May 31, 2024

QIAGEN GmbH
% Selina Salthouse
Regulatory Affairs Manager
QIAGEN Manchester Ltd
CityLabs 2.0, 200 Hathersage Road
Manchester, Manchester M13 0BH, United Kingdom

Re: K220062
Trade/Device Name: QIAstat-Dx Gastrointestinal Panel 2
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal microorganism multiplex nucleic acid-based assay
Regulatory Class: Class II
Product Code: PCH
Dated: April 4, 2023
Received: April 4, 2023

Dear Selina Salthouse:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Noel J. Gerald -S

Noel J. Gerald, Ph.D.
Branch Chief
Bacterial Respiratory and Medical Countermeasures Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K220062

Device Name
QIAstat-Dx Gastrointestinal Panel 2

Indications for Use *(Describe)*

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	Reported	Reported
• Astrovirus	Reported	Reported
• Norovirus GI/GII	Reported	Reported
• Rotavirus A	Reported	Reported
• Campylobacter (C. jejuni, C. coli and C. upsaliensis)	Reported	Reported
• Shigella/Enteroinvasive Escherichia coli (EIEC)	Reported	Reported
• Enteropathogenic Escherichia coli (EPEC)	Reported	Not Reported
• Enterotoxigenic Escherichia coli (ETEC) lt/st	Reported	Reported
• Shiga-like toxin-producing Escherichia coli (STEC) stx1/stx2 (including specific identification of E. coli O157 serogroup within STEC)	Reported	Not Reported
• Salmonella	Reported	Reported
• Plesiomonas shigelloides	Reported	Reported
• Yersinia enterocolitica	Reported	Reported
• Cryptosporidium	Reported	Reported
• Cyclospora cayetanensis	Reported	Reported
• Entamoeba histolytica	Reported	Reported
• Giardia lamblia*	Reported	Reported

*(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 assay test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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5.0 510(k) SUMMARY

General Information

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Date Prepared: May 30, 2024

Device Name: QIAstat-Dx® Gastrointestinal Panel 2

Trade Name: QIAstat-Dx® Gastrointestinal Panel 2

Common Name: QIAstat-Dx® Gastrointestinal Panel 2

Classification: 21 CFR 866.3990 - Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System

Product Code: PCH

Predicate Device

Manufacturer	Product Name	510(k) No.
BioFire Diagnostics LLC	FilmArray® Gastrointestinal (GI) Panel	K140407

Device Description

QIAstat-Dx is based on single-test cartridges with pre-packaged reagents including both wet and dry chemistry to handle the sample preparation and detection steps for the presence of a range of selected analytes by PCR technology. After insertion of the sample, the QIAstat-Dx assay cartridge is processed by the QIAstat-Dx Analyzer 1.0.

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 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. When the test is finished, the cartridge is removed by the user and discarded. The QIAstat-Dx Analyzer 1.0 automatically interprets test results and displays a summary on the analyzer display screen. The results can be printed using a connected printer if needed. The detected analytes are displayed in red. For other analytes tested, they are displayed in green if not detected or in gray if not applicable or invalid. The analyzer will report if an error occurs during processing, in which case the test will fail and no results will be provided (screen will show "FAIL").

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 Para-Pak C&S or FecalSwab transport 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 by the company but not provided with the QIAstat-Dx Gastrointestinal Panel 2.

QIAGEN provides an 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.

The RCA will provide the results to the Application Software (SW). The Application SW will store the information related to a given result in the database and will display a summary of detected analytes and the result for the IC. All POSITIVE analytes will be listed as "DETECTED PATHOGENS". The screen will also display the complete list of all "TESTED PATHOGENS", including positive, negative, not applicable or invalid analytes. All results for tests executed on each QIAstat-Dx Analyzer 1.0 are stored and can be reviewed in a specific archive on each QIAstat-Dx Analyzer 1.0.

Specimen collection and transport materials

Stool samples are collected and resuspended in Para-Pak C&S or FecalSwab transport medium following the manufacturer's instructions (Para-Pak C&S (Meridian Bioscience) or FecalSwab (COPAN)).

Intended 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 *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 assay test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

Comparison of the QIAstat-Dx® Gastrointestinal Panel 2 and the Predicate Device

The QIAstat-Dx Gastrointestinal Panel 2 is substantially equivalent to the predicate device:

- K140407: BioFire Diagnostics LLC FilmArray® Gastrointestinal (GI) Panel

Similarities and differences between the QIAstat-Dx Gastrointestinal Panel 2 and the predicate device are shown in Table 5.1.

Table 5.1: Comparison of the QIAstat-Dx Gastrointestinal Panel 2 with the predicate device

Characteristic	Device	Predicate
Name	QIAstat-Dx Gastrointestinal Panel 2	BioFire Diagnostics, LLC. FilmArray Gastrointestinal (GI) Panel
510(k) No.	K220062	K140407
Regulation	21 CFR 866.3990	21 CFR 866.3990
Product Code	PCH	PCH
Device Class	Class II	Class II
Similarities		
Intended 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 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: • 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*	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), 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*

Characteristic	Device	Predicate
	• 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 *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 assay test or non-infectious causes such as ulcerative colitis,	• 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, 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.

Characteristic	Device	Predicate
	irritable bowel syndrome, or Crohn's 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

Characteristic	Device	Predicate
		identification of acute gastroenteritis in the context of outbreaks.
Specimen Type	Preserved stool in Para-Pak C&S or FecalSwab transport media	Stool in Cary-Blair Medium
Analyte Detected	RNA/DNA	RNA/DNA
Organism Detected	See above	See above
Amplification and Detection Technology	PCR	PCR
Assay Controls	One internal control in each cartridge to control for sample processing that is subjected to all nucleic acid extraction and amplification steps similar to patient samples. 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.	Two controls are included in each reagent pouch to control for 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.
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: a generic *Vibrio* target which differentiates *Vibrio cholerae , Clostridium difficile (C. difficile)* toxin A/B, Enteroaggregative *Escherichia coli* (EAEC) and Sapovirus.
Nucleic Acid Extraction	Extraction of nucleic acids using silica membrane	Extraction of nucleic acids using magnetic beads

Characteristic	Device	Predicate
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
Operational	The sample is loaded straight into the cartridge.	The FilmArray® Gastrointestinal Panel has to rehydrate the cartridge before use
Amplification and Detection Instrument System	QIAstat-Dx Analyzer 1.0	FilmArray Instrument

Performance Characteristics – Non-Clinical Studies

Sensitivity (Limit of Detection)

The Analytical Sensitivity, or Limit of Detection (LoD), is defined as the lowest concentration at which ≥95% of the tested samples generate a positive call.

