hominis	1.0E+06 cells/mL
	Campylobacter lari*	1.0E+06 CFU/mL
	Campylobacter mucosalis	1.0E+06 CFU/mL
	Campylobacter rectus	1.0E+06 cells/mL
	Chlamydia trachomatis	1.0E+06 cells/mL
	Citrobacter freundii	1.0E+06 CFU/mL

	Clostridium difficile non-toxigenic	1.0E+06 CFU/mL
	Clostridium perfringens	1.0E+06 cells/mL
	Clostridium septicum	1.0E+06 CFU/mL
	Clostridium tetani	1.0E+06 CFU/mL
	Corynebacterium genitalium	1.0E+06 cells/mL
	Enterobacter aerogenes	1.0E+06 CFU/mL
	Enterobacter cloacae	1.0E+06 CFU/mL
	Enterococcus faecalis	1.0E+06 cells/mL
	Enterococcus faecium	1.0E+06 CFU/mL
	Escherichia fergusonii	1.0E+06 CFU/mL
	Escherichia hermannii	1.0E+06 cells/mL
	Escherichia vulneris	1.0E+06 CFU/mL
	Faecalibacterium prausnitzii	1.0E+06 CFU/mL
	Gardnerella vaginalis	1.0E+06 cells/mL
	Haemophilus influenzae	1.0E+06 CFU/mL
	Hathewaya histolytica (Clostridium)	1.0E+06 CFU/mL
	Helicobacter pylori	1.0E+06 cells/mL
	Klebsiella pneumoniae	1.0E+06 CFU/mL
	Lactobacillus casei	1.0E+06 CFU/mL
	Listeria monocytogenes	1.0E+06 cells/mL
	Proteus mirabilis	1.0E+06 CFU/mL
	Proteus vulgaris	1.0E+06 CFU/mL
	Pseudomonas aeruginosa	1.0E+06 cells/mL
	Staphylococcus aureus	1.0E+06 CFU/mL
	Staphylococcus aureus subsp. aureus	1.0E+06 CFU/mL
	Staphylococcus epidermidis	1.0E+06 cells/mL
	Streptococcus agalactiae	1.0E+06 CFU/mL
	Streptococcus pyogenes	1.0E+06 CFU/mL
Fungi	*Aspergillus fumigatus*	1.0E+05 cells/mL
	Candida albicans	1.0E+05 CFU/mL
	Saccharomyces boulardii	1.0E+05 CFU/mL
	Saccharomyces cerevisiae	1.0E+05 copies/mL
Parasite	*Babesia microti*	N/A**
	Blastocystis hominis	1.0E+05 cells/mL
	Giardia muris	1.0E+05 cysts/mL
	Toxoplasma gondii	1.0E+05 cells/mL
	Trichomonas tenax	1.0E+05 cells/mL
Viruses	Adenovirus C:2	1.0E+05 $TCID_{50}$/mL
	Adenovirus B:34	1.0E+05 $TCID_{50}$/mL
	Adenovirus B3	1.0E+05 $TCID_{50}$/mL
	Adenovirus E:4a	1.0E+05 $TCID_{50}$/mL
	Adenovirus serotype 1	1.0E+05 $TCID_{50}$/mL
	Adenovirus serotype 5	1.0E+05 $TCID_{50}$/mL
	Adenovirus serotype 8	1.0E+05 $TCID_{50}$/mL

	Bocavirus Type 1	1.0E+05 copies/mL
	Coronavirus 229E	1.0E+05 U/mL
	Coxsackievirus B3	1.0E+05 $TCID_{50}$/mL
	Cytomegalovirus	1.0E+05 $TCID_{50}$/mL
	Enterovirus 6 (Echovirus)	1.0E+05 U/mL
	Enterovirus 68	1.0E+05 $TCID_{50}$/mL
	Herpes Simplex Virus Type 2	1.0E+05 PFU/mL
	Rhinovirus 1A	1.0E+05 $TCID_{50}$/mL

*Positive results generated for Campylobacter
**The stock concentration for *Babesia microti* is expressed as 32 % parasitemia (Number of infected cells/Total cells*100)

In silico analysis of potential cross-reactivity predicted that the following microorganisms may cross-react with QIAstat-Dx Gastrointestinal Panel 2 targets (Table 5).

Table 5: Potential Cross-Reactivity Based On *in silico* Analysis

QIAstat-Dx Gastrointestinal Panel 2 Target	Potential cross-reactive microorganisms
Enteropathogenic *E. coli* (EPEC)	*Shigella boydii*[1,2,3], *Escherichia albertii*[1,2]
Campylobacter.	*Campylobacter lari*[4], *Campylobacter helveticus*[4]
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	*Shigella sonnei*[1,3], *Shigella Dysenteriae*[1,3], *Acinetobacter haemolyticus*[1,5], *Citrobacter freundi*[1,5], *Enterobacter cloacae*[1,5], *Aeromonas caviae*[1,5], *Escherichia albertii* [1,5]
E. coli O157	Non-STEC *E.coli* O157 strains[6]

[1]Predicted cross-reactivity identified by *in silico* analysis reflects sequences that can be acquired between species by horizontal gene transfer.

[2]Rare or less common *eae* intimin carrier organisms

[3]On-panel target.

[4]In vitro testing of *Campylobacter lari* and *Campylobacter helveticus* strains at high concentration confirmed potential cross-reactivity of these Campylobacter species with the QIAstat-Dx Gastrointestinal Panel 2 assay.

[5]Rare or less common s*tx* toxins producers

[6]*E. coli* O157 will only be reported by the QIAstat-Dx Gastrointestinal Panel 2 assay when there is a positive amplification for the *E. coli* (STEC) design according to the calling algorithm. An infrequent case of an *E. coli* (STEC) and an *E. coli* O157 co-infection will not be differentiated from a single infection caused by a STEC O157:H7 strain.

Interfering Substances Study:

The effect of potentially interfering substances on the performance of the QIAstat-Dx Gastrointestinal Panel 2 was evaluated. Thirty-five (35) potentially interfering substances were spiked into the sample mixes at a level predicted to be above the concentration of the substance

likely to be found in stool specimens. Substances included endogenous, exogenous as well as technique-specific substances.

Testing included samples containing negative clinical stool matrix in Para-Pak C&S medium with and without the addition of each potentially interfering substance. Samples containing organism mixes with one strain for each targeted pathogen were tested at a concentration of 3x LoD. Testing was performed in triplicates. Additionally, for endogenous substances, negative specimens (stool matrix in Para-Pak C&S medium matrix with no organism mix) were spiked with only the test substance to evaluate the potential for false positive results due to the test substance itself.

For the majority of substances tested, no interference was observed, with the exceptions of mucin, calcium carbonate, nonoxynol-9, and Rotavirus reassortants which demonstrated interference at high concentrations.

The mucin at 5% w/v sample generated unexpected false positives results for the *Yersinia* target. These results were investigated by testing the interfering substance with an FDA-cleared method that also detected *Yersinia* in the sample, demonstrating that the unexpected positive results were not due to mucin interference.

Calcium carbonate at concentrations above 0.5% w/v was found to generate false negative results for all the QIAstat-Dx Gastrointestinal Panel 2 targets and the internal control.

Nonoxynol-9 at concentrations above 0.02% v/v was found to generate false negative results for detection of *Entamoeba histolytica*.

As expected, Rotavirus reassortants WC3:2-5, R574(9) and WI79-4,9 used in Rotavirus A vaccines generated positive results for Rotavirus A in the QIAstat-Dx Gastrointestinal Panel 2. Final concentrations without interference (i.e., no false positive results for Rotavirus) for WC3:2-5, R574(9) and WI79-4,9 were 8.89×10^{-5} $TCID_{50}$/mL and 1.10 PFU/mL, respectively.

The strains used for sample mixes tested in the study are presented in Table 6**.** Results from testing the 35 endogenous and exogenous substances evaluated are provided in Table 7.

Table 6: Interfering Substances Study, Microorganisms/Viruses Evaluated

Mix	Target	Strain	Supplier	Catalog ID	Final Concentration tested (3x LoD)
1	*Giardia lamblia*	Portland-1	ATCC	30888	1.91E+03 cells/mL
	Enteropathogenic *E. coli* (EPEC)	B171-8 (O111:NM)	ZeptoMetrix	0801747	7.75E+03 CFU/mL
	Yersinia enterocolitica	Z036	ZeptoMetrix	0801734	6.21E+03 CFU/mL
	Salmonella	*Salmonella enterica* Serovar Typhimurium; Z005	ZeptoMetrix	0801437	1.36E+04 CFU/mL

Mix	Target	Strain	Supplier	Catalog ID	Final Concentration tested (3x LoD)
	Adenovirus F40/F41	Adenovirus Type 41 (Tak)	ZeptoMetrix	0810085CF	1.50E-01 $TCID_{50}$/mL
2	Norovirus GI/GII	Norovirus GII-recombinant	ZeptoMetrix	0810087CF	3.15E+01 $TCID_{50}$/mL
	Entamoeba histolytica	HM-1:IMSS	ATCC	30459	6.60E-01 cells/mL
	Campylobacter .	*Campylobacter jejuni* Z086	ZeptoMetrix	0801650	4.98E+03 CFU/mL
	Shiga-like toxin-producing *E. coli* (STEC) O157	O157:H7; EDL933	ZeptoMetrix	0801622	6.84E+03 CFU/mL
3	*Cryptosporidium*	*Cryptosporidium parvum*, Iowa isolate	Waterborne	P102C	1.88E+03 oocysts/mL
	Plesiomonas shigelloides	Z130	ZeptoMetrix	0801899	6.87E+03 CFU/mL
	Rotavirus A	69M	ZeptoMetrix	0810280CF	1.31E+03 U/mL
4	Astrovirus	(Type 8) ERE IID 2371	ZeptoMetrix	0810277CF	3.51E+01 U/mL
	Cyclospora cayetanensis	n/a	LACNY	LAC2825	1.37E+04 copies/mL
	Enterotoxigenic *E. coli* (ETEC) *lt*/*st*	ETEC; ST+, LT+	ZeptoMetrix	0801624	1.70E+03 CFU/mL
	Enteroinvasive *E. coli* (EIEC)/*Shigella*	CDC EDL 1282; Escherichia coli serotype O29:NM	ATCC	43892	1.24E+02 CFU/mL
5	Negative (IC)	n/a	n/a	n/a	n/a

Table 7: Interfering Substances Study Results

Substance tested	Concentration tested	Result
Endogenous substances		
Bovine and ovine bile	12% w/v	No Interference
Cholesterol	1.5% w/v	No Interference
Fatty acids (palmitic acid)	0.2% w/v	No Interference
Fatty acids (stearic acid)	0.4% w/v	No Interference
Human genomic DNA	20 µg/mL 5 ug/mL	No Interference No Interference
Human stool (overfill of transport medium vial)	300 mg/mL	No Interference
Human urine	50% v/v	No Interference
Human whole blood with Na Citrate	40% v/v	No Interference

Substance tested	Concentration tested	Result
Mucin from bovine submaxillary	5% w/v 2.5% w/v	**Interference**[1] No Interference
Triglycerides	5% w/v	No Interference
Exogenous substances		
Bacitracin	250U/mL	No Interference
Bisacodyl	0.3% w/v	No Interference
Bismuth subsalicylate	0.35% w/v	No Interference
Calcium carbonate (TUMS Extra Strength 750)	5%w/v 0.5% w/v	**Interference** No Interference
Docusate sodium	2.5% w/v	No Interference
Doxycycline hydrochloride	0.05% w/v	No Interference
Glycerin	50% v/v	No Interference
Hydrocortisone	0.5% w/v	No Interference
Loperamide hydrochloride	0.078% w/v	No Interference
Magnesium hydroxide	0.1% w/v	No Interference
Metronidazole	1.5% w/v	No Interference
Mineral oil	50% v/v	No Interference
Naproxen sodium	0.7% w/v	No Interference
Nonoxynol-9	1.2% v/v 0.6% v/v 0.3% v/v 0.15% v/v 0.075% v/v 0.02% v/v	**Interference** **Interference** **Interference** **Interference** **Interference** No Interference
Nystatin	10000 USP units/mL	No Interference
Phenylephrine hydrochloride	0.075% w/v	No Interference
Sodium phosphate	5% w/v	No Interference
Vaccine components		
Rotavirus reassortant WC3:2-5, R574(9) - VR 2195	8.89×10^{-3} $TCID_{50}$/mL 8.89×10^{-4} $TCID_{50}$/mL 8.89×10^{-5} $TCID_{50}$/mL	**Interference** **Interference** No Interference
Rotavirus reassortant WI79-4,9 - VR 2415	1.10×10^{2} pfu/mL 1.10×10^{1} pfu/mL 1.10 pfu/mL	**Interference** **Interference** No Interference
Technique-specific Substances, Transport Media		
Bleach	0.5% v/v	No Interference
Ethanol	0.2% v/v	No Interference

Substance tested	Concentration tested	Result
Puritan Fecal Opti-Swab Collection &Transport System with Cary-Blair Medium[2]	100%	No Interference
Puritan PurSafe DNA/RNA Preservative[2]	100%	No Interference
Sigma Fecal Transwab[2]	1 swab/2mL Cary Blair	No Interference

[1]This substance was tested by another FDA-cleared test that also detected *Yersinia* positive signals.
[2]**Note: Performance has not established for these transport media**

***Microbial Interference*:**

A microbial interference study was conducted to assess whether non-target microorganisms/viruses can interfere with the detection of panel targets. Clinically relevant and challenging concentrations of non-target organisms (1.0E+06 x 10^6 CFU/mL for bacteria, 1.0E +05 cells/mL for yeast and 1.0E+05 $TCID_{50}$/mL for viruses) were individually co-spiked with each of the targeted pathogens (Table 6 above) at 3x LoD. Samples were prepared in negative stool matrix in Para-Pak C&S transport medium. Testing was performed in triplicate. Microbial interference was not observed as all combinations and replicates tested successfully detected all the QIAstat-Dx Gastrointestinal Panel 2 targets. Results from the study are presented in Table 8.

Table 8: Microbial Interference Testing Results

Non-Target Microrganism/Virus Tested	Concentration tested	Result
Aeromonas hydrophila	1.0E+06 units/mL	No Interference
Bacteroides vulgatus	1.0E+06 units/mL	No Interference
Bifidobacterium bifidum	1.0E+06 units/mL	No Interference
Enterovirus Species D, Serotype EV-D68	1.0E+05 units/mL	No Interference
Non-pathogenic *E. coli*	1.0E+06 units/mL	No Interference
Helicobacter pylori	1.0E+06 units/mL	No Interference
Saccharomyces cerevisiae (deposited as *S. boulardii*)	1.0E+05 units/mL	No Interference

Competitive Interference

The potential for competitive interference was evaluated for a subset of analytes detected by the QIAstat-Dx Gastrointestinal Panel 2. The samples were prepared as a combination of two targeted pathogens in stool matrix (negative pooled stools resuspended in Para-Pak C&S transport medium) with one pathogen spiked at a high concentration (50x LoD) and the other spiked at a low concentration (3x LoD). Testing was conducted in triplicate. No false positives or false negative results were observed during the study. Results are presented in Table 9.

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Table 9: QIAstat-Dx Gastrointestinal Panel 2 Detection Rate Results for Competitive Interference

Mix	Pathogen	Concentration tested x LoD	Concentration tested	Detection rate	Coinfection Detected
Norovirus 50x - Rotavirus 3x	Norovirus GI/GII	50x	4.5E+05 copies/mL	3/3	Yes
	Rotavirus A	3x	1.7E+04 copies/mL	3/3	Yes
Norovirus 3x - Rotavirus 50x	Norovirus GI/GII	3x	2.7E+04 copies/mL	3/3	Yes
	Rotavirus A	50x	2.9E+05 copies/mL	3/3	Yes
Giardia 50x - Adenovirus 3x	Giardia lamblia	50x	7.2 E+05 copies/mL	3/3	Yes
	Adenovirus F40/F41	3x	2.9E+03 copies/mL	3/3	Yes

*Target only applicable for Para-Pak C&S samples

Analytical Reactivity (Inclusivity)

Analytical Reactivity (Inclusivity) testing was conducted to assess the ability of the QIAstat-Dx Gastrointestinal panel 2 to detect gastrointestinal pathogen isolates/strains that were selected based on clinical relevance as well as temporal, geographical and phylogenetic diversity. Samples were prepared with pathogen mixes in negative stool matrix collected in Para-Pak C&S transport medium. Based on the previously established LoD for each assay target, three serial log dilutions were tested with four replicates per concentration and per strain/isolate. The lowest concentration for which all four replicates were detected was identified and two more replicates were tested for a total of six sample replicates. Most pathogen strains evaluated (104/114) were detected at ≤3-fold of the corresponding LoD reference strain. Less than 100% detection was observed for one strain each of ETEC, EIEC/*Shigella* and Rotavirus and two strains each of STEC (one STEC O157), Adenovirus and Norovirus when tested at a concentration of <3x LoD. Testing of these strains at 10x LoD generated the expected positive resulted for all replicates. For each strain evaluated in the study, the lowest concentration (based on a factor of LoD) that generated positive results for 6/6 replicates are presented in tables 10-26 below. LoD reference strains are shown in bold for each targeted analyte.

An additonal evaluation of inclusivity for the QIAstat-Dx GI Panel 2 targets was conducted using *in silico* analysis of bacterial, viral, and parasite strains with available sequences in the database. All single primers and probes used for detection of each assay target included in the QIAstat-Dx Gastrointestinal Panel 2 were analyzed to predict the reactivity of the panel using BLAST. Further analysis of the predicted reactivity was performed through alignment

analysis including both entire genome sequences and specific target gene sequences available from the identified BLAST accession hits. Results from *in silico* analysis are presented in Table 27 below.

Based on the results observed for inclusivity *in vitro* (wet) testing and *in silico* analysis, the QIAstat-Dx GI Panel 2 primers and probes are inclusive for each targeted analyte for clinically relevant strains, including species, subspecies, subtypes, serotypes or serovars, as applicable.

Table 10: Inclusivity, *Campylobacter* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Factor of LoD
Campylobacter	***Campylobacter coli***	**76-GA2 [LMG 21266]**	**ATCC**	**43478[1]**	**1x LoD**
	Campylobacter coli	Z293	ZeptoMetrix	0804272	1x LoD
	Campylobacter coli	CIP 7080 [1407, CIP 70.80]	ATCC	335591	3x LoD
	Campylobacter jejuni	**Z086**	**ZeptoMetrix**	**0801650[1]**	**1x LoD**
	Campylobacter jejuni	subsp. *jejuni* RM3193	ATCC	BAA-1234*	0.1x LoD
	Campylobacter jejuni subsp. jejuni	O:19 HL7; D3180	ATCC	BAA-218	0.1x LoD
	Campylobacter jejuni subsp. jejuni	AS-83-79	ATCC	33291	0.1x LoD
	Campylobacter jejuni subsp. doylei	NCTC 11951	ATCC	49349	0.1x LoD
	Campylobacter upsaliensis	**NCTC 11541**	**ZeptoMetrix**	**0801999[1]**	**1x LoD**
	Campylobacter upsaliensis	RM 3195 (1994)	ATCC	BAA-1059	0.3x LoD
	Campylobacter upsaliensis	NCTC 11541 [C231]	ATCC	43954	1x LoD

[1]Strain tested during LoD verification study.

Table 11: Inclusivity, *Plesiomonas shigelloides* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Factor of LoD
Plesiomonas shigelloides	***Plesiomonas shigelloides***	**Z130**	**ZeptoMetrix**	**0801899[1]**	**1x LoD**
	Plesiomonas shigelloides	GNI 14	ATCC	51903	1x LoD

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Factor of LoD
	Plesiomonas shigelloides	CDC 3085-55 [Bader M51, NCIB 9242, NCTC 10360, RH 798]	ATCC	14029[1]	0.3x LoD

[1]Strain tested during LoD verification study.

Table 12: Inclusivity, *Salmonella* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Factor of LoD
Salmonella	***Salmonella enterica***	**Serovar Typhimurium Z005**	**ZeptoMetrix**	**0801437[1]**	**1x LoD**
	Salmonella enterica	Subsp. Enterica, serovar Bareilly	NCTC	NC05745	1x LoD
	Salmonella enterica	Subsp. Enterica, serovar typhi, Z152	ZeptoMetrix	0801933	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Enteridis, CDC K-1891 [ATCC 25928]	ATCC	13076	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Infantis, MZ1479 [SARB27]	ATCC	BAA-1675	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Montevideo, G4639	ATCC	BAA-710	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Javiana	NCTC	NC06495	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Thompson	NCTC	NC08496	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Saintpaul	ATCC	9712	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Berta	NCTC	NC05770	0.1x LoD
	Salmonella enterica	Subsp. Salame, II NCTC 10310 [JT945, SS140/61]	ATCC	700151	0.1x LoD
	Salmonella enterica	Subsp. diarizonae IIIb, 62	ATCC	29934	0.1x LoD
	Salmonella enterica	Subsp. houtenae IV, CIP 82.32 [264.66]	ATCC	43974	0.1x LoD
	Salmonella enterica	Subsp. Indica VI, CIP 102501 [F. Kauffmann 1240]	ATCC	43976	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Agona, CDC 873 [CDC 1111-61]	ATCC	51957	0.1x LoD

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Factor of LoD
	Salmonella enterica	Subsp. Enterica, serovar Muenchen, 54	ATCC	8388	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Oranienburg, E1093	ATCC	9239	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Paratyphi B var. Java, CDC 5	ATCC	51962	0.1x LoD
	Salmonella bongori	CIP 82.33 [1224.72]	ATCC	43975	0.3x LoD
	Salmonella enterica	Subsp. Enterica, serovar Choleraesius,NCTC 5735 [1348, K.34]	ATCC	13312[1]	0.3x LoD
	Salmonella enterica	Subsp. Enterica, serovar Newport, C487-69	ATCC	27869	0.3x LoD
	Salmonella enterica	Subsp. Enterica, 4, 5, 12:7 serovar Typhimurium	NCTC	NC13952	0.3x LoD
	Salmonella enterica	Subsp. Enterica, serovar Braenderup	ATCC	700136	0.3x LoD
	Salmonella enterica	Subsp. Enterica, serovar Anatum	NCTC	NC05779	0.3x LoD
	Salmonella enterica	Subps. arizonae IIIa, NCTC 7311 [CDAI 426]	ATCC	700156	0.3x LoD
	Salmonella enterica	Subsp. Enterica, serovar Heidelberg, [16]	ATCC	8326	0.3x LoD
	Salmonella enterica	Subsp. Enterica, serovar Mississippi, CDC 2012K-0487	ATCC	BAA-2739	0.3x LoD

[1] Strain tested during LoD verification study

Table 13: Inclusivity, *Yersinia enterocolitica* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Yersinia enterocolitica	***Yersinia enterocolitica***	**Z036**	**ZeptoMetrix**	**0801734[1]**	**1x LoD**
	Yersinia enterocolitica	NTCC 11175, Biotype 4, serotype 3 (O:3)	ATCC	700822*	1x LoD
	Yersinia enterocolitica	33114 [CCUG 11291, CCUG 12369, CIP 80.27, DSM 4780, LMG 7899, NCTC 12982], Biovar 1, O:8	ATCC	9610	1x LoD
	Yersinia enterocolitica	0:9	ATCC	55075	3x LoD

[1] Strain tested during LoD verification study

Table 14: Inclusivity, Enteropathogenic *E. coli* (EPEC) strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Enteropathogenic *E. coli* (EPEC)	**Enteropathogenic *E. coli* (EPEC)**	**O111:NM**	**ZeptoMetrix**	**0801747[1]**	**1x LoD**
	Enteropathogenic *E. coli* (EPEC)	7.1493,O84:H28	ZeptoMetrix	0801938	1x LoD
	Enteropathogenic *E. coli* (EPEC)	Stoke W,O111:K58(B4):H-	ATCC	33780	1x LoD

[1] Strain tested during LoD verification study

Table 15: Inclusivity, Enterotoxigenic *E. coli* (ETEC) strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Enterotoxigenic *E. coli* (ETEC) lt/st	**Enterotoxigenic *E. coli* (ETEC) lt/st**	**ST+, LT+**	**ZeptoMetrix**	**0801624[1]**	**1x LoD**
	Enterotoxigenic *E. coli* (ETEC) lt/st	H10407,O78:H11,LT(+)/ctx A11(+)	ATCC	35401[1]	0.3x LoD
	Enterotoxigenic *E. coli* (ETEC) lt/st	O27:H7,ST (+)/ LT (-)	SSI Diagnostica	82173	0.1x LoD
	Enterotoxigenic *E. coli* (ETEC) lt/st	O115:H15,ST (+)/ LT (-)	SSI Diagnostica	82174	3x LoD
	Enterotoxigenic *E. coli* (ETEC) lt/st	O169:H-,ST (-)/LT (+)	SSI Diagnostica	82172	10x LoD[2]

[1]Strain tested during LoD verification study

[2]Testing at a lower concentration resulted in a detection rate of <100%

Table 16: Inclusivity, Enteroinvasive *E. coli* (EIEC)/*Shigella* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Enteroinvasive *E. coli (EIEC)/ Shigella*	**Enteroinvasive *E. coli* (EIEC)**	**CDC EDL 1282, O29:NM**	**ATCC**	**43892[1]**	**1x LoD**
	Enteroinvasive *E. coli* (EIEC)	O172:H-	SSI Diagnostica	82171	3x LoD
	Shigella sonnei	**NCDC 1120-66**	**ATCC**	**25931[1]**	**1x LoD**
	Shigella boydii (Serogroup C)	Z131	ZeptoMetrix	0801900	1x LoD
	Shigella flexneri (Serogroup B)	AMC 43-G-68 [EVL 82, M134]	ATCC	9199	1x LoD
	Shigella flexneri (Serogroup B)	Z046	ZeptoMetrix	0801757	1x LoD
	Shigella sonnei (Serogroup D)	WRAIR I virulent	ATCC	29930	1x LoD

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
	Shigella sonnei (Serogroup D)	Z004	ZeptoMetrix	0801627	3x LoD
	Shigella boydii (Serogroup C)	AMC 43-G-58 [M44 (Type 170)]	ATCC	9207	10x LoD[2]

[1]Strain tested during LoD verification study

[2]Testing at a lower concentration resulted in a detection rate of <100%

Table 17: Inclusivity, Shiga-like toxin-producing *E. coli* (STEC) (*stx2*-carrier strains)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog	Times LoD
Shiga-like toxin producing *E. coli* (STEC) - *stx1*/2	**Shiga-like toxin producing *E. coli* (STEC) - stx1/stx2**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622[1]**	**1x LoD**
	Shiga-like toxin producing *E. coli* (STEC) - *stx1*	O26:H4,*stx1* (+)	ZeptoMetrix	0801748[1]	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - *stx1*/*stx2*	Reference ATCC® 35150 (EDL 931),O157:H7,*stx1* (+), *stx2* (+)	Microbiologics	617	3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - *stx1*	Reference CDC 00-3039,O45:H2,unknown	Microbiologics	1098	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - *stx1*	O103:H2,*stx1* (+)	SSI Diagnostica	82170	3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - *stx1*/*stx2*	O22:H8,*stx1*c (+), *stx2*b (+)	SSI Diagnostica	91350	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - *stx2*	O92,O107:K+:H48, *stx2*d (+)	SSI Diagnostica	91352	10x LoD[2]
	Shiga-like toxin producing *E. coli* (STEC) - *stx2*	O101:K32:H-,*stx2*e (+)	SSI Diagnostica	91354	0.3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - *stx1*/*stx2*	O128ac:H-,*stx2*f (+)	SSI Diagnostica	91355	10x LoD[2]
	Shiga-like toxin producing *E. coli* (STEC) - *stx2*	O26:H11,*stx2*a (+)	SSI Diagnostica	95211	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - *stx1*	O8 ,*stx1*d (+)	SSI Diagnostica	91349	1x LoD

[1]Strain tested during LoD verification study

[2]Testing at a lower concentration resulted in a detection rate of <100%

Table 18: Inclusivity, Shiga-like toxin producing *E. coli* (STEC) O157 strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalogue ID	Times LoD
Shiga-like toxin producing *E. coli* (STEC) O157	**Shiga-like toxin producing *E. coli* (STEC) - O157**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622[1]**	**1x LoD**
	Shiga-like toxin producing *E. coli* (STEC) O157	O128ac:H-,*stx2*f (+)	SSI Diagnostica	91355[2]	10x LoD[3]
	Shiga-like toxin producing *E. coli* (STEC) O157	Reference ATCC 35150 (EDL 931), O157:H7, *stx1* (+), *stx2* (+)	Microbiologics	617	1x LoD

[1]Strain tested during LoD verification study

[2]The *E. coli* strain 91355 from SSI Diagnostica is reported as following in its catalog: *vtx2f*+, *eae*+. However, it was found to amplify for *E. coli* O157 in both QIAstat-Dx and FilmArray device

[3]Testing at a lower concentration resulted in a detection rate of <100%

Table 19: Inclusivity, *Cryptosporidium* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Cryptosporidium	***Cryptosporidium parvum***	**Iowa isolate**	**Waterborne**	**P102C[1]**	**1x LoD**
	Cryptosporidium hominis	n/a	Public Health Wales	Clinical sample; UKM 84[1]	0.01x LoD
	Cryptosporidium parvum	–	ATCC	PRA-67DQ (isolated genomic DNA)	<0.01 LoD
	Cryptosporidium meleagridis	–	Public Health Wales	Clinical sample; UKMEL 14	<0.01 LoD