The LoD for each of the QIAstat-Dx Gastrointestinal Panel 2 target pathogenic organisms was assessed, using in total 36 pathogens strains, by analyzing serial dilutions of analytical samples prepared from culture isolates from commercial suppliers (e.g., ZeptoMetrix® and ATCC®) or clinical samples positive for target analytes commercially unavailable. Each sample tested was prepared in human stool matrix, which consists of a pool of previously tested negative clinical stool specimens resuspended in Para-Pak C&S (Meridian Biosciences) transport medium following the manufacturer's instructions collection device. A matrix equivalency study between Para-Pak C&S and FecalSwab transport media was conducted to support the conclusions in the section, except for EPEC, STEC stx1/stx2 and STEC O157 that are only applicable for Para-Pak C&S resuspended samples.

Individual LoD values for each QIAstat-Dx Gastrointestinal Panel 2 target is shown in Table 5.2.

Table 5.2: LoD concentration details for the 36 pathogen strains tested with QIAstat-Dx Gastrointestinal Panel 2

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen Strain	Supplier & Catalog ID	Dilution from the stock	Detection Rate	Concentration (molecular units) #	Concentration (microbiological units)
Campylobacter	*Campylobacter coli* 76-GA2 [LMG 21266]	ATCC 43478	1.00E-06	20/20	5802 copies/mL	1.2 CFU/mL

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen Strain	Supplier & Catalog ID	Dilution from the stock	Detection Rate	Concentration (molecular units) #	Concentration (microbiological units)
	Campylobacter coli CIP 7080	ATCC 33559	1.00E-05	20/20	8941 copies/mL	0.6 CFU/mL
	Campylobacter jejuni Z086	ZeptoMetrix 0801650	1.00E-06	20/20	14491 copies/mL	1660 CFU/mL
	Campylobacter jejuni subsp. Jejuni RM3193	ATCC BAA-1234	1.00E-06	19/20	7210 copies/mL	110 CFU/mL
	Campylobacter upsaliensis NCTC 11541	ZeptoMetrix 0801999	3.16E-06	20/20	56165 copies/mL	2259.4 CFU/mL
	Campylobacter upsaliensis RM3195	ATCC BAA-1059	1.00E-06	19/20	7631 copies/mL	35 CFU/mL
Plesiomonas shigelloides	Bader	ATCC 14029	3.16E-07	19/20	116 copies/mL	2.7 CFU/mL
	Z130	ZeptoMetrix 0801899	3.16E-07	20/20	481 copies/mL	2291 CFU/mL
Salmonella	*Salmonella enterica* Serovar Typhimurium Z005	ZeptoMetrix 0801437	3.16E-07	20/20	1441 copies/mL	4518.8 CFU/mL
	Salmonella enterica Serovar choleraseus	ATCC 13312	3.16E-07	20/20	647 copies/mL	91.6 CFU/mL
Yersinia enterocolitica	subsp. *enterocolitica* NTCC 11175, Biotype 4, serotype 3	ATCC 700822	3.16E-07	20/20	2496 copies/mL	120.1 CFU/mL
	Z036	ZeptoMetrix 0801734	1.00E-07	20/20	719 copies/mL	2070 CFU/mL
Enteropathogenic *E. coli* (EPEC)*	*Escherichia coli* O111:NM (EPEC)	ZeptoMetrix 0801747	3.16E-07	20/20	1817 copies/mL	2581.7 CFU/mL
	Escherichia coli 7.1493; EPEC; O84:H28	ZeptoMetrix 0801938	1.00E-06	20/20	29021 copies/mL	1190 CFU/mL
Enterotoxigenic *E. coli* (ETEC) *lt/st*	*Escherichia coli* ETEC; ST+, LT+	ZeptoMetrix 0801624	1.00E-07	20/20	855 copies/mL	567 CFU/mL
	Escherichia coli H10407, O78:H11	ATCC 35401	3.16E-08	19/20	367 copies/mL	10.1 CFU/mL

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen Strain	Supplier & Catalog ID	Dilution from the stock	Detection Rate	Concentration (molecular units) #	Concentration (microbiological units)
***Shigella*/ Enteroinvasive *E. coli* (EIEC)**	*Escherichia coli* CDC EDL 1282, O29:NM	ATCC 43892	1.00E-08	20/20	1431 copies/mL	41.3 CFU/mL
	Shigella sonnei NCDC 1120-66	ATCC 25931	3.16E-08	20/20	488 copies/mL	0.2 CFU/mL
Shiga-like toxin-producing *E.coli* (STEC) *stx1/stx2**	*Escherichia coli* O26:H4	ZeptoMetrix 0801748	3.16E-07	STEC stx1/stx2: 20/20	2012 copies/mL	726.8 CFU/mL
Shiga-like toxin-producing *E. coli* (STEC) O157*	*Escherichia coli* O157:H7; EDL933	ZeptoMetrix 0801622	3.16E-07	STEC stx1/stx2: 19/20 O157: 19/20	1217 copies/mL	2281.5 CFU/mL
Cryptosporidium	*Cryptosporidium parvum* – Iowa isolate	Waterborne® P102C	1.00E-05	20/20	661 copies/mL	n/a
	Cryptosporidium hominis	Public Health Wales UKM 84	3.16E-03	20/20	357 copies/mL	n/a
Cyclospora cayetanensis	N/A	LACNY-Clinical sample LAC2825	1.00E-04	19/20	53 copies/mL	n/a
	N/A	LACNY Clinical sample LAC2827	3.16E-04	20/20	137 copies/mL	n/a
Entamoeba histolytica	HM-1:IMSS (Mexico City 1967)	ATCC 30459	1.00E-06	20/20	7 copies/mL	0.2 cells/mL
	HK-9 (Korea)	ATCC 30015	1.00E-07	19/20	1 copy/mL	0.13 cells/mL
Giardia lamblia	WB (Bethesda)	ATCC 30957	3.16E-05	19/20	11850 copies/mL	790 cells/mL
	Portland-1	ATCC 30888	1.00E-04	20/20	14500 copies/mL	635 cells/mL
Adenovirus F40/F41	Type 40 (Dugan)	ZeptoMetrix 0810084CF	1.00E-06	20/20	11726 copies/mL	0.1 TCID50/mL
	Type 41 (Tak)	ZeptoMetrix 0810085CF	1.00E-07	19/20	979 copies/mL	0.05 TCID50/mL
Astrovirus	ERE IID 2371 (type 8)	Zeptometrix 0810277CF	1.00E-04	20/20	11586371 copies/mL	11.7 TCID50/mL
	ERE IID 2868 (type 4)	Zeptometrix 0810276CF	3.16E-06	19/20	52184 copies/mL	1.3 TCID50/mL