[1] Strain tested during LoD verification study

Table 20: Inclusivity, *Cyclospora cayetanensis* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalogue ID	Times LoD
Cyclospora cayetanensis	***Cyclospora cayetanensis***	**n/a**	**Clinical sample**	**LAC2825[1]**	**1x LoD**
	Cyclospora cayetanensis	n/a	Clinical sample	LAC2827[1]	1x LoD
	Cyclospora cayetanensis	–	ATCC	PRA-3000SD	1x LoD

[1] Strain tested during LoD verification study

Table 21: Inclusivity, *Entamoeba histolytica* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalogue ID	Times LoD
Entamoeba histolytica	***Entamoeba histolytica***	**HM-1:IMSS (Mexico City 1967)**	**ATCC**	**30459[1]**	**1x LoD**
	Entamoeba histolytica	HK-9 (Korea)	ATCC	30015[1]	1x LoD
	Entamoeba histolytica	–	Vall d'Hebrón	Clinical sample	1x LoD

[1] Strain tested during LoD verification study

Table 22: Inclusivity, *Giardia lamblia* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalogue ID	Times LoD
Giardia lamblia	***Giardia lamblia***	**Portland -1 (Portland, OR, 1971)**	**ATCC**	**30888[1]**	**1x LoD**
	Giardia lamblia	WB (Bethesda, MD, 1979)	ATCC	30957[1]	1x LoD
	Giardia intestinalis	H3 isolate	Waterborne	P101	1x LoD

[1] Strain tested during LoD verification study

Table 23: Inclusivity, Adenovirus F40/F41 strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Adenovirus F40/F41	**Human Adenovirus F41**	**Tak**	**ZeptoMetrix**	**0810085CF[1]**	**1x LoD**
	Human Adenovirus F41	Tak (73-3544)	ATCC	VR-930	10x LoD[2]
	Human Adenovirus F40	Dugan [79-18025]	ATCC	VR-931	10x LoD[2]
	Human Adenovirus Type 40	Dugan	ZeptoMetrix	0810084CF[1]	3x LoD

[1]Strain tested during LoD verification study

[2]Testing at a lower concentration resulted in a detection rate of <100%

Table 24: Inclusivity, Astrovirus strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Astrovirus	**Human Astrovirus**	**ERE IID 2371 (type 8)**	**ZeptoMetrix**	**0810277CF[1]**	**1x LoD**
	Human Astrovirus	HAstV-1	Universitat de Barcelona	Clinical sample; 160521599	1x LoD

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
	Human Astrovirus	ERE IID 2868 (type 4)	ZeptoMetrix	0810276CF[1]	1x LoD
	Human Astrovirus	HAstV-3	Universitat de Barcelona	Clinical sample; 151601306	1x LoD

[1] Strain tested during LoD verification study

Table 25: Inclusivity, Norovirus GI/GII strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Norovirus GI/GII	**Human Norovirus Genogroup 1**	**Recombinant GI.1**	**ZeptoMetrix**	**0810086CF[1]**	**1x LoD**
	Human Norovirus Genogroup 1	–	Indiana University Health	Clinical sample; IU3156	1x LoD
	Human Norovirus Genogroup 1	–	Indiana University Health	Clinical sample; IU3220	1x LoD
	Human Norovirus Genogroup 1	–	TriCore Reference Laboratories	Clinical sample; TC4274	3x LoD
	Human Norovirus Genogroup 2	**Recombinant GII.4**	**ZeptoMetrix**	**0810087CF[1]**	**1x LoD**
	Human Norovirus Genogroup 2	GII.2	Vall d'Hebrón	Clinical sample; 198058327	1x LoD
	Human Norovirus Genogroup 2	GII.4	Universitat de Barcelona	Clinical sample; N26.2TA	1x LoD
	Human Norovirus Genogroup 2	–	Lacny Hospital	Clinical sample; LAC2019	1x LoD
	Human Norovirus Genogroup 2	–	Nationwide Children's Hospital	Clinical sample; NWC6063	1x LoD
	Human Norovirus Genogroup 2	GII.6	QIAGEN Barcelona (STAT-Dx)	Clinical sample; GI 12	3x LoD
	Human Norovirus Genogroup 2	–	Lacny Hospital	Clinical sample; LAC2133	10x LoD[2]
	Human Norovirus Genogroup 2	–	Lacny Hospital	Clinical sample; LAC2074	10x LoD[2]

[1]Strain tested during LoD verification study

[2]Testing at a lower concentration resulted in a detection rate of <100%

Table 26: Inclusivity, Rotavirus A strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Rotavirus A	**Human Rotavirus A**	**69M**	**ZeptoMetrix**	**0810280CF[1]**	**1x LoD**
	Human Rotavirus A	Wa, G1P1A[8]	ZeptoMetrix	0810041CF[1]	1x LoD
	Human Rotavirus A	DS-1, G2P1B[4]	ATCC	VR-2550	1x LoD
	Human Rotavirus A	Va70	ZeptoMetrix	0810281CF	1x LoD
	Human Rotavirus A	RRV	ZeptoMetrix	0810530CF	10x LoD[2]

[1]Strain tested during LoD verification study

[2]Testing at a lower concentration resulted in a detection rate of <100%

Table 27: Microorganisms/Viruses with Predicted Reactivity Based on *In silico* Analysis

QIAstat-Dx GI Panel 2 Target	Microorganisms/viruses with predicted reactivity
Bacteria	
Campylobacter	*Campylobacter coli*, Campylobacter jejuni, Campylobacter jejuni* subsp. *jejuni*, *Campylobacter jejuni* subsp. *doylei, Campylobacter upsaliensis*
Salmonella	*Salmonella bongori**, *Salmonella enterica* subsp. *salamae* II (e.g. serovar 55:k:z39), *Salmonella enterica* subsp. *arizonae* IIIa (e.g. serovar 63:g:z51), *Salmonella enterica* subsp. *diarizonae* IIIb (e.g. serovar 47:l,v:z), *Salmonella enterica* subsp. *houtenae* IV (e.g. serovar 43:z4), *Salmonella enterica* subsp. *indica* VI.
	Salmonella enterica subsp. *enterica* (up to 92 different serovars including Agona, Anatum, Bareilly, Choleraesuis, Enteritidis, Heidelberg, Infantis, Kentucky, Montevideo, Newport, Paratyphi A*, Senftenberg, Tennessee, Thompson, Typhi, Typhimurium, Weltevreden*)
Plesiomonas shigelloides	*Plesiomonas shigelloides* (e.g. strains NCTC10360, ATCC 14029T, R4605035)
Yersinia enterocolitica	*Yersinia enterocolitica, Yersinia enterocolitica* subsp. *palearctica, Yersinia enterocolitica* subsp. *enterocolitica*
Enteroinvasive *E. coli* (EIEC)/*Shigella*	Enteroinvasive *E. coli* (EIEC), *Escherichia coli* sp., *Shigella flexneri, Shigella dysenteriae, Shigella boydii, Shigella sonnei.*
Enteropathogenic *E. coli* (EPEC) β	Enteropathogenic *E. coli* (EPEC) (e.g. including serotypes OUT: HND, OUT:H6, OUT:H34, OUT:H21, O55:H7, O119:HNM, O117)
Enterotoxigenic *E. coli* (ETEC) &	Enterotoxigenic *E. coli* (ETEC) (including H10407 and E24377A strains and serotypes O169:H41, O25:H42, O148:H28, O6:H16) carrier of: Heat-labile enterotoxin gene subtype LT-I and Heat-stable enterotoxin gene variant Sta, subtypes STp and STh
Shiga-like toxin-producing *E. coli* (STEC) - *stx1*/*stx2* β	Shiga-like toxin-producing *E. coli* (STEC) including O157:H7 and O157:NM serotype and non-O157 serotypes (O111:NM, O111:H-, O26:H11, O145:NM, O145:H28, O45:H2,

QIAstat-Dx GI Panel 2 Target	Microorganisms/viruses with predicted reactivity
	O26:H11, ONT:NM, O104:H4, O121:H19, O145:H34, O113:H21, ONT:H-, O128:H2, OUT:HNM, O124:HNM *E. coli* strains carrier of: *stx1*a, *stx1*c, *stx1*d, *stx2*a, *stx2*b, *stx2*c, *stx2*d, *stx2*d, *stx2*e, *stx2*f, *stx2*g, *stx2*h, *stx2*i, *stx2*j, *stx2*k and *stx2*l. Other stx-carrying bacteria: *Shigella sonnei*, *Shigella dysenteriae*
Shiga-like toxin-producing *E. coli* (STEC) O157 [β]	*Escherichia coli* O157 including: STEC O157:H7 strains (e.g. EDL933) and *E. coli* O157: non-H7 groups including non-Shiga-toxigenic *E. coli* O157 bacteria (e.g. serotype O157:H45)
Parasites	
Cryptosporidium[#]	Common *Cryptosporidium* species involved in human disease: *C. parvum, C. hominis.* Less common *Cryptosporidium* species involved in human infections: *C. meleagridis, C. felis , C. bovis, C. viatorum, C. ubiquitum,C. tyzzeri, C. cuniculus, Cryptosporidium sp. Chipmunk genotype I, C. canis*.* Rare or non-human species: *Cryptosporidium wrairi*
Cyclospora cayetanensis	*Cyclospora cayetanensis* (including strains LG, CY9, NP20 and NP21) *
Entamoeba histolytica	*Entamoeba histolytica* (e.g. strains HM-1: IMSS, EHMfas1, HK-9)*
Giardia lamblia	*Giardia lamblia* (aka *Giardia duodenalis*, *Giardia intestinalis*)*
Viruses	
Adenovirus	Human Adenovirus F40/41
Astrovirus[$]	Human Astrovirus (including types 1, 2, 3, 4, 5, 6, 7, 8)
Norovirus GI/GII	Norovirus genogroup II genotypes: GII.1, GII.2, GII.3*, GII.4*, GII.5, GII.6, GII.7, GII.8, GII.9, GII.10, GII.12, GII.13, GII.14, GII.16, GII.17, GII.20, GII.21, GII.22, GII.23, GII.24*, GII.25, GII.26, GII.27, GII.NA1 and GII.NA2*
	Norovirus genogroup I genotypes: GI.1, GI2, GI.3*, GI.4*, GI.5, GI.6*, GI.7*, GI.8, GI.9.
Rotavirus	Rotavirus A including genotypes: G1P[8]*, G2P[4]*, G3P[8]*, G4P[8]*, G9P[6], G9P[8]*, G12P[6]* and G12P[8]*

* Certain sequences are predicted to be detected with reduced sensitivity due to the presence of a reduced number of mismatches at critical positions of the primer-probe design.

[&]The assay is not predicted to detect bacteria carrier of Heat-labile enterotoxin gene subtype LT-II and/or of Heat-stable enterotoxin gene variant Stb e.

[#]The assay is not predicted to detect other *Cryptosporidium spp*. less involved in human disease: *C. andersoni* and *C. muris*.

[$] The assay is not predicted to detect Human Astrovirus types MLB1-3 and VA1-5.

[β] Only applicable for samples with Para-Pak C&S collection device

4. Assay Reportable Range:

   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Sample Stability

A study was conducted to evaluate the stability of stool specimens collected in Para-Pak C&S or FecalSwab transport media that are stored under claimed storage conditions (i.e., up to four days at room temperature (15°C to 25°C) or four days at refrigerated conditions (2°C to 8°C)). After storage under each storage condition, samples were tested with QIAstat-Dx Gastrointestinal Panel 2. For Para-Pak C&S, at least one strain was evaluated for each reportable analyte (26 strains). For FecalSwab, 11 representative bacterial, viral, and parasite strains were evaluated. Individual samples were prepared by spiking mixtures of up to seven different analytes at 2.5x LoD into human stool matrix in Para-Pak C&S and FecalSwab transport media. The study acceptance criteria to support claims for each storage condition required at least 90% (e.g., ≥9/10) of test replicates to generate a positive signal for each spiked pathogen. Sample replicates were tested fresh as well as after each day of storage at room temperature or refrigerated conditions.

Overall, all tested conditions resulted in a detection rate of ≥90% for all strains and storage conditions except for the following:

- For Para-Pak C&S, one strain of *Cyclospora cayetanensis* showed <90% detection at several time points for both refrigerated and room temperature storage. These results were found to be due to samples that were prepared at levels below the LoD. Testing of newly prepared samples generated the expected positive results for each test condition evaluated.
- For Para-Pak C&S, one strain of Enteroinvasive *E. coli* (EIEC)/*Shigella* (EIEC, ATCC 43892) *s*howed <90% detection at multiple time points for samples stored at room temperature. Retesting of additional replicates for this strain generated the expected positive results. An additional *Shigella strain* (*Shigella sonnei* (ATCC ref. 25931) was also evaluated and generated acceptable results for all test conditions.

Overall results of the sample stability study support the following storage conditions for stool specimens resuspended in Para-Pak C&S and FecalSwab transport media before testing with the QIAstat-Dx Gastrointestinal Panel 2:

- Room temperature up to 4 days at 15–25˚C
- Refrigerated up to 4 days at 2–8˚C

Assay Controls

External Controls

External Controls are not provided with the QIAstat-Dx Gastrointestinal Panel 2. The following information regarding external control testing is included in the Instructions for Use:

- All external quality control requirements and testing should be performed in accordance with local, state, and federal regulations or accreditation organizations and should follow the user's laboratory standard quality control procedures.

- Blank controls are not applicable to the device because it is a single test disposable cartridge. Regular testing of negative and positive external controls is recommended by the company but controls are not provided with the QIAstat-Dx Gastrointestinal Panel 2. Use transport media as the external Negative Control, and previously characterized positive samples or negative sample spiked with well characterized target organisms as external positive controls.

Internal Control

The QIAstat-Dx Gastrointestinal Panel Cartridge includes a full process Internal Control, which is titered *Schizosaccharomyces pombe*. *Schizosaccharomyces pombe* is a yeast (fungus) that is included in the cartridge in dried form and is rehydrated upon sample loading. This Internal Control material verifies all steps of the analysis process, including sample homogenization, lysis of viral and cellular structures (by means of chemical and mechanical disruption), nucleic acid purification, reverse transcription, and real-time PCR. A passed result for the Internal Control indicates that all processing steps performed by the QIAstat-Dx Gastrointestinal Panel Cartridge were successful. A failed result of the Internal Control does not negate any positive results for detected and identified targets, but it does invalidate all negative results in the analysis. Therefore, the test should be repeated if the Internal Control signal is negative.

Expected Values:

See Section VII(E) below

6. Detection Limit:

To establish the limit of detection (LoD) for each of the QIAstat-Dx Gastrointestinal Panel 2 targets, a total of 36 pathogen strains were evaluated in samples prepared from serial dilutions of culture isolates or positive clinical samples. Samples were prepared in human stool matrix, consisting of a pool of previously tested negative clinical stool specimens resuspended in Para-Pak C&S transport medium.

The LoD was established as the lowest concentration at which ≥95% of replicates are detected. A minimum of 20 replicates were tested to confirm the LoD for each strain. Additional testing of samples with concentrations below the LoD (0.3x LoD) demonstrated <95% detection, confirming the LoD results. LoD results for each microorganism/virus tested are presented in Table 28.

Table 28: LoD Study Results

Target Organism/ Analyte	Strain	Source/ Catalog ID	LoD concentration (microbiological units)	LoD concentration (molecular units)[1]	LoD Detection rate	Mean Ct Value
Campylobacter coli	*Campylobacter coli* 76-GA2 [LMG 21266]	ATCC 43478	**1.2 CFU/mL**	**5802 copies/mL**	20/20	35.6
Campylobacter coli	*Campylobacter coli* CIP 7080	ATCC 33559	**0.6 CFU/mL**	**8941 copies/mL**	20/20	34.5
Campylobacter jejuni	*Campylobacter jejuni* Z086	ZeptoMetrix 801650	**1660 CFU/mL**	**14491 copies/mL**	20/20	33.3
Campylobacter jejuni	*Campylobacter jejuni* subsp. jejuni RM3193	ATCC BAA-1234	**110 CFU/mL**	**7210 copies/mL**	19/20	36.3
Campylobacter upsaliensis	*Campylobacter upsaliensis* NCTC 11541	ZeptoMetrix 801999	**2259 CFU/mL**	**56165 copies/mL**	20/20	34.7
Campylobacter upsaliensis	*Campylobacter upsaliensis* RM3195	ATCC BAA-1059 9689	**35.0 CFU/mL**	**7631 copies/mL**	19/20	36.1
Plesiomonas shigelloides	Bader	ATCC 14029	**2.7 CFU/mL**	**116 copies/mL**	19/20	34.6
Plesiomonas shigelloides	Z130	ZeptoMetrix 801899	**2291 CFU/mL**	**481 copies/mL**	20/20	33.7
Salmonella enterica	*Salmonella enterica* Serovar Typhimurium Z005	ZeptoMetrix 801437	**4518 CFU/mL**	**1441 copies/mL**	20/20	32.5
Salmonella enterica	*Salmonella enterica* Serovar cholerasuis	ATCC 13312	**91.6 CFU/mL**	**647 copies/mL**	20/20	33.1
Yersinia enterocolitica	Z036	ATCC 700822	**120 CFU/mL**	**2496 copies/mL**	20/20	34.5
Yersinia enterocolitica	subsp. *enterocolitica* NTCC 11175, Biotype 4, serotype 3	ZeptoMetrix 801734	**2070 CFU/mL**	**719 copies/mL**	20/20	35.0
Shigella/Enteroinvasive *E. coli* (EIEC)	*Escherichia coli* CDC EDL 1282, O29:NM	ATCC 43892	**41 CFU/mL**	**1431 copies/mL**	20/20	35.4
Shigella/Enteroinvasive *E. coli* (EIEC)	*Shigella sonnei* NCDC 1120-66	ATCC 25931	**0.2 CFU/mL**	**488 copies/mL**	20/20	34.8

Target Organism/ Analyte	Strain	Source/ Catalog ID	LoD concentration (microbiological units)	LoD concentration (molecular units)[1]	LoD Detection rate	Mean Ct Value
Enteropathogenic *E. coli* (EPEC)	*Escherichia coli* O111:NM (EPEC)	ZeptoMetrix 801747	**2581 CFU/mL**	**1817 copies/mL**	20/20	35.5
Enteropathogenic *E. coli* (EPEC)	*Escherichia coli* 7.1493; EPEC; O84:H28	ZeptoMetrix 801938	**1190.0 CFU/mL**	**29021 copies/mL**	20/20	35.1
Enterotoxigenic *E. coli* (ETEC) lt/st	*Escherichia coli* ETEC; ST+, LT+	ZeptoMetrix 801624	**567 CFU/mL**	**855 copies/mL**	20/20	33.7
Enterotoxigenic *E. coli* (ETEC) lt/st	*Escherichia coli* H10407, O78:H11	ATCC 35401	**10.1 CFU/mL**	**367 copies/mL**	19/20	34.2
Shiga-like toxin-producing *E.coli* (STEC)	*Escherichia coli* O26:H4	ZeptoMetrix 801748	**726.8 CFU/mL**	**2012 copies/mL**	20/20	36.1
Shiga-like toxin-producing E.coli (STEC)	*Escherichia coli* O157:H7; EDL933	ZeptoMetrix 801622	**2281 CFU/mL**	**1217 copies/mL**	19/20	35.3
Cryptosporidium parvum	*Cryptosporidium parvum* – Iowa isolate	Waterborne P102C	**N/A**	**661 copies/mL**	20/20	28.3
Cryptosporidium hominis	*Cryptosporidium hominis*	Clinical Sample UKM 84	**N/A**	**357 copies/mL**	20/20	32.4
Cyclospora cayetanensis	N/A	Clinical Sample LAC2825	**N/A**	**53 copies/mL**	19/20	35.3
Cyclospora cayetanensis	N/A	Clinical Sample LAC2827	**N/A**	**137 copies/mL**	20/20	35.0
Entamoeba histolytica	HM-1:IMSS (Mexico City 1967)	ATCC 30459	**0.2 cells/mL**	**7 copies/mL**	20/20	36.6
Entamoeba histolytica	HK-9 (Korea)	ATCC 30015	**0.13 cells/mL**	**1 copy/mL**	19/20	37.0
Giardia lamblia	WB (Bethesda)	ATCC 30957	**790 cells/mL**	**11850 copies/mL**	19/20	32.1
Giardia lamblia	Portland-1	ATCC 30888	**635.0 cells/mL**	**14500 copies/mL**	20/20	31.2
Adenovirus F40	Dugan	ZeptoMetrix 0810084CF	**0.1 $TCID_{50}$/mL**	**11726 copies/mL**	20/20	35.7
Adenovirus F41	Type 41 (Tak)	ZeptoMetrix 0810085CF	**0.05 $TCID_{50}$/mL**	**979 copies/mL**	19/20	36.6

Target Organism/ Analyte	Strain	Source/ Catalog ID	LoD concentration (microbiological units)	LoD concentration (molecular units)[1]	LoD Detection rate	Mean Ct Value
Astrovirus	ERE IID 2371 (type 8)	ZeptoMetrix 0810277CF	**11.7 $TCID_{50}$/mL**	**11586371 copies/mL**	20/20	32.4
Astrovirus	ERE IID 2868 (type 4)	ZeptoMetrix 0810276CF	**1.3 $TCID_{50}$/mL**	**52184 copies/mL**	19/20	32.4
Norovirus GI	GI.1 (recombinant)	ZeptoMetrix 0810086CF	**891.1 $TCID_{50}$/mL**	**24629 copies/mL**	19/20	33.6
Norovirus GII	GII.4 (recombinant)	ZeptoMetrix 0810087CF	**10.5 $TCID_{50}$/mL**	**8998 copies/mL**	20/20	33.6
Rotavirus A	WA	ZeptoMetrix 0810041CF	**14.1 $TCID_{50}$/mL**	**5201 copies/mL**	19/20	36.4
Rotavirus A	69M	ZeptoMetrix 0810280CF	**436 $TCID_{50}$/mL**	**5787 copies/mL**	19/20	36.9

[1] Pathogen titer from clinical samples was determined by molecular units (copies/mL) using in-house developed and validated qPCR assays. Pathogen titer of clinical samples is not provided in microbiological units.

7. Carryover/Cross-Contamination Study:

A study was performed to evaluate the potential for carryover or cross-contamination between consecutive runs when using the QIAstat-Dx Gastrointestinal Panel 2 on the QIAstat-Dx Analyzer 1.0. Samples were prepared in stool matrix in Para-Pak C&S transport medium. Testing included alternating high-positive samples (1.0E+06 CFU/mL for bacteria, 1.0E+05 $TCID_{50}$ for viruses, or 1.0E+05 organisms/mL for parasites) and negative samples. A total of 31 positive samples and 31 negative samples were tested over five days using two QIAstat-Dx Analyzer 1.0 instruments. Results from testing generated the expected results for all positive and negative samples, demonstrating no carryover or cross-contamination for the QIAstat-Dx Gastrointestinal Panel 2 when run on the QIAstat-Dx analyzer. Analyte mixes used to prepare high positive samples are presented in Table 29.

Table 29: Carryover Study, Analytes Tested

Sample	Pathogen/Target	Concentration tested
Positive Mix 1	Norovirus GI	1.0E+05 $TCID_{50}$/mL
	Plesiomonas shigelloides	1.0E+06 CFU/mL
	Shiga-like toxin-producing *E. coli* (STEC) (*stx1*+)	1.0E+06 CFU/mL
	Cryptosporidium parvum	1.0E+05 oocysts/mL

Positive Mix 2	*Campylobacter jejuni*	1.0E+06 CFU/mL
Negative mix	None	N/A

8. Transport Media Equivalency Study:

A transport media equivalency study was conducted to assess potential performance differences between Para-Pak C&S and FecalSwab transport devices. Specimens tested in the study were prepared using raw clinical stool specimens that were known positive for representative analytes detected by the QIAstat-Dx Gastrointestinal Panel 2. Each positive raw stool was inoculated into both Para-Pak C&S and FecalSwab transport media in accordance with the instuctions for use for each transport device. Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated based on comparison to the expected result. Results from testing demonstrated equivalent performance between the two transport media, when compared to the expected positive and negative results for each analyte evaluated. Results for positive and negative agreement are presented in Tables 30 and 31 respectively.

Table 30: Transport Media Equivalency, Clinical Specimens - PPA

	Para-Pak C&S			**FecalSwab**		
Target	**Detected/ Total**	**PPA**	**Confidence Interval 95%**	**Detected/ Total**	**PPA**	**Confidence Interval 95%**
Adenovirus F40/F41*	0 / 0	n/a	n/a	0 / 0	n/a	n/a
*Cryptosporidium**	0 / 1	0.00%	0.00%- 97.50%	1 / 1	100%	2.50%-100%
Enteropathogenic *E. coli* (EPEC)	58 / 61	95.08%	86.29%-98.97%	57 / 61	93.44%	84.05%-98.18%
Giardia lamblia	47 / 48	97.92%	88.93%-99.95%	47 / 48	97.92%	88.93%-99.95%
Norovirus GI/GII*	8 / 8	100%	63.06%-100%	7 / 8	87.50%	47.35%-99.68%

*Low sample size; therefore, equivalence for these analytes was based on the testing of contrived specimens

Table 31: Transport Media Equivalency, Clinical Specimens - NPA

	Para-Pak C&S			FecalSwab		
Target	**#Not Detected**	**NPA**	**Confidence Interval**	**#Not Detected**	**NPA**	**Confidence Interval**
Adenovirus F40/F41	174 / 174	100%	97.90% 100%	174 / 174	100%	97.90%-100%
Cryptosporidium	171 / 173	98.84%	95.89%-99.86%	170 / 173	98.27%	95.02%-99.64%
Enteropathogenic *E. coli* (EPEC)	52 / 58	89.66%	78.83%-96.11%	49 / 58	84.48%	72.58%-92.65%
Giardia lamblia	123 / 127	96.85%	92.13%-99.14%	124 / 127	97.64%	93.25%-99.51%
Norovirus GI/GII	164 / 164	100%	97.78%-100%	164 / 164	100%	97.78%-100%

Additonal testing was conducted using individual negative stool matrices spiked with selected target analytes at concentrations of 2x, 5x and 10x LoD. Positive stool samples were inoculated into Para-Pak C&S and FecalSwab transport devices. Testing demonstrated equivalent performance for all analytes for contrived clinical specimens in Para-Pak C&S and FecalSwab transport devices. Results from testing demonstrated equivalent analytical performance between Para-Pak C&S and FecalSwab transport media. Results for positive and negative agreement for testing of contrived specimens are presented in Tables 32 and 33 respectively.

Table 32: Transport Media Equivalency, Contrived Specimens - PPA

	Para-Pak C&S				FecalSwab			
Target	**Detected/ Total**	**PPA**	**Lower**	**Upper**	**Detected/ Total**	**PPA**	**Lower**	**Upper**
Adenovirus F40/F41	35 / 36	97.22%	85.47%	99.93%	35 / 36	97.22%	85.47%	99.93%
Norovirus GI/GII	36 / 36	100%	90.26%	100%	36 / 36	100%	90.26%	100%
Cryptosporidium	36 / 36	100%	90.26%	100%	36 / 36	100%	90.26%	100%

Table 33: Transport Media Equivalency, Contrived Specimens - NPA

	Para-Pak C&S				FecalSwab			
Target	**Not Detected**	**NPA**	**Lower**	**Upper**	**Not Detected**	**NPA**	**Lower**	**Upper**
Adenovirus F40/F41	167 / 168	99.40%	96.73%	99.98%	168 / 168	100%	97.83%	100%
Norovirus GI/GII	168 / 168	100%	97.83%	100%	168 / 168	100%	97.83%	100%
Cryptosporidium	168 / 168	100%	97.83%	100%	167 / 168	99.40%	96.73%	99.98%

9. Fresh Versus Frozen Study:

Para-Pak C&S Medium

To support inclusion of frozen archived Para-Pak C&S clinical specimens in the clinical study, an equivalency study was conducted to demonstrate equivalent performance between fresh and frozen specimens. Samples for the study were prepared by spiking different pathogen strains at low concentrations (i.e., 2x LoD) into stool sample matrix in Para-Pak C&S transport medium. Fresh (non-stored) samples were tested immediately after preparation. Frozen sample aliquots were stored at -70 to -80ºC or -15 to -25°C) and for at least 46 days and subjected to two freeze and thaw cycles prior testing. Results from the study demonstrated equivalent performance between fresh and frozen stool samples in Para-Pak C&S medium. Results are presented in Table 34.