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen Strain	Supplier & Catalog ID	Dilution from the stock	Detection Rate	Concentration (molecular units) #	Concentration (microbiological units)
Norovirus GI/GII	GI.1 (recombinant)	ZeptoMetrix 0810086CF	3.16E-05	19/20	24629 copies/mL	891.1 TCID50/mL
	GII.4 (recombinant)	ZeptoMetrix 0810087CF	1.00E-05	20/20	8998 copies/mL	10.5 TCID50/mL
Rotavirus A	Wa	ZeptoMetrix 0810041CF	1.00E-04	19/20	5201 copies/mL	14.1 TCID50/mL
	69M	ZeptoMetrix 0810280CF	3.16E-05	19/20	5787 copies/mL	436.1 TCID50/mL

Molecular unit titers were determined using in-house developed and validated qPCR assays.
* Target applicable for Para-Pak C&S samples only.

Analytical Reactivity (Inclusivity)

Analytical Reactivity (Inclusivity) was evaluated with gastrointestinal pathogen isolates/strains that were selected based on clinical relevance and genetic, temporal and geographical diversity. Based on *in vitro* (wet) testing and *in silico* analysis, the QIAstat-Dx Gastrointestinal Panel 2 primers and probes are specific and inclusive for clinically prevalent and relevant strains for each pathogen tested.

In vitro **testing**

QIAstat-Dx Gastrointestinal Panel 2 is inclusive for 100% (114 out of 114) of the pathogen strains tested *in vitro*. Most pathogen strains evaluated in laboratory testing were detected at ≤ 3-fold (104/114) of the corresponding LoD reference strain which is written in bold (refer to Table 5.3 to **Error! Reference source not found.**). 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 at 3x LoD. Testing of these strains at 10x LoD generated the expected positive resulted for all replicates.

Table 5.3: Inclusivity test results for *Campylobacter* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Campylobacter	***Campylobacter coli***	**76-GA2 [LMG 21266]**	**ATCC**	**43478***	**1x LoD**
	Campylobacter coli	Z293	ZeptoMetrix	804272	1x LoD
	Campylobacter coli	CIP 7080 [1407, CIP 70.80]	ATCC	33559*	3x LoD
	Campylobacter jejuni	**Z086**	**ZeptoMetrix**	**0801650***	**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***	**1x LoD**
	Campylobacter upsaliensis	RM 3195 (1994)	ATCC	BAA-1059*	0.3x LoD
	Campylobacter upsaliensis	NCTC 11541 [C231]	ATCC	43954	1x LoD

* Strain tested during LoD verification study

Table 5.4: Inclusivity test results for *Plesiomonas shigelloides* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Plesiomonas shigelloides	***Plesiomonas shigelloides***	**Z130**	**ZeptoMetrix**	**0801899***	**1x LoD**
	Plesiomonas shigelloides	GNI 14	ATCC	51903	1x LoD
	Plesiomonas shigelloides	CDC 3085-55 [Bader M51, NCIB 9242, NCTC 10360, RH 798]	ATCC	14029*	0.3x LoD

* Strain tested during LoD verification study

Table 5.5: Inclusivity test results for *Salmonella* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Salmonella	***Salmonella enterica***	**Serovar Typhimurium Z005**	**ZeptoMetrix**	**0801437***	**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,	ATCC	9712	0.1x LoD

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
		serovar Saintpaul			
	Salmonella enterica	Subsp. Enterica, serovar Berta	NCTC	NC05770	0.1x LoD
	Salmonella enterica	Subps. Salame, II NCTC 10310 [JT945, SS140/61]	ATCC	700151	0.1x LoD
	Salmonella enterica	Subps. diarizonae IIIb, 62	ATCC	29934	0.1x LoD
	Salmonella enterica	Subps. houtenae IV, CIP 82.32 [264.66]	ATCC	43974	0.1x LoD
	Salmonella enterica	Subps. 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
	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,	ATCC	51962	0.1x LoD

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
		serovar Paratyphi B var. Java, CDC 5			
	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*	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,	ATCC	BAA-2739	0.3x LoD

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
		serovar Mississippi, CDC 2012K-0487			

* Strain tested during LoD verification study

Table 5.6: Inclusivity test results for *Yersinia enterocolitica* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Yersinia enterocolitica	***Yersinia enterocolitica***	**Z036**	**ZeptoMetrix**	**08017 34***	**1x LoD**
	Yersinia enterocolitica	NTCC 11175, Biotype 4, serotype 3 (O:3)	ATCC	70082 2*	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	O:9	ATCC	55075	3x LoD

* Strain tested during LoD verification study

Table 5.7: Inclusivity test results for Enteropathogenic *E. coli* (EPEC) strains. Only applicable for Para-Pak C&S samples.

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Enteropathogenic *E. coli* (EPEC)	**Enteropathogenic *E. coli* (EPEC)**	**O111:NM**	**ZeptoMetrix**	**080174 7***	**1x LoD**
	Enteropathogenic *E. coli* (EPEC)	7.1493,O84:H28	ZeptoMetrix	080193 8*	1x LoD
	Enteropathogenic *E. coli* (EPEC)	Stoke W,O111:K58(B4):H-	ATCC	33780	1x LoD

* Strain tested during LoD verification study

Table 5.8: Inclusivity test results for Enterotoxigenic *E. coli* (ETEC) strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Enterotoxigenic *E. coli* (ETEC) lt/st	**Enterotoxigenic *E. coli* (ETEC) lt/st**	**ST+, LT+**	**ZeptoMetrix**	**0801624 ***	**1x LoD**
	Enterotoxigenic *E. coli* (ETEC) lt/st	H10407,O78:H11,LT(+) / ctx A11(+)	ATCC	35401*	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[#]

* Strain tested during LoD verification study
[#] Testing at a lower concentration resulted in a detection rate of <100%.

Table 5.9: Inclusivity test results for Enteroinvasive *E. coli* (EIEC)*/Shigella* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Enteroinvasive E. coli (EIEC)/Shigella	**Enteroinvasive *E. coli* (EIEC)**	**CDC EDL 1282, O29:NM**	**ATCC**	**43892***	**1x LoD**
	Enteroinvasive *E. coli* (EIEC)	O172:H-	SSI Diagnostica	82171	3x LoD
	Shigella sonnei	**NCDC 1120-66**	**ATCC**	**25931***	**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
	Shigella sonnei (Serogroup D)	Z004	ZeptoMetrix	0801627	3x LoD
	Shigella boydii (Serogroup C)	AMC 43-G-58 [M44 (Type 170)]	ATCC	9207	10x LoD#

* Strain tested during LoD verification study
# Testing at a lower concentration resulted in a detection rate of <100%.