Table 34: Fresh/Frozen Study, Para-Pak C&S, 2x LoD

Organism Type	Pathogen		Timepoint	Storage time point	Detection rate
Bacteria	*Campylobacter jejuni*		TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	42/42
	Clostridium difficile		TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	42/42
	Enteropathogenic *E. coli* (EPEC)		TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	40/40
	Shiga-like toxin-producing *E. coli* (STEC) O157	O157	TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	42/42
		Stx1	TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	42/42
		Stx2	TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	42/42
	Salmonella enterica		TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	41/41
	Vibrio parahaemolyticus		TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	41/41
	Yersinia enterocolitica		TP0 (0 days)	Fresh	40/40
			TP1 (57 days)	Frozen -15 to -25ºC	40/40
			TP1 (48 days)	Frozen -70 to -80ºC	41/41
Parasites	*Entamoeba histolytica*		TP0 (0 days)	Fresh	41/41
			TP1 (57 days)	Frozen -15 to -25ºC	41/41
			TP1 (48 days)	Frozen -70 to -80ºC	38/40
	Giardia lamblia		TP0 (0 days)	Fresh	40/40

Organism Type	Pathogen	Timepoint	Storage time point	Detection rate
		TP1 (57 days)	Frozen -15 to -25ºC	40/40
		TP1 (48 days)	Frozen -70 to -80ºC	42/42
Viruses	Adenovirus F40	TP0 (0 days)	Fresh	40/40
		TP1 (57 days)	Frozen -15 to -25ºC	40/40
		TP1 (48 days)	Frozen -70 to -80ºC	41/41
	Norovirus GI	TP0 (0 days)	Fresh	40/40
		TP1 (57 days)	Frozen -15 to -25ºC	40/40
		TP1 (48 days)	Frozen -70 to -80ºC	41/41
	Rotavirus A	TP0 (0 days)	Fresh	41/41
		TP1 (57 days)	Frozen -15 to -25ºC	40/41
		TP1 (48 days)	Frozen -70 to -80ºC	40/40

FecalSwab Medium

To support inclusion of frozen archived FecalSwab clinical specimens in the clinical study, an equivalency study was conducted to demonstrate equivalent performance between fresh and frozen specimens. Samples for the study were prepared by spiking different pathogen strains into stool sample matrix in FecalSwab transport medium. Fresh (non-stored) samples were tested immediately after preparation. Frozen sample aliquots were stored at -70 to -80ºC for at least 46 days and subjected to two freeze/thaw cycles prior testing. Results from the study demonstrated equivalent performance between fresh and frozen samples prepared in FecalSwab transport medium. Results are presented in Table 35.

Table 35: Fresh/Frozen Study, FecalSwab, 2x LoD

Pathogen	Time Point	Sample type	Conc	Detection Rate	Detection Rate Overall	% Agreement
Campylobacter jejuni (ZeptoMetrix, 0801650)	TP0 (0 days)	Fresh	2x LoD	41/41	71/71	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	40/40	70/70	100
	TP1 (46 days)		5x LoD	30/30		
Salmonella enterica (ZeptoMetrix, 0801437)	TP0 (0 days)	Fresh	2x LoD	40/40	70/70	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	41/41	72/72	100
			5x LoD	31/31		
Yersinia enterocolitica (ZeptoMetrix, 0801734)	TP0 (0 days)	Fresh	2x LoD	40/40	70/70	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	41/41	72/72	100
			5x LoD	31/31		
Enteropathogenic *E. coli* (EPEC) (ZeptoMetrix, 0801747)	TP0 (0 days)	Fresh	2x LoD	40/40	70/70	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	41/41	72/72	100
			5x LoD	31/31		

Shiga-like toxin-producing *E. coli* (STEC) O157:H7 – O157 (ZeptoMetrix, 0801622)	TP0 (0 days)	Fresh	2x LoD	41/41	71/71	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	40/40	70/70	100
	TP1 (46 days)		5x LoD	30/30		
Shiga-like toxin-producing *E. coli* (STEC) O157:H7 – *stx1*/*stx2* (ZeptoMetrix, 0801622)	TP0 (0 days)	Fresh	2x LoD	41/41	71/71	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	40/40	70/70	100
	TP1 (46 days)		5x LoD	30/30		
Entamoeba histolytica (ATCC, 30459)	TP0 (0 days)	Fresh	2x LoD	41/41	71/71	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	40/40	70/70	100
	TP1 (46 days)		5x LoD	30/30		
Giardia lamblia (ATCC, 30957)	TP0 (0 days)	Fresh	2x LoD	40/40	70/70	100
			5x LoD	30/30		
	TP1 (46 days)	Frozen -80ºC	2x LoD	40/40	70/70	100
			5x LoD	30/30		
Adenovirus F40 (ZeptoMetrix, 0810085CF)	TP0 (0 days)	Fresh	2x LoD	40/40	70/70	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	41/41	72/72	100
			5x LoD	31/31		
Norovirus GII (ZeptoMetrix, 0810087CF)	TP0 (0 days)	Fresh	2x LoD	40/40	70/70	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	41/41	72/72	100
			5x LoD	31/31		
Rotavirus A (ZeptoMetrix, 0810280CF)	TP0 (0 days)	Fresh	2x LoD	40/40	70/70	100
			5x LoD	30/30		
	TP1 (47 days)	Frozen -80ºC	2x LoD	41/41	72/72	100
			5x LoD	31/31		

10. Sample Volume Equivalency Study: 200 µL versus 100 µL sample volumes:

When an error message is generated by the QIAstat-Dx analyzer indicating that the sample concentration is too high, the user is instructed to repeat testing with half the sample volume (100 µL). This error is most likely to occur when transport media containers are overfilled. To assess the potential for an impact to assay performance when testing the lower sample volume, an equivalency study was conducted comparing testing with a 100 µL sample input volume to the standard sample input volume (200 µL). Samples were prepared by spiking analyte mixes containing one strain for each targeted analyte at 1x LoD in stool matrix resuspended in Para-Pak C&S transport medium. Results from the study demonstrated equivalent results between the two sample input volumes and for all assay targets, with the exception of Adenovirus F40/F41, *Cyclospora cayetanensis*, *Entamoeba histolytica*, STEC, and *E. coli* O157. For these targets, additional testing was conducted to determine the assay LoD for samples tested using the 100 µL sample volume. For these four analytes, the LoD

when testing a 100 µL sample volume was 3.16x higher than the LoD for samples tested with a 200 µL sample volume. Results from the study are presented in Table 36 below.

The following limitation statements are included in the package insert to warn the user of the risk of overfilling Para-Pak C&S or FecalSwab collection devices and the potential for lower assay sensitivity for the four analytes when testing with the 100 µL sample volume:

- The overfilling of Para-Pak C&S or FecalSwab collection devices can result in a failed test with an error indicating ' Sample concentration too high'.
- The assay sensitivity to detect *Cyclospora cayetanensis*, Adenovirus F40/F41, *Entamoeba histolytica* and the Shiga-like toxin-producing *Escherichia coli* (STEC) might be reduced up to 3.16-fold when using half-input sample volume (100 µL) workflow.

Table 36: LoD Equivalency: 200 and 100 µL Sample Volumes

LoD (200 µL)					LoD (100 µL)		
Target	**Catalog ID.**	**Ct mean**	**Detection rate**	**Conclusion**	**Ct mean**	**Detection rate**	**Conclusion**
Campylobacter jejuni	ZeptoMetrix 0801650	**32.9**	20/20	Equivalent	**34.7**	20/20	Equivalent
Clostridium difficile toxin A/B	ATCC 9689	**32.5**	20/20	Equivalent	**33.5**	20/20	Equivalent
Plesiomonas shigelloides	ZeptoMetrix 0801899	**34.8**	20/20	Equivalent	**35.2**	20/20	Equivalent
Salmonella	ZeptoMetrix 0801437	**33.5**	20/20	Equivalent	**34.5**	20/20	Equivalent
Vibrio cholerae	ZeptoMetrix 0801902	**33.9**	20/20	Equivalent	**34.9**	20/20	Equivalent
Vibrio parahaemolyticus	ATCC 17802	**32.2**	20/20	Equivalent	**33.3**	20/20	Equivalent
Vibrio vulnificus	ATCC 27562	**32.2**	20/20	Equivalent	**33.6**	20/20	Equivalent
Yersinia enterocolitica	ZeptoMetrix 0801734	**34.9**	20/20	Equivalent	**35.7**	20/20	Equivalent
Enteroaggregative *E. coli* (EAEC)	ZeptoMetrix 801919	**33.1**	20/20	Equivalent	**34**	20/20	Equivalent
Enteropathogenic *E. coli* (EPEC)	ZeptoMetrix 0801747	**34.8**	20/20	Equivalent	**35.9**	20/20	Equivalent
Enterotoxigenic *E. coli* (ETEC) *lt*/*st*	ZeptoMetrix 0801624	**33.3**	20/20	Equivalent	**34.3**	19/20	Equivalent
Enteroinvasive E. coli (EIEC)/*Shigella*	ATCC 43892	**34.6**	20/20	Equivalent	**36.4**	19/20	Equivalent
Shiga-like toxin-producing *E. coli* (STEC) *stx1*	ZeptoMetrix 0801622	**35.3**	20/20	Equivalent	**36.6**	20/20	Equivalent
Shiga-like toxin-producing *E. coli* (STEC) *stx2*	ZeptoMetrix 0801622	**35.1**	20/20	Equivalent	**36.4**	**18/20**	**Not equivalent**
		n/a	n/a	n/a	**34.7**	20/20	**New LoD* with half-volume input**

LoD (200 µL)					LoD (100 µL)		
Target	**Catalog ID.**	**Ct mean**	**Detection rate**	**Conclusion**	**Ct mean**	**Detection rate**	**Conclusion**
Shiga-like toxin-producing *E. coli* (STEC) O157	ZeptoMetrix 0801622	**35.3**	19/20	Equivalent	**36.2**	**16/20**	**Not equivalent**
		n/a	n/a	n/a	**34.7**	20/20	**New LoD* with half-volume input**
Cryptosporidium	Waterborne P102C	**29.4**	20/20	Equivalent	**31.7**	19/20	Equivalent
Cyclospora cayetanensis	Clinical sample LAC2825	**35.5**	19/20	Equivalent	**36.6**	**17/20**	**Not equivalent**
		n/a	n/a	n/a	**35.6**	20/20	**New LoD* with half-volume input**
Entamoeba histolytica	ATCC 30459	**35.7**	20/20	Equivalent	**36.2**	**17/20**	**Not equivalent**
		n/a	n/a	n/a	**35**	20/20	**New LoD* with half-volume input**
Giardia lamblia	ATCC 30888	**28.7**	20/20	Equivalent	**31.5**	20/20	Equivalent
Adenovirus F40/F41	ZeptoMetrix 0810085CF	**36.4**	19/20	Equivalent	**36.9**	**13/20**	**Not equivalent**
		n/a	n/a	n/a	**36**	20/20	**New LoD* with half-volume input**
Astrovirus	ZeptoMetrix 0810277CF	**31.1**	20/20	Equivalent	**32.1**	20/20	Equivalent
Norovirus GI/GII	ZeptoMetrix 0810087CF	**32.7**	19/20	Equivalent	**33.5**	20/20	Equivalent
Rotavirus A	ZeptoMetrix 0810280CF	**36.2**	20/20	Equivalent	**36.6**	19/20	Equivalent

*New LoD for testing 100 µL sample volume was determined to be 3.16x higher than the LoD for testing 200 µL sample volume.

The study results support that the risk to clinical performance when testing the reduced sample volume is likely to be minimal as the new LoD was only 3.16-fold higher for the five analytes listed above.

11. Assay Cut-Off:

The QIAstat Dx Gastrointestinal Panel 2 includes defined Ct value cutoffs for each assay target. The Ct cutoffs are included as automatic calculations in the assay definition file (ADF).

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable.

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

Prospective Clinical Study:

The clinical performance of QIAstat-Dx Gastrointestinal Panel 2 was established during a multi-center international prospective study conducted at thirteen clinical settings representative of different geographical areas within the US and Europe (nine sites in US and four sites in Europe) between May and July 2021. All study sites were hospital-associated or independent clinical diagnostics laboratories that perform routine diagnostics of gastrointestinal infections. A total of 1939 prospectively collected evaluable stool specimens (stool in Para-Pak C&S (Meridian Bioscience) or FecalSwab (COPAN) transport media) were obtained from patients with clinical indications of diarrhea caused by gastrointestinal infection. For the 1939 evaluable specimens tested in the prospective study, Table 37 provides a summary of specimen distribution across all study sites and Table 38 includes a summary of patient demographic information.

Table 37: Prospective Clinical Study, Specimen Distribution

	No. of prospective specimens		
Site/Country	**FecalSwab**	**Para-Pak C&S**	**Total**
Germany	293	46	339
Denmark	293	0	293
Spain	247	0	247
France	63	0	63
USA site 1	0	186	186
USA site 2	0	43	43
USA site 3	282	0	282
USA site 4	0	177	177
USA site 5	44	0	44
USA site 6	0	39	39
USA site 7[a]	0	0	0
USA site 8	0	131	131
USA site 9	0	95	95
Total	1222	717	1939

[a]Specimens from Site 7 were excluded from the analysis because they were collected with a device other than Para-Pak C&S or FecalSwab.

Table 38: Demographic data for enrolled prospective specimens

Demographic data	FecalSwab		Para-Pak C&S	
	N	**%**	**N**	**%**
Gender				
Female	628	32.4	442	22.8
Male	594	30.6	275	14.2
Age Group				
0-5 years	182	9.4	31	1.6
6-21 years	121	6.2	38	2.0
22-49 years	290	15.0	215	11.1
50+ years	629	32.4	426	22.0
Not Reported	0	0.0	7	0.4
Patient population				
Emergency room	46	2.4	29	1.5
Hospitalized	342	17.6	143	7.4
Immunocompromised	3	0.2	0	0.0
Outpatient	491	25.3	325	16.8
No information available	340	17.5	220	11.3
No. Days between Symptom Onset and QIAstat-Dx Testing				
> 7 days	89	4.6	0	0.0
≤ 7 days	146	7.5	16	0.8
Not Reported	987	50.9	701	36.2

The performance of the QIAstat-Dx Gastrointestinal Panel 2 was evaluated for each panel test result using one FDA-cleared test as comparator for the most analytes. A composite comparator consisting of either three independent FDA-cleared test methods or two independent FDA-cleared tests methods and two validated PCR assays followed by bi-directional sequencing was used for Norovirus GI/GII, ETEC, STEC and *Giardia lamblia* (Table 39).

Table 39: QIAstat-Dx Gastrointestinal Panel 2 Clinical studies comparator method

QIAstat-Dx Gastrointestinal Panel 2 target	Comparator method
Adenovirus F40/F41	FDA-cleared molecular assay
Astrovirus	FDA-cleared molecular assay
Norovirus GI/GII	Composite of three FDA-cleared molecular assays
Rotavirus A	FDA-cleared molecular assay
Campylobacter (C. jejuni, C. coli and *C. upsaliensis)*	FDA-cleared molecular assay
Shigella/Enteroinvasive Escherichia coli (EIEC)	FDA-cleared molecular assay
*Enteropathogenic Escherichia coli (EPEC)**	FDA-cleared molecular assay
Enterotoxigenic Escherichia coli (ETEC) lt/st	Composite of three FDA-cleared molecular assays
*Shiga-like toxin- producing Escherichia coli (STEC) stx1/stx2**	Composite of three FDA-cleared molecular assays
*E. coli O157 serogroup**	FDA-cleared molecular assay
Salmonella	FDA-cleared molecular assay
Plesiomonas shigelloides	FDA-cleared molecular assay
Yersinia enterocolitica	FDA-cleared molecular assay
Cryptosporidium	FDA-cleared molecular assay
Cyclospora cayetanensis	FDA-cleared molecular assay
Entamoeba histolytica	FDA-cleared molecular assay
Giardia lamblia	Composite of two FDA-cleared molecular assays and two validated PCR/Sequencing assays**

*Targets evaluated in Para-Pak C&S only

** For *Giardia lamblia*, each of the two PCR comparator assays used were well-characterized and validated nucleic acid amplification tests (NAAT) followed by bi-directional sequencing analysis. Both assays were designed to amplify different sequences than those targeted by the QIAstat-Dx Gastrointestinal Panel 2. Positive results required to generate sequences from bi-directional sequencing with at least 200 bases of

adequate quality that by BLAST analyses matched a sequence of the expected organism or gene from NCBI GenBank database with at least 95% query coverage and at least 95% identity compared to the reference.

Where a composite comparator was used, the result was determined as the majority of the three individual test results (i.e., a positive composite comparator result is based on positive results for at least two comparator tests and a negative composite comparator result is based on negative results for at least two comparator tests). If insufficient pathogen positive sample was available to obtain a majority test result, a worst-case model was applied in the PPA calculation. In this model, the PPA was calculated including all observed true positive and false negative samples between QIAstat-Dx Gastrointestinal Panel 2 and the composite comparator, while the following procedure was used for the samples where it was not possible to conduct testing with the complete comparator due to insufficient sample volume:

- Samples that were negative for the QIAstat-Dx Gastrointestinal Panel 2 and positive for one comparator test, negative (or insufficient volume) for a second comparator, and insufficient volume for a third comparator were included in the calculations as worst-case false negatives.

- Samples that were positive for the QIAstat-Dx Gastrointestinal Panel 2 and positive for one comparator test, negative (or insufficient volume) for a second comparator, and insufficient volume for the third comparator, were considered as worst-case false positives and therefore excluded from the PPA calculations.

The PPA was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both the QIAstat-Dx Gastrointestinal Panel 2 and comparator method showed a positive result for the specific target, and false negative (FN) indicates that the QIAstat-Dx Gastrointestinal Panel 2 result was negative while the comparator method result was positive. The NPA was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the QIAstat-Dx Gastrointestinal Panel 2 and the comparator method showed negative results, and a false positive (FP) indicates that the QIAstat-Dx Gastrointestinal Panel 2 result was positive when the comparator method result was negative. The exact binomial two-sided 95% confidence intervals for PPA and NPA were calculated.

Discrepancies between the QIAstat-Dx Gastrointestinal Panel 2 and the comparator methods were investigated for the analytes where the QIAstat-Dx Gastrointestinal Panel 2 test result was compared to one FDA-cleared method. Discrepant result analyses are footnoted in the clinical performance summary table.

Results for the prospective clinical study are presented in Table 40.

Table 40: QIAstat-Dx Gastrointestinal Panel 2 Clinical Performance, Prospective study

		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Medium Brand**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Viruses							
Adenovirus F40/F41	FecalSwab	5 / 6 [1]	83.3	43.7-97.0	1216 / 1216	100	99.7-100
	Para-Pak C&S	1 / 2 [2]	50.0	9.5-90.6	703 / 704 [2]	99.9	99.2-100
Astrovirus	FecalSwab	3 / 3	100	43.9-100	1219 / 1219	100	99.7-100
	Para-Pak C&S	6 / 6	100	61.0-100	700 / 700	100	99.5-100
Norovirus GI/GII	FecalSwab	31 / 33 [3]	93.9	80.4-98.3	493 / 495 [3]	99.6	98.6-100
	Para-Pak C&S	14 / 18[4]	77.8	54.8-91.0	399 / 399 [4]	100	99.1-100
Rotavirus A	FecalSwab	21 / 23[5]	91.3	73.2-97.6	1197 / 1199[5]	99.8	99.4-100
	Para-Pak C&S	3 / 3	100	43.9-100	702 / 703 [6]	99.9	99.2-100
Bacteria							
Campylobacter	FecalSwab	65 / 67 [7]	97.0	89.8-99.2	1151 / 1155[7]	99.7	99.1-99.9
	Para-Pak C&S	30 / 31 [8]	96.8	83.8-99.4	675 / 677 [8]	99.7	98.9-99.9
Plesiomonas shigelloides	FecalSwab	0 / 0	N/A	N/A	1220 / 1222 [9]	99.8	99.4-100
	Para-Pak C&S	5 / 6 [10]	83.3	43.7-97.0	698 / 700 [10]	99.7	99.0-99.9
Salmonella	FecalSwab	14 / 16 [11]	87.5	64.0-96.5	1206 / 1206	100	99.7-100
	Para-Pak C&S	19 / 20 [12]	95.0	76.4-99.1	688 / 688	100	99.4-100
Yersinia enterocolitica	FecalSwab	15 / 16 [13]	93.8	71.7-99.0	1199/1206 [13]	99.4	98.8-99.7
	Para-Pak C&S	3 / 3	100	43.9-100	698 / 703 [14]	99.3	98.4-99.7
Diarrheagenic *E. coli*/*Shigella*							
Enteropathogenic *E. coli* (EPEC)	Para-Pak C&S	57 / 65	87.7	77.6-93.6	632 / 632	100	99.4-100
Enterotoxigenic *E. coli* (ETEC) lt/st	FecalSwab	9 / 10 **[15]**	90.0	59.6-99.2	427 / 430 **[15]**	99.3	98.0-99.8
	Para-Pak C&S	9 / 10 **[16]**	90.0	59.6-99.2	390 / 395 **[16]**	98.7	97.1-99.5
Shiga-like toxin *E. coli* (STEC) stx1/stx2	Para-Pak C&S	5 / 6 **[17]**	83.3	43.7-97.0	397 / 400 **[17]**	99.3	97.8-99.7
***E. coli* O157**	Para-Pak C&S	0 / 0	N/A	N/A	5 / 5	100	56.6-100
	FecalSwab	10 / 10	100	72.3-100	1212 / 1212	100	99.7-100

		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Medium Brand**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Shigella/Enteroinvasive *E. Coli* (EIEC)	Para-Pak C&S	2 / 2	100	34.2-100	703 / 704 [18]	99.9	99.2-100
Parasites							
Cryptosporidium	FecalSwab	2 / 4	50.0	15.0-85.0	1218 / 1218	100	99.7-100
	Para-Pak C&S	6 / 6	100	61.0-100	699 / 700 [19]	99.9	99.2-100
Cyclospora cayetanensis	FecalSwab	3 / 3	100	43.9-100	1219 / 1219	100	99.7-100
	Para-Pak C&S	18 / 19 [20]	94.7	75.4-99.1	687 / 687	100	99.4-100
Entamoeba histolytica	FecalSwab	0 / 0	N/A	N/A	1222 / 1222	100	99.7-100
	Para-Pak C&S	0 / 0	N/A	N/A	706 / 706	100	99.5-100
Giardia lamblia	FecalSwab	6 / 8 [21]	75.0	40.9-92.9	434 / 441 [21]	98.4	96.8-99.2
	Para-Pak C&S	1 / 1	100	20.7-100	406 / 406 [22]	100	99.1-100

[1] Adenovirus F40/41 was not detected in the single false negative specimen (0/1) in FecalSwab using a different FDA-cleared test method.

[2] Adenovirus F40/41 was not detected in the single false negative specimen (0/1) and in the single false positive specimen (0/1) in Para-Pak C&S using a different FDA-cleared test method.

[3] Ten (10) FecalSwab samples positive for Norovirus GI/GII by both QIAstat-Dx and one FDA-cleared comparator were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. The sample size for NPA is smaller for Norovirus GI/GII as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[4] Two (2) Para-Pak C&S samples positive for Norovirus GI/GII by both QIAstat-Dx and one FDA-cleared comparator were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. One (1) Para-Pak C&S sample negative by QIAstat-Dx and positive with one FDA-cleared comparator with insufficient volume for complete composite comparator testing were classed as false negative in the PPA calculations. The sample size for NPA is smaller for Norovirus GI/GII as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[5] Rotavirus A was detected in one of the two false negative specimens (1/2) and was not detected in the two false positive specimens (0/2) in FecalSwab using a different FDA-cleared test method.

[6] Rotavirus A was not detected in the single false positive specimen (0/1) in Para-Pak C&S using a different FDA-cleared test method.

[7] *Campylobact*er was not detected in the two false negative specimens (0/2) and was detected in three of the four false positive specimens (3/4) in FecalSwab using a different FDA-cleared test method.

[8] *Campylobacter* was not detected in the single false negative specimens (0/1) and was detected in one of the two false positive specimens (1/2) in Para-Pak C&S using a different FDA-cleared test method.

[9] *Plesiomonas shigelloides* was not detected in the two false positive specimens (0/2) in FecalSwab using a different FDA-cleared test method.

[10] *Plesiomonas shigelloides* was not detected in the single false negative specimen (0/1) and was not detected in the two false positive specimens (0/2) in Para-Pak C&S using a different FDA-cleared test method.

[11] *Salmonella* was not detected in the two false negative specimens (0/2) in FecalSwab using a different FDA-cleared test method.

[12] *Salmonella* was not detected in the single false negative specimen (0/1) in Para-Pak C&S using a different FDA-cleared test method.

[13] *Yersinia enterocolitica* was not detected in the single false negative specimen (0/1) and was not detected in the seven false positive specimens (0/7) in FecalSwab using a different FDA-cleared test method.

[14] *Yersinia enterocolitica* was not detected in the five false positive specimens (0/5) using a different FDA-cleared test method.

[15]Six (6) FecalSwab samples positive for ETEC by both QIAstat-Dx and the primary FDA-cleared comparator were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. The sample size for NPA is smaller for ETEC as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[16] Three (3) Para-Pak C&S samples positive for ETEC by both QIAstat-Dx and one FDA-cleared comparator were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. The sample size for NPA is smaller for ETEC as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[17] One (1) FecalSwab sample positive for STEC by both QIAstat-Dx and one FDA-cleared comparator was excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. The sample size for NPA is smaller for STEC as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[18] *Shigella*/Enteroinvasive E. coli (EIEC) was detected in the single false positive specimen (1/1) in Para-Pak C&S using a different FDA-cleared test method.

[19] *Cryptosporidium* was not detected in the single false positive specimen (0/1) in Para-Pak C&S using PCR followed by bi-directional sequence analysis.

[20] For *Cyclospora cayetanensis* there was one (1) false negative specimen in Para-Pak C&S that was not further investigated by discrepant analyses.

[21] Two (2) FecalSwab samples positive for *Giardia lamblia* by both QIAstat-Dx and one FDA-cleared comparator were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. Two (2) FecalSwab samples negative by QIAstat-Dx and positive with one FDA-cleared comparator with insufficient volume for complete composite comparator testing were classed as false negative in the PPA calculations. The sample size for NPA is smaller for Giardia lamblia as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[22]the sample size for NPA is smaller for *Giardia lamblia* as only a portion of the samples with a negative result in QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

The QIAstat-Dx Gastrointestinal Panel 2 reported multiple organism detections (i.e., mixed infections) for a total of 15 and 29 prospective specimens in FecalSwab and Para-Pak C&S, respectively. This represents 7.0% of positive specimens (15/213) in FecalSwab and 17.0% of positive specimens (29/171) in Para-Pak C&S. Most multiple detections in FecalSwab specimens (14/15; 93%) contained two organisms, while 6.7% (1/15) contained three organisms. In Para-Pak C&S specimens, most multiple detections (22/29; 75.9%) contained two organisms, while 24.1% (7/29) contained three organisms. The most common multiple organism detections are presented in Table 41 and Table 42 below.

Table 41: Most Prevalent Multiple Detection Combinations (≥2 instances) as Determined by the QIAstat-Dx Gastrointestinal Panel 2 in the Prospective Clinical study in FecalSwab specimens

Multiple Detection Combination	Number of FecalSwab Specimens
Campylobacter + Norovirus GI/GII	2
Enterotoxigenic *E. coli* (ETEC) lt/st + Norovirus GI/GII	3
Campylobacter + Rotavirus A	4

Table 42. Most Prevalent Multiple Detection Combinations (≥2 instances) as Determined by the QIAstat-Dx Gastrointestinal Panel 2 in the Prospective Clinical study in Para-Pak C&S specimens

Multiple Detection Combination	Number of Para-Pak C&S Specimens
Campylobacter + Enteropathogenic *E. coli* (EPEC)	3
Enteropathogenic *E. coli* (EPEC) + *Salmonella*	3
Enteropathogenic *E. coli* (EPEC) + Enterotoxigenic *E. coli* (ETEC) lt/st	4

The analytes most commonly found in mixed infections in FecalSwab specimens were *Campylobacter* (9), Norovirus GI/GII (7), Rotavirus (4) and ETEC (3)) as shown in Table 43, while the analytes most commonly found in mixed infections in Para-Pak C&S specimens were EPEC (17), ETEC (8), *Campylobacter* (7), Norovirus GI/GII (5), Rotavirus (4) and STEC (5) as shown in Table 44.

Table 43: Prevalence of Analytes in Mixed Infections in FecalSwab specimens as determined by the QIAstat-Dx Gastrointestinal Panel 2

Analyte	N	%
Adenovirus F40/F41	1	3.2
Astrovirus	1	3.2
Campylobacter	9	29.0
Enterotoxigenic *E. coli* (ETEC) lt/st	3	9.7
Giardia lamblia	2	6.5
Norovirus GI/GII	7	22.6
Plesiomonas shigelloides	1	3.2
Rotavirus A	4	12.9
Shigella/Enteroinvasive *E. coli* (EIEC)	2	6.5
Yersinia enterocolitica	1	3.2

Table 44: Prevalence of Analytes in Mixed Infections in Para-Pak C&S specimens as determined by the QIAstat-Dx Gastrointestinal Panel 2

Analyte	N	%
Adenovirus F40/F41	1	1.5
Astrovirus	1	1.5
Campylobacter	7	10.8
Cryptosporidium	2	3.1
Cyclospora cayetanensis	2	3.1
Enteropathogenic *E. coli* (EPEC)	17	26.2
Enterotoxigenic *E. coli* (ETEC) lt/st	8	12.3
Giardia lamblia	1	1.5

Analyte	N	%
Norovirus GI/GII	5	7.7
Plesiomonas shigelloides	7	10.8
Rotavirus A	1	1.5
Salmonella	4	6.2
Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*	5	7.7
Shigella/Enteroinvasive *E. coli* (EIEC)	3	4.6
Yersinia enterocolitica	1	1.5

Tables 45-48 include details on prospective clinical specimens (FecalSwab and Para-Pak C&S) with multiple organisms detected by the QIAstat-Dx Gastrointestinal Panel 2 and comparator methods.