Table 5.10: Inclusivity test results for Shiga-like toxin-producing *E. coli* (STEC) (*stx1/stx2*-carrier strains). Only applicable for Para-Pak C&S samples.

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Shiga-like toxin producing *E. coli* (STEC) *stx1/stx2*	**Shiga-like toxin producing *E. coli* (STEC) - stx1/stx2**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622 ***	**1x LoD**
	Shiga-like toxin producing *E. coli* (STEC) - stx1	O26:H4,stx1 (+)	ZeptoMetrix	0801748 *	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1/stx2	Reference ATCC® 35150 (EDL 931),O157:H7,stx 1 (+), stx2 (+)	Microbiologics	617	3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	Reference CDC 00-3039,O45:H2,unk nown	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,stx1c (+), stx2b (+)	SSI Diagnostica	91350	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O92,O107:K+:H4 8,stx2d (+)	SSI Diagnostica	91352	10x LoD#
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O101:K32:H-,stx2e (+)	SSI Diagnostica	91354	0.3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1/stx2	O128ac:H-,stx2f (+)	SSI Diagnostica	91355	10x LoD#
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O26:H11,stx2a (+)	SSI Diagnostica	95211	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	O8 ,stx1d (+)	SSI Diagnostica	91349	1x LoD

* Strain tested during LoD verification study
# Testing at a lower concentration resulted in a detection rate of <100%.

Table 5.11: Inclusivity test results for Shiga-like toxin producing *E. coli* (STEC) O157 strains. Only applicable for Para-Pak C&S samples.

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Shiga-like toxin producing *E. coli* (STEC) O157	**Shiga-like toxin producing *E. coli* (STEC) O157**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622***	**1x LoD**
	Shiga-like toxin producing *E. coli* (STEC) O157	O128ac:H-,stx2f (+)	SSI Diagnostica	91355 #	10x LoD$
	Shiga-like toxin producing *E. coli* (STEC) O157	Reference ATCC® 35150 (EDL 931), O157:H7, stx1 (+), stx2 (+)	Microbiologics	617	1x LoD

* Strain tested during LoD verification study
# 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
$Testing at a lower concentration resulted in a detection rate of <100%.

Table 5.12: Inclusivity test results for *Cryptosporidium* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Cryptosporidium	***Cryptosporidium parvum***	**Iowa isolate**	**Waterborne**	**P102C***	**1x LoD**
	Cryptosporidium hominis	n/a	Public Health Wales	Clinical sample; UKM 84*	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

* Strain tested during LoD verification study

Table 5.13: Inclusivity test results for *Cyclospora cayetanensis* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Cyclospora cayetanensis	***Cyclospora cayetanensis***	**n/a**	**LACNY**	**Clinical sample, LAC2825***	**1x LoD**
	Cyclospora cayetanensis	n/a	**LACNY**	Clinical sample, LAC2827*	1x LoD
	Cyclospora cayetanensis	–	ATCC	PRA-3000SD	1x LoD

* Strain tested during LoD verification study

Table 5.14: Inclusivity test results for *Entamoeba histolytica* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Entamoeba histolytica	***Entamoeba histolytica***	**HM-1:IMSS (Mexico City 1967)**	**ATCC**	**30459***	**1x LoD**
	Entamoeba histolytica	HK-9 (Korea)	ATCC	30015*	1x LoD
	Entamoeba histolytica	–	Hospital Vall d'Hebrón	Clinical sample; 1	1x LoD

* Strain tested during LoD verification study

Table 5.15: Inclusivity test results for *Giardia lamblia* strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Giardia lamblia	***Giardia lamblia***	**Portland -1 (Portland, OR, 1971)**	**ATCC**	**30888***	**1x LoD**
	Giardia lamblia	WB (Bethesda, MD, 1979)	ATCC	30957*	1x LoD
	Giardia intestinalis	H3 isolate	Waterborne	P101	1x LoD

* Strain tested during LoD verification study

Table 5.16: Inclusivity test results for Adenovirus F40/F41 strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Adenovirus F40/F41	**Human Adenovirus F41**	**Tak**	**ZeptoMetrix**	**0810085CF***	**1x LoD**
	Human Adenovirus F41	Tak (73-3544)	ATCC	VR-930	10x LoD#
	Human Adenovirus F40	Dugan [79-18025]	ATCC	VR-931	10x LoD#
	Human Adenovirus F 40	Dugan	ZeptoMetrix	0810084CF*	3x LoD

* Strain tested during LoD verification study
# Testing at a lower concentration resulted in a detection rate of <100%.

Table 5.17: Inclusivity test results for Astrovirus strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Astrovirus	**Human Astrovirus**	**ERE IID 2371 (type 8)**	**ZeptoMetrix**	**0810277CF***	**1x LoD**
	Human Astrovirus	HAstV-1	Universitat de Barcelona	Clinical sample; 160521599	1x LoD
	Human Astrovirus	ERE IID 2868 (type 4)	ZeptoMetrix	0810276CF*	1x LoD
	Human Astrovirus	HAstV-3	Universitat de Barcelona	Clinical sample; 151601306	1x LoD

* Strain tested during LoD verification study

Table 5.18: Inclusivity test results for Norovirus GI/GII strains

QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Norovirus GI/GII	**Human Norovirus Genogroup 1**	**Recombinant GI.1**	**ZeptoMetrix**	**0810086CF***	**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***	**1x LoD**
	Human Norovirus Genogroup 2	GII.2	Hospital Vall d'Hebrón	Clinical sample; 198058327	1x LoD
	Human Norovirus Genogroup 2	GII.4	Universitat de Barcelona	Clinicial sample; N26.2TA	1x LoD
	Human Norovirus Genogroup 2	–	LACNY	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	Clinical sample; LAC2133	10x LoD#
	Human Norovirus Genogroup 2	–	LACNY	Clinical sample; LAC2074	10x LoD#

* Strain tested during LoD verification study
# Testing at a lower concentration resulted in a detection rate of <100%.