Table 45: Multi-Detections for QIAstat-Dx Gastrointestinal Panel 2, FecalSwab

Analyte 1	Analyte 2	Analyte 3	# Multi-Detections	# FP analytes by QIAStat	Discrepant Analyte(s)
Adenovirus F40/F41	*Campylobacter*		1	0	N/A
Astrovirus	Giardia lamblia		1	1	*Giardia lamblia*
Campylobacter	*Giardia lamblia*	*Shigella*/Enteroinvasive *E. coli* (EIEC)	1	1	*Shigella*/Enteroinvasive *E. coli* (EIEC)
Campylobacter	Norovirus GI/GII		2	0	N/A
Campylobacter	*Plesiomonas shigelloides*		1	1	*Plesiomonas shigelloides*
Campylobacter	Rotavirus A		4	2	Rotavirus A
Enterotoxigenic *E. coli* (ETEC) lt/st	Norovirus GI/GII		3	1	Norovirus GI/GII
Norovirus GI/GII	*Shigella*/Enteroinvasive *E. coli* (EIEC)		1	1	Norovirus GI/GII
Norovirus GI/GII	*Yersinia enterocolitica*		1	1	*Yersinia enterocolitica*
Total Multi-Detections			**15**	**12**	
Double Detections			14	11	
Triple Detections			1	1	

Table 46: Multi-Detections for Comparator Methods, FecalSwab

Analyte 1	Analyte 2	Analyte 3	# Multi-Detections	# FN analytes by QIAStat	Discrepant Analyte(s)
Adenovirus F40/F41	*Campylobacter*		1	0	N/A
Adenovirus F40/F41	Norovirus GI/GII		1	1	Adenovirus F40/F41
Campylobacter	*Shigella*/Enteroinvasive *E. coli* (EIEC)		1	0	N/A
Campylobacter	Norovirus GI/GII		2	0	N/A
Campylobacter	Norovirus GI/GII	Rotavirus A	1	1	Norovirus GI/GII
Campylobacter	Rotavirus A		2	0	N/A
Cryptosporidium	Norovirus GI/GII		1	1	*Cryptosporidium*
Enterotoxigenic *E. coli* (ETEC) lt/st	Norovirus GI/GII		2	0	N/A
Total Multi-Detections			**11**	**3**	
Double Detections			10	2	
Triple Detections			1	1	

Table 47: Multi-Detections for QIAstat-Dx Gastrointestinal Panel 2, Para-Pak C&S

Distinct Multi-Detection Combinations Detected by QIAstat Dx GI2 Panel					
Analyte 1	**Analyte 2**	**Analyte 3**	**# Multi-Detections**	**# FP analytes by QIAStat**	**Discrepant Analyte(s)**
Adenovirus F40/F41	Enteropathogenic E. coli (EPEC)		1	0	N/A
Astrovirus	*Campylobacter*		1	0	N/A
Campylobacter	Enteropathogenic *E. coli* (EPEC)		3	0	N/A
Campylobacter	Enteropathogenic *E. coli* (EPEC)	*Shigella*/Enteroinvasive *E. coli* (EIEC)	1	1	*Shigella*/Enteroinvasive *E. coli* (EIEC)
Campylobacter	*Plesiomonas shigelloides*		1	1	*Plesiomonas shigelloides*
Campylobacter	*Plesiomonas shigelloides*	Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*	1	1	Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*
Cryptosporidium	*Cyclospora cayetanensis*		1	1	*Cryptosporidium*
Cryptosporidium	*Plesiomonas shigelloides*	Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*	1	0	N/A
Cyclospora cayetanensis	Enteropathogenic *E. coli* (EPEC)		1	0	N/A
Enteropathogenic *E. coli* (EPEC)	Enterotoxigenic *E. coli* (ETEC) lt/st		4	3	Enterotoxigenic *E. coli* (ETEC) lt/st
Enteropathogenic *E. coli* (EPEC)	Enterotoxigenic *E. coli* (ETEC) lt/st	Norovirus GI/GII	1	1	Enterotoxigenic *E. coli* (ETEC) lt/st + Norovirus GI/GII

Enteropathogenic *E. coli* (EPEC)	Enterotoxigenic *E. coli* (ETEC) lt/st	*Shigella*/Enteroinvasive *E. coli* (EIEC)	1	0	N/A
Enteropathogenic *E. coli* (EPEC)	Norovirus GI/GII		1	0	N/A
Enteropathogenic *E. coli* (EPEC)	*Salmonella*		3	0	N/A
Enteropathogenic *E. coli* (EPEC)	*Yersinia enterocolitica*		1	1	*Yersinia enterocolitica*
Enterotoxigenic *E. coli (*ETEC) lt/st	*Plesiomonas shigelloides*	Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*	1	1	*Plesiomonas shigelloides* + Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*
Enterotoxigenic *E. coli* (ETEC) lt/st	Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*		1	1	Enterotoxigenic *E. coli* (ETEC) lt/st
Giardia lamblia	Norovirus GI/GII		1	0	N/A
Norovirus GI/GII	*Plesiomonas shigelloides*	*Shigella*/Enteroinvasive *E. coli* (EIEC)	1	0	N/A
Norovirus GI/GII	Shiga-like toxin *E. coli (*STEC) *stx1*/*stx2*		1	1	Norovirus GI/GII + Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*
Plesiomonas shigelloides	Rotavirus A		1	0	N/A
Plesiomonas shigelloides	*Salmonella*		1	0	N/A
Total Multi-Detections			**29**	**12**	
Double Detections			22	8	
Triple Detections			7	4	

Table 48: Multi-Detections for Comparator Methods, Para-Pak C&S

Analyte 1	Analyte 2	Analyte 3	# Multi-Detections	# FN analytes by QIAStat	Discrepant Analyte(s)
Adenovirus F40/F41	Enteropathogenic *E. coli* (EPEC)		2	1	Adenovirus F40/F41
Astrovirus	*Campylobacte*r	Enteropathogenic E. coli (EPEC)	1	1	Enteropathogenic *E. coli* (EPEC)
Campylobacter	Enteropathogenic *E. coli* (EPEC)		4	0	N/A
Campylobacter	Enteropathogenic *E. coli* (EPEC)	Plesiomonas shigelloides	1	1	Enteropathogenic *E. coli* (EPEC)
Campylobacter	Enterotoxigenic *E. coli* (ETEC) lt/st		1	1	Enterotoxigenic *E. coli* (ETEC) lt/st
Cryptosporidium	*Plesiomonas shigelloides*	Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*	1	0	N/A

Cyclospora cayetanensis	Enteropathogenic *E. coli* (EPEC)		3	2	Enteropathogenic *E. coli* (EPEC)
Cyclospora cayetanensis	Enterotoxigenic *E. coli (*ETEC) lt/st		1	1	*Cyclospora cayetanensis*
Enteropathogenic *E. coli* (EPEC)	Enterotoxigenic *E. coli* (ETEC) lt/st		1	0	N/A
Enteropathogenic *E. coli* (EPEC)	Enterotoxigenic *E. coli* (ETEC) lt/st	*Shigella*/Enteroinvasive *E. coli* (EIEC)	1	0	N/A
Enteropathogenic *E. coli* (EPEC)	Norovirus GI/GII		2	1	Enteropathogenic *E. coli* (EPEC)
Enteropathogenic *E. coli* (EPEC)	*Plesiomonas shigelloides*		1	1	Enteropathogenic E. coli (EPEC)
Enteropathogenic *E. coli* (EPEC)	*Salmonella*		3	0	N/A
Enterotoxigenic *E. coli* (ETEC) lt/st	*Salmonella*		1	1	*Salmonella*
Giardia lamblia	Norovirus GI/GII		1	0	N/A
Norovirus GI/GII	*Plesiomonas shigelloides*	*Shigella*/Enteroinvasive *E. coli* (EIEC)	1	0	N/A
Plesiomonas shigelloides	Rotavirus A		1	0	N/A
Plesiomonas shigelloides	*Salmonella*		1	0	N/A
Total Multi-Detections			**27**	**10**	
Double Detections			22	8	
Triple Detections			5	2	

Prospective Archived Specimen Testing:

Additional prospectively archived stool specimens were collected for Norovirus GI/GII (81 specimens) and STEC (18 specimens). Specimens were prospectively collected from four different sites (3 US and 1 EU). All positive specimens (i.e., all-comer positives) by standard of care testing for Norovirus (GI/GII) or STEC were archived for inclusion in the study. An additional 20 negative specimens were included in the study for blinding purposes. Testing included a total of 119 total specimens. Of the initial 81 specimens positive for Norovirus by standard of care at the collection site, 77 specimens were determined to be positive for Norovirus by the composite comparator method. Of the initial 18 specimens positive for STEC by standard of care, 13 specimens were determined to be positive by the composite comparator method. The remaining specimens that were not confirmed to be positive by the composite comparator were included in the analysis as negative specimens. Results from the prospective archived specimen testing are presented in Table 49.

Table 49: QIAstat-Dx Gastrointestinal Panel 2, Clinical Performance, Prospective Archived Specimen Testing

		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Medium Brand**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Norovirus GI/GII	FecalSwab	48 / 49 [1]	98.0	89.3 - 99.6	2 / 4	50.0	15.0-85.0
	Para-Pak C&S	26 / 28 [2]	92.9	77.4 - 98.0	37 / 38	97.4	86.5-99.5
Shiga-like toxin *E. coli* (STEC) stx1/stx2	Para-Pak C&S	12 / 13 [3, 4]	92.3	66.7 - 98.6	51 / 52	98.1	89.9-99.7

[1] For Norovirus GI/GII four out of the eighty-one (4/81) prospectively archived samples (positive by standard of care) were negative by the composite comparator and therefore included as negative samples in the NPA calculations

[2]One (1) Para-Pak C&S sample negative by QIAstat-Dx and positive with one FDA-cleared comparator with insufficient volume for complete composite comparator testing was classed as false negative in the PPA calculations.

[3] For STEC five out of the eighteen (5/18) prospectively archived samples (positive by standard of care) were negative by the composite comparator and therefore included as negative samples in the NPA calculations.

[4] One (1) Para-Pak C&S sample positive for STEC in both QIAstat-Dx and one FDA-cleared comparator was excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing.

Retrospective Archived Study (Pre-selected specimens)

To supplement the results of the prospective clinical study, a total of 750 preselected frozen archived retrospective specimens were evaluated. These specimens served to increase the sample size for analytes that showed low prevalence in the prospective study or that were less represented in a particular sample type (Para-Pak C&S or FecalSwab). Specimens were selected based on standard of care result and were excluded from the data analysis if the comparator methods did not confirm the preselected target as positive. Performance for the QIAstat Gastrointestinal Panel 2 for retrospectively tested archived specimens is presented in Table 50.

Table 50. Clinical Performance in the Retrospective study

		Positive Percent Agreement			Negative Percent Agreement		
	Medium Brand	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Viruses							
Adenovirus F40/F41	FecalSwab	23 / 26 [1]	88.5	71.0-96.0	203 / 203	100	98.1-100
	Para-Pak C&S	29 / 29	100	88.3-100	39 / 39	100	91.0-100
Astrovirus	FecalSwab	2 / 3 [2]	66.7	20.8-93.9	191 / 191	100	98.0-100
	Para-Pak C&S	0 / 0	N/A	N/A	14 / 14	100	78.5-100
Norovirus GI/GII	FecalSwab	28 / 32 [3]	87.5	71.9-95.0	74 / 75 [3]	98.7	92.8-99.8
	Para-Pak C&S	27 / 29	93.1	78.0-98.1	86 / 86 [4]	100	95.7-100
Rotavirus A	FecalSwab	8 / 9 [5]	88.9	56.5-98.0	185 / 185	100	98.0-100
	Para-Pak C&S	2 / 2 [6]	100	34.2-100	12 / 12	100	75.8-100
Bacteria							
Campylobacter	FecalSwab	31 / 31	100	89.0-100	161 / 163 [6]	98.8	95.6-99.7
	Para-Pak C&S	3 / 3	100	43.9-100	11 / 11	100	74.1-100
	FecalSwab	2 / 2	100	34.2-100	192 / 192	100	98.0-100

		Positive Percent Agreement			Negative Percent Agreement		
	Medium Brand	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Plesiomonas shigelloides	Para-Pak C&S	33 / 36 [7]	91.7	78.2-97.1	117 / 117	100	96.8-100
Salmonella	FecalSwab	30 / 31 [8]	96.8	83.8-99.4	161 / 163 [8]	98.8	95.6-99.7
	Para-Pak C&S	1 / 1	100	20.7-100	13 / 13	100	77.2-100
Yersinia enterocolitica	FecalSwab	32 / 34 [9]	94.1	80.9-98.4	160 / 160	100	97.7-100
	Para-Pak C&S	1 / 1	100	20.7-100	14 / 14	100	78.5-100
Diarrheagenic *E. coli*/*Shigella*							
Enteropathogenic *E. coli* (EPEC)	Para-Pak C&S	60 / 65[10]	92.3	83.2-96.7	42 / 42	100	91.6-100
Enterotoxigenic *E. coli* (ETEC) lt/st	FecalSwab	22 / 24 [11]	91.7	74.2-97.7	85 / 86 [11]	98.8	93.7-99.8
	Para-Pak C&S	23 / 24	95.8	79.8-99.3	61 / 61 [12]	100	94.1-100
Shiga-like toxin *E. coli* (STEC) stx1/stx2	Para-Pak C&S	60 / 64	93.8	85.0-97.5	44 / 44 [13]	100	92.0-100
***E. coli* O157**	Para-Pak C&S	39 / 42 [14]	92.9 %	80.1-99.4	16 / 16	100	80.6-100
***Shigella*/Enteroinvasive *E. coli* (EIEC)**	FecalSwab	22 / 24 [15]	91.7	74.2-97.7	170 / 170	100	97.8-100
	Para-Pak C&S	0 / 0	N/A	N/A	14 / 14	100	78.5-100
Parasites							
Cryptosporidium	FecalSwab	6 / 6	100	61-100	186 / 188 [16]	98.9	96.2-99.7
	Para-Pak C&S	26 / 26	100	87.1-100	117 / 117	100	96.8-100
Cyclospora cayetanensis	FecalSwab	1 / 1	100	20.7-100	193 / 193	100	98.1-100
	Para-Pak C&S	1 / 1	100	20.7-100	13 / 13	100	77.2-100
Entamoeba histolytica	FecalSwab	0 / 0	N/A	N/A	194 / 194	100	98.1-100
	Para-Pak C&S	0 / 0	N/A	N/A	14 / 14	100	76.5-100
Giardia lamblia	FecalSwab	29 / 31 [17]	93.6	79.3-98.2	46 / 48 [18]	95.8	86.0-98.9
	Para-Pak C&S	27 / 28 [17]	96.4	82.3-99.4	92 / 92 [17]	100	96.0-100

[1] Adenovirus F40/41 was detected in one of the three false negatives (1/3) in FecalSwab using a different FDA-cleared test method

[2] Astrovirus was detected in the single false negative specimen (1/1) in FecalSwab using a different FDA-cleared test method.