Table 5.19: Inclusivity test results for Rotavirus A strains

QIAs QIAstat-Dx Gastrointestinal Panel 2 Target	Pathogen	Strain	Supplier	Catalog ID	x-fold LoD detected
Rotavirus A	**Human Rotavirus A**	**69M**	**Zepto Metrix**	**0810280 CF***	**1x LoD**
	Human Rotavirus A	Wa, G1P1A[8]	ZeptoMetrix	0810041 CF*	1x LoD
	Human Rotavirus A	DS-1, G2P1B[4]	ATCC	VR-2550	1x LoD
	Human Rotavirus A	Va70	ZeptoMetrix	0810281 CF	1x LoD
	Human Rotavirus A	RRV	ZeptoMetrix	0810530 CF	10x LoD#

* Strain tested during LoD verification study
# Testing at a lower concentration resulted in a detection rate of <100%.

In silico **analysis**

In silico analysis of potential reactivity showed that the following organisms (including species, subspecies, subtypes, serotypes or serovars) are predicted to be detected with the QIAstat-Dx Gastrointestinal Panel 2 (Table 5.20).

Table 5.20: Organisms with predicted reactivity based on *in silico* analysis

QIAstat-Dx GI Panel 2 Target	Organisms 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, 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:

QIAstat-Dx GI Panel 2 Target	Organisms with predicted reactivity
	stx1a, stx1c, stx1d, stx2a, stx2b, stx2c, stx2d, stx2d, stx2e, stx2f, stx2g, stx2h, stx2i, stx2j, stx2k and stx2l. 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 small 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] Target only applicable for Para-Pak C&S samples.

Analytical Specificity (Cross-Reactivity and Exclusivity)

The analytical specificity study was carried out by *in vitro* testing and *in silico* analysis to assess the potential cross-reactivity of the QIAstat-Dx Gastrointestinal Panel 2. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity and Off-panel organisms were tested to evaluate cross-reactivity with organisms not covered by the panel content. The On-panel and Off-panel organisms tested are shown in Table 5.21 and Table 5.22, respectively.

Samples were prepared by single spiking organisms into negative stool resuspended in ParaPak C&S media at the highest concentration possible based on the organism stock, preferably at 10^5 $TCID_{50}$/ml for viral, 10^5 cells/ml for parasite targets and 10^6 CFU/ml for bacterial targets. The pathogens were tested in 3 replicates. There was no intra-panel or Off-panel cross-reactivity for all pathogens tested *in vitro*, except for 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.

Table 5.21: List of Analytical Specificity On-Panel Pathogens Tested

Type	Pathogen
Bacteria	*Campylobacter coli*
	Campylobacter jejuni
	Campylobacter upsaliensis
	Escherichia coli (EPEC)*
	Escherichia coli (ETEC lt/st)
	Escherichia coli (STEC)*
	Plesiomonas shigelloides
	Salmonella enterica
	Shigella sonnei
	Yersinia enterocolitica
Parasites	*Cryptosporidium parvum*
	Cyclospora cayetanensis
	Entamoeba histolytica
	Giardia lamblia
Viruses	Adenovirus F41
	Astrovirus
	Norovirus GI
	Norovirus GII
	Rotavirus

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

Table 5.22: List of Analytical Specificity Off-Panel Pathogens Tested

Type	Pathogen (Potential Cross-Reactant)
Bacteria	*Abiotrophia defectiva*
	Acinetobacter baumannii
	Aeromonas hydrophila
	Arcobacter cryaerophilus
	Bacillus subtilis
	Bifidobacterium bifidum
	Campylobacter fetus
	Campylobacter gracilis
	Campylobacter helveticus (Cross-reactive for Campylobacter target)
	Campylobacter hominis
	Campylobacter lari (Cross-reactive for Campylobacter target)
	Campylobacter mucosalis
	Campylobacter rectus
	Citrobacter freundii
	Clostridium difficile non-toxigenic
	Clostridium perfringens
	Clostridium septicum
	Clostridium tetani
	Corynebacterium genitalium
	Enterobacter aerogenes
	Enterobacter cloacae
	Enterococcus faecalis
	Enterococcus faecium
	Escherichia fergusonii
	Escherichia hermannii
	Escherichia vulneris
	Faecalibacterium prausnitzii
	Gardnerella vaginalis
	Haemophilus influenzae
	Hathewaya histolytica (Clostridium)
	Helicobacter pylori
	Klebsiella pneumoniae
	Lactobacillus casei
	Listeria monocytogenes
	Proteus mirabilis
	Proteus vulgaris
	Pseudomonas aeruginosa
	Staphylococcus aureus
	Staphylococcus aureus subsp. aureus
	Staphylococcus epidermidis
	Streptococcus agalactiae
	Streptococcus pyogenes

Type	Pathogen (Potential Cross-Reactant)
Fungi	*Aspergillus fumigatus*
	Candida albicans
	Saccharomyces boulardii
	Saccharomyces cerevisiae
Parasite	*Babesia microti*
	Blastocystis hominis
	Chlamydia trachomatis
	Giardia muris
	Toxoplasma gondii
	Trichomonas tenax
Viruses	Adenovirus C:2
	Adenovirus B:34
	Adenovirus B3
	Adenovirus E:4a
	Adenovirus serotype 1
	Adenovirus serotype 5
	Adenovirus serotype 8
	Bocavirus Type 1
	Coronavirus 229E
	Coxsackievirus B3
	Cytomegalovirus
	Enterovirus 6 (Echovirus)
	Enterovirus 68
	Herpes Simplex Virus Type 2
	Rhinovirus 1A

In silico predictions of potential cross-reactions show that the following cross-reactions may occur when testing stool samples with the QIAstat-Dx Gastrointestinal Panel 2 (Table 5.23)

Table 5.23: Potential Cross-Reactions Based On *in silico* Analysis

QIAstat-Dx Gastrointestinal Panel 2 Target	Design Abbreviation	Potential cross-reactive organisms
Enteropathogenic *E. coli* (EPEC) [7]	EPEC_eae	*Shigella boydii*[1,2,3], *Escherichia albertii*[1,2]
Campylobacter.	Cupsal_cpn60	*Campylobacter lari*[4], *Campylobacter helveticus*[4]
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* [7]	STEC_stx1	*Shigella sonnei*[1,3], *Shigella dysenteriae*[1,3]
	STEC_stx2	*Acinetobacter haemolyticus*[1,5], *Citrobacter freundi*[1,5], *Enterobacter cloacae*[1,5] *Aeromonas caviae*[1,5]
	STEC_stx2f	*Escherichia albertii*[1,5]
E. coli O157 [7]	O157	Non-STEC *E. coli* O157 strains[6]

[1]Note that these potential cross-reactions identified by *in-silico* analysis reflects sequences which 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 Stx toxins producers
[6]*E. coli* O157 reported by the QIAstat-Dx Gastrointestinal Panel will only be called 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 they will not be differentiated from a single infection caused by an STEC O157:H7 strain.
[7] Target only applicable for Para-Pak C&S samples.