[3] Two (2) FecalSwab samples positive for Norovirus GI/GII by both QIAstat-Dx and one FDA-cleared comparator were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. The sample size for NPA is smaller for Norovirus GI/GII as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study.

[4] The sample size for NPA is smaller for Norovirus GI/GII in Para-Pak C&S as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study

[5] Rotavirus A was detected in the single false negative specimen (1/1) in FecalSwab using a different FDA-cleared test method.

[6] *Campylobacter* was detected in one of the two false positive specimens (1/2) in FecalSwab using a different FDA-cleared test method.

[7] *Plesiomonas shigelloides* was detected in one of the three false negative specimens (1/3) in Para-Pak C&S using a different FDA-cleared test method.

[8] *Salmonella* was not detected in the single false negative specimen (0/1) and was not detected in the two false positive specimens (0/2) in FecalSwab using a different FDA-cleared test method.

[9] *Yersinia enterocolitica* was not detected in the two false negative specimens (0/2) in FecalSwab using a different FDA-cleared test method.

[10] Enteropathogenic *E. coli* (EPEC) was detected in all three false negative specimens (3/3) in Para-Pak C&S using PCR followed by bi-directional sequence analysis. There were two (2) other false negative specimens that were not further investigated by discrepant analyses.

[11] Ten (10) FecalSwab samples positive for ETEC in both QIAstat-Dx and one FDA-cleared comparator were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. One (1) FecalSwab sample negative by QIAstat-Dx and positive with one FDA-cleared comparator with insufficient volume for complete composite comparator testing was classed as false negative in the PPA calculations. The sample size for NPA is smaller for ETEC as only a portion of the samples with a negative result by QIAstat-Dx and one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study.

[12] The sample size for NPA is smaller for ETEC in Para-Pak CS&S as only a portion of the samples with a negative result by QIAstat-Dx and one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study).

[13] The sample size for NPA is smaller for STEC in Para-Pak C&S as only a portion of the samples with a negative result by QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study[1414] *Shigella*/Enteroinvasive *E. coli* (EIEC) was detected in one of the two false negative specimens (1/2) in FecalSwab using a different FDA-cleared test method.

[14] *E. coli* O157 was not detected in two false negative specimens (0/2) in Para-Pak C&S using a different FDA-cleared test method. There was one (1) false negative specimen in Para-Pak C&S that was not further investigated by discrepant analyses.

[15] *Shigella*/Enteroinvasive *E. coli* (EIEC) was detected in one of the two false negative specimens (1/2) in FecalSwab using a different FDA-cleared test method.

[16]*Cryptosporidium* was not detected in the two false positive specimens (0/2) in FecalSwab using PCR followed by bi-directional sequence analysis

[17] Four (4) samples positive for *Giardia lamblia* by both QIAstat-Dx and one FDA-cleared comparator were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. Two (2) FecalSwab samples negative by QIAstat-Dx and positive with one FDA-cleared comparator with insufficient volume for complete composite comparator testing was classed as false negative in the PPA calculations. The sample size for NPA is smaller for *Giardia lamblia* as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study.

[18] One (1) Para-Pak C&S samples positive for *Giardia lamblia* in both QIAstat-Dx and primary FDA-cleared comparator (were excluded from the PPA calculations because the samples did not have sufficient volume for complete composite comparator testing. One (1) Para-Pak C&S sample negative by QIAstat-Dx and positive with one FDA-cleared comparator with insufficient volume for complete composite comparator testing was classed as false negative in the PPA calculations. The sample size for NPA is smaller for *Giardia lamblia* as only a portion of the samples with a negative result by QIAstat-Dx and by one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study.

For the clinical study, the proportion of failed or invalid runs on initial attempt and following repeat testing are summarized in Table 51**.** The failure rates due to instrument, invalid result (IC failure), 'sample too concentrated' and other run failures are summarized in Table 52**.**

Table 51: Failure Rates Summary

Transport medium	Study	Initial Runs			Final Runs		
		N / Total	%	95% CI	N / Total	%	95% CI
FecalSwab	**Prospective**	16 / 1227	1.3	0.7 - 2.1	3 / 1227	0.2	0.1 - 0.7
	Prospective Archived	0 / 53	0.0	0.0 - 6.7	0 / 53	0.0	0.0 - 6.7
	Retrospective	11 / 366	3.0	1.5 - 5.3	5 / 366	1.4	0.4 - 3.2
	Total	**27 / 1646**	**1.6**	**1.1 - 2.4**	**8 / 1646**	**0.5**	**0.2 - 1.0**
Para-Pak	**Prospective**	66 / 740	8.9	7.0 - 11.2	21 / 740	2.8	1.8 - 4.3

Transport medium	Study	Initial Runs			Final Runs		
		N / Total	%	95% CI	N / Total	%	95% CI
	Prospective Archived	3 / 66	4.5	0.9 - 12.7	0 / 66	0.0	0.0 - 5.4
	Retrospective	46 / 454	10.1	7.5 - 13.3	25 / 454	5.5	3.6 - 8.0
	Total	**115 / 1260**	**9.1**	**7.6 - 10.9**	**46 / 1260**	**3.7**	**2.7 - 4.8**

Table 52: Summary of Run Failure Types

Transport Medium	Study	Failure Reason	Initial Runs		Final Runs	
			N / Total	%	N / Total	%
FecalSwab	**Prospective**	Instrument	0 / 1227	0.0	0 / 1227	0.0
		Invalid [1]	0 / 1227	0.0	0 / 1227	0.0
		Sample too Concentrated [2]	5 / 1227	0.4	0 / 1227	0.0
		Other [3]	11 / 1227	0.9	3 / 1227	0.2
	Prospective Archived	Instrument	0 / 53	0.0	0 / 53	0.0
		Invalid	0 / 53	0.0	0 / 53	0.0
		Sample too Concentrated	0 / 53	0.0	0 / 53	0.0
		Other	0 / 53	0.0	0 / 53	0.0
	Retrospective	Instrument	1 / 366	0.3	0 / 366	0.0
		Invalid	1 / 366	0.3	0 / 366	0.0
		Sample too Concentrated	0 / 366	0.0	0 / 366	0.0
		Other	9 / 366	2.5	5 / 366	1.4
Para-Pak C&S	**Prospective**	Instrument	9 / 740	1.2	2 / 740	0.3
		Invalid	5 / 740	0.7	5 / 740	0.7
		Sample too Concentrated	35 / 740	4.7	7 / 740	0.9
		Other	17 / 740	2.3	7 / 740	0.9
	Prospective Archived	Instrument	0 / 66	0.0	0 / 66	0.0
		Invalid	1 / 66	1.5	0 / 66	0.0
		Sample too Concentrated	1 / 66	1.5	0 / 66	0.0
		Other	1 / 66	1.5	0 / 66	0.0
	Retrospective	Instrument	1 / 454	0.2	0 / 454	0.0
		Invalid	10 / 454	2.2	6 / 454	1.3
		Sample too Concentrated	10 / 454	2.2	2 / 454	0.4
		Other	25 / 454	5.5	17 / 454	3.7

[a] Run failures re [a] Internal Control failures with at least one analyte detected and the other analytes reported as 'invalid'

[b] Run failures related to 'sample concentration too high'. These specimens were repeated with 100 microliters as instructed in the package insert

[c] Run failures related to workflow checkpoints.

Contrived Specimen Testing

Prospective and retrospective specimen testing generated low numbers of positive specimens for at least one specimen type for Astrovirus, Rotavirus A, *Plesiomonas shigelloides*, *Yersinia enterocolitica*, *Shigella*/EIEC, *Cryptosporidium*, *Cyclospora cayetanensis* and *Entamoeba histolytica*. To supplement the prospective and retrospective specimens test results for these targets, an evaluation of contrived specimens was performed. Each contrived specimen was prepared using an individual residual stool specimen matrix in Para-Pak C&S transport medium that had previously tested negative by QIAstat-Dx Gastrointestinal Panel 2 and comparator methods. At least, 50% of the contrived specimens were spiked at concentrations slightly above the Limit of Detection (2x LoD) and the remainder were spiked at concentrations of 5x and 10x LoD, using quantified strains for each pathogen. A minimum of 50 contrived specimens were tested for each analyte evaluated. The analyte status of each contrived specimen was blinded to the operators conducting the testing. Results are summarized in Table 53.

Table 53. Test Results Summary for Contrived Specimens

Assay Target		Positive Percent Agreement (PPA)		
	Medium Brand	Fraction	Percentage	95% CI
Astrovirus	FecalSwab	33 / 34	97.1	85.1-99.5
	Para-Pak C&S	34 / 34	100	89.8-100
Rotavirus A	FecalSwab	35 / 35	100	90.1-100
	Para-Pak C&S	34 / 35	97.1	85.5-99.5
Plesiomonas shigelloides	FecalSwab	33 / 33	100	89.6-100
	Para-Pak C&S	34 / 35	97.1	85.5-99.5
Yersinia enterocolitica	FecalSwab	34 / 34	100	89.8-100
	Para-Pak C&S	34 / 35	97.1	85.5-99.5
***Shigella*/EIEC**	FecalSwab	35 / 35	100	90.1-100
	Para-Pak C&S	34 / 34	100	89.8-100
Cryptosporidium	FecalSwab	27 / 27	100	87.5-100
	Para-Pak C&S	31 / 31	100	89.0-100
Cyclospora cayetanensis	FecalSwab	26 / 26	100	87.1-100
	Para-Pak C&S	30 / 30	100	88.6-100
Entamoeba histolytica	FecalSwab	35 / 35	100	90.1-100
	Para-Pak C&S	34 / 35	97.1	85.5-99.5

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The number and percentage of positive results as determined by the QIAstat-Dx Gastrointestinal Panel 2 in the prospective clinical evaluation, stratified by age group, are presented in Table 54. Overall, the QIAstat-Dx Gastrointestinal Panel 2 detected at least one organism in 17.4% (213/1222) and 23.8% (171/717) of the prospectively collected stool specimens in FecalSwab and Para-Pak C&S, respectively.

Table 54: Expected values Summary by Age Group for the Prospective Clinical study as determined by the QIAstat-Dx Gastrointestinal Panel 2

Pathogen	Medium Brand	Overall	0-5 years	6-21 years	22-49 years	50+ years	Not Reported
Viruses							
Adenovirus F40/F41	FecalSwab	5 (0.4%)	3 (1.7%)	2 (1.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Para-Pak C&S	2 (0.3%)	1 (3.2%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)
Astrovirus	FecalSwab	3 (0.2%)	3 (1.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Para-Pak C&S	6 (0.8%)	2 (6.5%)	0 (0.0%)	3 (1.4%)	1 (0.2%)	0 (0.0%)
Norovirus GI/GII	FecalSwab	43 (3.5%)	22 (12.1%)	1 (0.8%)	14 (4.8%)	6 (1.0%)	0 (0.0%)
	Para-Pak C&S	16 (2.3%)	3 (9.7%)	1 (2.8%)	3 (1.4%)	9 (2.2%)	0 (0.0%)
Rotavirus A	FecalSwab	23 (1.9%)	13 (7.1%)	2 (1.7%)	7 (2.4%)	1 (0.2%)	0 (0.0%)
	Para-Pak C&S	4 (0.6%)	2 (6.5%)	0 (0.0%)	0 (0.0%)	2 (0.5%)	0 (0.0%)
Bacteria							
Campylobacter	FecalSwab	69 (5.6%)	25 (13.7%)	7 (5.8%)	17 (5.9%)	20 (3.2%)	0 (0.0%)
	Para-Pak C&S	30 (4.2%)	2 (6.5%)	0 (0.0%)	10 (4.7%)	18 (4.3%)	0 (0.0%)
Plesiomonas shigelloides	FecalSwab	2 (0.2%)	0 (0.0%)	0 (0.0%)	2 (0.7%)	0 (0.0%)	0 (0.0%)
	Para-Pak C&S	7 (1.0%)	1 (3.2%)	0 (0.0%)	4 (1.9%)	2 (0.5%)	0 (0.0%)
Salmonella	FecalSwab	14 (1.1%)	5 (2.7%)	4 (3.3%)	3 (1.0%)	2 (0.3%)	0 (0.0%)
	Para-Pak C&S	17 (2.4%)	4 (12.9%)	0 (0.0%)	3 (1.4%)	10 (2.4%)	0 (0.0%)
Yersinia enterocolitica	FecalSwab	22 (1.8%)	3 (1.6%)	2 (1.7%)	9 (3.1%)	8 (1.3%)	0 (0.0%)
	Para-Pak C&S	8 (1.1%)	0 (0.0%)	0 (0.0%)	4 (1.9%)	4 (1.0%)	0 (0.0%)
Diarrheagenic *E. coli*/*Shigella*							
Enteropathogenic *E. coli* (EPEC)	Para-Pak C&S	56 (7.9%)	9 (29.0%)	2 (5.6%)	18 (8.4%)	27 (6.5%)	0 (0.0%)
Enterotoxigenic *E. coli* (ETEC) lt/st	FecalSwab	18 (1.5%)	2 (1.1%)	2 (1.7%)	11 (3.8%)	3 (0.5%)	0 (0.0%)
	Para-Pak C&S	17 (2.4%)	1 (3.2%)	0 (0.0%)	7 (3.3%)	9 (2.2%)	0 (0.0%)
Shiga-like toxin *E. coli* (STEC) stx1/stx2	Para-Pak C&S	9 (1.3%)	0 (0.0%)	0 (0.0%)	6 (2.8%)	3 (0.7%)	0 (0.0%)
***E. coli* O157**	Para-Pak C&S	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Shigella/Enteroinvasive *E. coli* (EIEC)	FecalSwab	10 (0.8%)	1 (0.5%)	0 (0.0%)	6 (2.1%)	3 (0.5%)	0 (0.0%)
	Para-Pak C&S	3 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	2 (0.5%)	0 (0.0%)

Pathogen	Medium Brand	Overall	0-5 years	6-21 years	22-49 years	50+ years	Not Reported
Parasites							
Cryptosporidium	FecalSwab	2 (0.2%)	0 (0.0%)	1 (0.8%)	1 (0.3%)	0 (0.0%)	0 (0.0%)
	Para-Pak C&S	7 (1.0%)	0 (0.0%)	1 (2.8%)	4 (1.9%)	2 (0.5%)	0 (0.0%)
Cyclospora cayetanensis	FecalSwab	3 (0.2%)	0 (0.0%)	1 (0.8%)	2 (0.7%)	0 (0.0%)	0 (0.0%)
	Para-Pak C&S	18 (2.5%)	0 (0.0%)	0 (0.0%)	6 (2.8%)	12 (2.9%)	0 (0.0%)
Giardia lamblia	FecalSwab	15 (1.2%)	3 (1.6%)	1 (0.8%)	7 (2.4%)	4 (0.6%)	0 (0.0%)
	Para-Pak C&S	1 (0.1%)	1 (3.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Entamoeba histolytica	FecalSwab	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Para-Pak C&S	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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