Interfering Substances

The effect of potentially interfering substances on the detectability of the QIAstat-Dx Gastrointestinal Panel 2 organisms was evaluated. Thirty-four (34) 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. Each organism was tested at 3x LoD and testing was performed in triplicates. Endogenous substances such as human whole blood, human genomic DNA and several pathogens were tested alongside exogenous substances like antibiotics, other gastrointestinal-related medications and different technique-specific substances.

For the vast majority of substances tested, no inhibition was observed, with the exceptions of mucin, calcium carbonate, nonoxynol-9 and Rotavirus reassortants, that may cause inhibition at high concentration.

Mucin at 5% w/v was found to generate false positives results for the *Yersinia* target. These signals were investigated by testing the interfering substance with and FDA-cleared method and they were confirmed to be present in the endogenous substance.

Calcium carbonate was found to interfere with the detection of all the QIAstat-Dx Gastrointestinal Panel 2 targets at concentrations above 0.5% w/v.

Nonoxynol-9 was found to interfere with the detection of *Entamoeba histolytica* at concentrations above 0.02% v/v.

As predicted, 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 $8.89x10^{-5}$ $TCID_{50}$/ml and 1.10 PFU/ml, respectively (see Table 5.24 for other concentrations tested).

Results from the 34 interfering substances that could be present or introduced in a stool specimen are provided in Table 5.24.

Table 5.24: Final highest concentrations per potentially interfering substance without observable interference on the QIAstat-Dx Gastrointestinal Panel 2

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	No Interference
	10 µg/mL	No Interference
	5 µg/mL	No Interference
Human stool (overfill of Cary Blair vial)	300 mg/mL	No Interference
Human urine	50% v/v	No Interference
Human whole blood with Na Citrate	40% v/v	No Interference
Mucin from bovine submaxillary	5% w/v	Interference#
	2.5% w/v	No Interference
Triglycerides	5% w/v	No Interference
Exogenous substances		
Bacitracin	250U/mL	No Interference
Bisacodyl	0.3% w/v	No Interference
	0.15% w/v	No Interference
Bismuth subsalicylate	0.35% w/v	No Interference
Calcium carbonate (TUMS® Extra Strength 750)	5% w/v	Interference
	0.5% w/v	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

Substance tested	Concentration tested	Result
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	Interference
	0.6% v/v	Interference
	0.3% v/v	Interference
	0.15% v/v	Interference
	0.075% v/v	Interference
	0.02% v/v	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} TCID50/mL	Interference
	8.89×10^{-4} TCID50/mL	Interference
	8.89×10^{-5} TCID50/mL	No Interference
Rotavirus reassortant WI79-4,9 - VR 2415	1.10×10^{2} pfu/mL	Interference
	1.10×10^{1} pfu/mL	Interference
	1.10 pfu/mL	No Interference
Technique-specific Substances, Transport Media		
Bleach	0.5% v/v	No Interference
Ethanol	0.2% v/v	No Interference
Puritan Fecal Opti-Swab Collection & Transport System with Cary-Blair Medium*	100%	No Interference
PurSafe DNA/RNA Preservative*	100%	No Interference
Sigma Fecal Transwab*	1 swab/2mL Cary Blair	No Interference

*Performance not stablished for this transport media
This substance was tested by another FDA-cleared that also detected Yersinia positive signals.

Microbial Interference

A microbial interference study was conducted to assess the inhibitory effects of select non-target organisms on the ability to detect the QIAstat-Dx Gastrointestinal Panel 2 targets. Clinically relevant and challenging concentrations of non-target organisms were individually mixed with negative clinical stool matrix with spiked targeted pathogens at 3x LoD. Testing was performed in triplicate. All combinations and replicates successfully detected all the QIAstat-Dx Gastrointestinal Panel 2 targets. See Table 5.25 for a list of the non-target organisms tested and the result summary.

Table 5.25: Final highest concentration without observable inhibitory effect

Substance tested	Concentration tested	Result
Non-target microorganisms		
Aeromonas hydrophila	1 x 10^6 units/mL	No Interference
Bacteroides vulgatus	1 x 10^6 units/mL	No Interference
Bifidobacterium bifidum	1 x 10^6 units/mL	No Interference
Enterovirus Specie D, Serotype EV-D68	1 x 10^5 units/mL	No Interference
Non-pathogenic *E. coli*	1 x 10^6 units/mL	No Interference
Helicobacter pylori	1 x 10^6 units/mL	No Interference
Saccharomyces cerevisiae (deposited as *S. boulardii*)	1 x 10^5 units/mL	No Interference

Competitive Interference

Competitive interference was tested in a subset of pathogens. No interference was observed when evaluating competitive interference by target pathogens when two QIAstat-Dx Gastrointestinal Panel target pathogens were tested by spiking samples with one pathogen target at 3x LoD and one at 50x LoD. Results from the pathogen targets tested are provided in Table 5.26.

Table 5.26: QIAstat-Dx Gastrointestinal Panel 2 Detection Rate Results for Competitive Interference

Mix	Pathogen	Final concentration (molecular units)*	Final concentration tested xLoD	Detection rate	Target detected
Norovirus 50x - Rotavirus 3x	Norovirus GI/GII	4.5E+05 copies/mL	50x	3/3	Yes
	Rotavirus A	1.7E+04 copies/mL	3x	3/3	Yes
Norovirus 3x - Rotavirus 50x	Norovirus GI/GII	2.7E+04 copies/mL	3x	3/3	Yes
	Rotavirus A	2.9E+05 copies/mL	50x	3/3	Yes
Giardia 50x - Adenovirus 3x	*Giardia lamblia*	7.2 E+05 copies/mL	50x	3/3	Yes
	Adenovirus F40/F41	2.9E+03 copies/mL	3x	3/3	Yes

* Molecular unit titers were determined using in house developed and validated qPCR assays.

Carryover

A carryover study was performed to evaluate the potential occurrence of cross-contamination between consecutive runs when using the QIAstat-Dx Gastrointestinal Panel 2 on the QIAstat-Dx Analyzer 1.0.

Pathogen samples of stool sample matrix, with alternating high-positive (10^5-10^6 organism/mL) and negative samples, were conducted on two QIAstat-Dx Analyzer 1.0.

No carryover between samples was observed in the QIAstat-Dx Gastrointestinal Panel 2, demonstrating that the system design and recommended sample handling and testing practices are effective in preventing false positive results due to carryover or cross-contamination between samples.

Sample Stability

Sample stability testing demonstrated that the QIAstat-Dx Gastrointestinal Panel 2 assay is capable of processing samples which are stored prior to analysis under conditions typically utilized for stool specimens according to the intended use.

The results of this study support the following recommendations for storage of stool resuspended in Para-Pak C&S and FecalSwab transport media before testing:

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

Reproducibility

Reproducibility testing of 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 potential variation introduced by sites, days, replicates, cartridge lots, operators, and QIAstat-Dx analyzers. For each site, testing was performed across 5 non-consecutive days with 6 replicates per day (leading to a total of 30 replicates per target, concentration and site), 4 QIAstat-Dx Analyzers (2 analyzers per operator and per site), and at least 2 operators on each testing day. A total of 5 sample mixes (two combined samples at 1x LoD and 3x LoD plus one negative sample) were prepared. For each mix, 6 replicates were tested and evaluated. shows the detection rate per target and concentration for each site of the Reproducibility study. In addition, data obtained at all three sites have been compiled to calculate the exact 2-sided 95% Confidence Interval by target and concentration.

Table 5.27 shows the detection rate per target and concentration for each site of the Reproducibility study. In addition, data obtained at all three sites have been compiled to calculate the exact 2-sided 95% Confidence Interval by target and concentration.

Table 5.27: Detection rate per target and concentration for each site of the Reproducibility study and exact 2-sided 95% Confidence Interval by target and concentration

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 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	29/30 96.67%	29/30 96.67%	29/30 96.67%	87/90[a] 96.67% (90.98-98.9%)
Campylobacter ZeptoMetrix 0801650	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30[b] 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Escherichia coli EPEC[d] ZeptoMetrix 0801747	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	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 - 100.00%)
Entamoeba histolytica ATCC 30459	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	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 - 100.00%)
Giardia lamblia[b] ATCC 30888	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)

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 - 100.00%)
	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 - 100.00%)
Rotavirus A[c] 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 - 100.00%)
Escherichia coli (STEC) O157:H7[d] ZeptoMetrix 0801622	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	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 - 100.00%)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Escherichia coli (STEC) *stx1/stx2*[d] ZeptoMetrix 0801622	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Salmonella enterica ZeptoMetrix 0801437	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	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 – 100.00%)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Yersinia enterocolitica ZeptoMetrix 0801734	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 – 100.00%)

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

[b] 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.

[c] 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 during an interim data evaluation (61/72, 84.7%) and was attributed to the sample manufacture 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.

[d]Target only applicable for Para-Pak C&S samples.

During the reproducibility study, potential variation introduced by sites, days, replicates, cartridge lots, operators, and QIAstat-Dx analyzers was analyzed showing no significant contribution to variability (Standard Deviation and Coefficient of Variation values below 1 and 5%, respectively) caused by any of the assessed variables (Table 5.28).

Table 5.28: Variability attributable to each variable according to ANOVA model for QIAstat-Dx Gastrointestinal Panel 2.

					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**	**61**	**11**	**36.71**	(0.0000, 0.00%)	(0.1487, 0.41%)	(0.0000, 0.00%)	(0.1824, 0.50%)	(0.0000, 0.00%)	(0.9172, 2.50%)	(0.9356, 2.55%)
	3x LoD	**88**	**2**	**35.95**	(0.3468, 0.96%)	(0.4463, 1.24%)	(0.1231, 0.34%)	(0.1295, 0.36%)	(0.1132, 0.31%)	(1.2073, 3.36%)	(1.3014, 3.62%)
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. 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

A repeatability study was conducted on the QIAstat-Dx Analyzer 1.0 instruments using a set of samples composed of low-concentrated analytes spiked into stool matrix (3x LoD and 1x LoD) and negative stool samples. QIAstat-Dx Gastrointestinal Panel 2 detected pathogens included in the positive samples were Adenovirus, *Campylobacter*, Enteropathogenic *E. coli* (EPEC), *Entamoeba histolytica, Giardia lamblia*, Norovirus GII, Rotavirus, *E. coli* O157, STEC stx1/stx2, *Salmonella enterica, and Yersinia enterocolitica*. Each sample was tested with the same instrument over 12 days. In total, 60 replicates of 1x LoD and 60 replicates of 3x LoD per each of the tested targets and 60 replicates of negative samples were run. Overall results showed a 93.33-100.00-% and 95.00-100.00% detection rate for 1x LoD and 3x LoD samples, respectively. Negative samples showed 100% of negative calls for all panel analytes.

Performance Characteristics - Clinical Studies

Expected values

In the prospective clinical performance evaluation of the QIAstat-Dx Gastrointestinal Panel 2, 1939 eligible specimens (stool preserved in Para-Pak C&S (Meridian Bioscience) or FecalSwab (COPAN)) were collected and tested at 13 clinical sites across 5 countries (4 sites in Europe and 9 sites in USA) from May 2021 to July 2021. The number and percentage of positive results as determined by the QIAstat-Dx Gastrointestinal Panel 2, stratified by age group, are presented in Table 5.29. Overall, the QIAstat-Dx Gastrointestinal Panel 2 detected at least 1 organism in 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 5.29).

Table 5.29: 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%)
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%)

Clinical Performance

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 USA and Europe (9 sites in USA and 4 sites in Europe) between May and July 2021. All study sites were hospital-associated or independent clinical diagnostics laboratories that perform routine diagnostics of GI infections. Table 5.30 provides a summary of prospective specimen's distribution across all study sites.

Table 5.30: Prospective Specimens Distribution Across the study sites

	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	0	0	0 [a]
USA site 8	0	131	131
USA site 9	0	95	95
Total	**1222**	**717**	**1939**

[a] the specimens from this site (148) were excluded from the analysis because they were collected with another device different to Para-Pak C&S or FecalSwab

Table 5.31: provides all demographic information for the 1,939 specimens evaluated in the prospective study.

Table 5.31: Demographic data for prospective evaluated specimens

Demographic data	**FecalSwab**		**Para-Pak C&S**	
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 5.32:). The composite endpoint was determined as the majority of the three results.

Table 5.32: QIAstat-Dx Gastrointestinal Panel 2 Clinical studies comparator method

QIAstat-Dx GI Panel 2 Test Result	Comparator Method
Adenovirus F40/41	One FDA-cleared test method
Astrovirus	
Rotavirus A	
Sapovirus	
Campylobacter	
Plesiomonas shigelloides	
Salmonella	
Yersinia enterocolitica	
E. coli O157 [a]	
Enteropathogenic *E. coli* (EPEC) [a]	
Shigella/Enteroinvasive *E. coli* (EIEC)	
Cryptosporidium	
Cyclospora cayetanensis	
Entamoeba histolytica	
Norovirus GI/GII	Composite of three FDA-cleared test methods
Enterotoxigenic *E. coli* (ETEC) *lt/st*	Composite of three FDA-cleared test methods
Shiga-like toxin- *E. coli* (STEC) *stx1*/*stx2* [a]	Composite of three FDA-cleared test methods
Giardia lamblia	Composite of two FDA-cleared test methods and two validated PCR tests followed by bi-directional sequencing [b]

[a] Targets evaluated in Para-Pak C&S specimens only.
[b] Each of the two PCR 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.

Additional prospective archived samples were collected for Norovirus GI/GII (81 samples) and STEC (18 samples). These were prospectively collected samples from four different collection sites (3 US and 1 EU), where only those positive for the pathogen by standard of care method were archived for analysis alongside 20 negative specimens.

In addition, to supplement the results of the prospective clinical studies, a total of 750 preselected archived frozen (retrospective) specimens were also evaluated. These specimens served to increase the sample size for analytes that showed low prevalence in the clinical prospective study or that were less represented in a particular sample type (Para-Pak C&S or FecalSwab). The same Comparator Methods detailed in Table 5.31 were used as confirmatory testing for the presence of the nucleic acids from the expected.

In total 2808 specimens (1939 prospective, 119 prospective archived and 750 retrospective) were evaluated in the clinical study. These specimens were collected using Para-Pak C&S (1217) or FecalSwab (1591).

The positive percentage agreement (PPA) and the negative percentage agreement (NPA) were calculated for the prospective and retrospective studies and for each sample type (Para-Pak C&S and FecalSwab) separately.

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 this 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, but the comparator method result was negative. The PPA and NPA exact binomial two-sided 95% confidence interval was calculated.

Where a composite comparator was used (Table 5.32), 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 and the composite comparator while for the samples where it was not possible to conduct testing with the complete comparator due to insufficient sample volume, the following was done:

- samples that were negative in QIAstat-Dx and positive for one comparator assay, 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 in QIAstat-Dx and positive in 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 in the PPA calculations.

The results of the clinical performance of the prospective, prospective archived and retrospective studies are summarized in Tables: Table 5.33: , Table 5.34 and Table 5.35, respectively.

Discrepancies between the QIAstat-Dx Gastrointestinal Panel 2 and the comparator methods were investigated for the analytes that the QIAstat-Dx Gastrointestinal Panel 2 test result was compared to one FDA-cleared method. Discrepancies analyses are footnoted on each clinical performance summary Table below (Table 5.33, and Table 5.35).

Table 5.33: Clinical Performance in the 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 [a]	83.3	43.7-97.0	1214 / 1214	100.0	99.7-100.0
	Para-Pak C&S	1 / 2 [b]	50.0	9.5-90.6	703 / 704 [b]	99.9	99.2-100.0
Astrovirus	FecalSwab	3 / 3	100.0	43.9-100.0	1219 / 1219	100.0	99.7-100.0
	Para-Pak C&S	6 / 6	100.0	61.0-100.0	700 / 700	100.0	99.5-100.0
Norovirus GI/GII	FecalSwab	31 / 33 [c]	93.9	80.4-98.3	493 / 495 [c]	99.6	98.6-100.0
	Para-Pak C&S	14 / 18 [d]	77.8	54.8-91.0	399 / 399 [d]	100.0	99.1-100.0
Rotavirus A	FecalSwab	21 / 23 [e]	91.3	73.2-97.6	1197 / 1199 [e]	99.8	99.4-100.0
	Para-Pak C&S	3 / 3	100.0	43.9-100.0	702 / 703 [f]	99.9	99.2-100.0
Bacteria							
Campylobacter	FecalSwab	65 / 67 [g]	97.0	89.8-99.2	1151 / 1155 [g]	99.7	99.1-99.9
	Para-Pak C&S	30 / 31 [h]	96.8	83.8-99.4	675 / 677 [h]	99.7	98.9-99.9
Plesiomonas shigelloides	FecalSwab	0 / 0	N/A	N/A	1220 / 1222 [i]	99.8	99.4-100.0
	Para-Pak C&S	5 / 6 [j]	83.3	43.7-97.0	698 / 700 [j]	99.7	99.0-99.9
Salmonella	FecalSwab	14 / 16 [k]	87.5	64.0-96.5	1206 / 1206	100.0	99.7-100.0
	Para-Pak C&S	19 / 20 [l]	95.0	76.4-99.1	688 / 688	100.0	99.4-100.0
Yersinia enterocolitica	FecalSwab	15 / 16 [m]	93.8	71.7-99.0	1199 / 1206 [m]	99.4	98.8-99.7
	Para-Pak C&S	3 / 3	100.0	43.9-100.0	698 / 703 [n]	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	100.0	99.4-100.0
Enterotoxigenic *E. coli* (ETEC) *lt*/*st*	FecalSwab	9 / 10 [o]	90.0	59.6-99.2	427 / 430 [o]	99.3	98.0-99.8
	Para-Pak C&S	9 / 10 [p]	90.0	59.6-99.2	390 / 395 [p]	98.7	97.1-99.5
Shiga-like toxin *E. coli* (STEC) *stx1*/*stx2*	Para-Pak C&S	5 / 6 [q]	83.3	43.6-97.0	397 / 400 [q]	99.3	97.8-99.7
***E. coli* O157**	Para-Pak C&S	0 / 0	N/A	N/A	5 / 5	100.0	56.6-100.0
***Shigella*/Enteroinvasive *E. coli* (EIEC)**	FecalSwab	10 / 10	100.0	72.3-100.0	1212 / 1212	100.0	99.7-100.0
	Para-Pak C&S	2 / 2	100.0	34.2-100.0	703 / 704 [r]	99.9	99.2-100.0
Parasites							
Cryptosporidium	FecalSwab	2 / 4	50.0	15.0-85.0	1218 / 1218	100.0	99.7-100.0
	Para-Pak C&S	6 / 6	100.0	61.0-100.0	699 / 700 [s]	99.9	99.2-100.0
Cyclospora cayetanensis	FecalSwab	3 / 3	100.00	43.9-100.0	1219 / 1219	100.0	99.7-100.0
	Para-Pak C&S	18 / 19 [t]	94.7	75.4-99.1	687 / 687	100.0	99.4-100.0

		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Medium Brand**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Entamoeba histolytica	FecalSwab	0 / 0	N/A	N/A	1222 / 1222	100.0	99.7-100.0
	Para-Pak C&S	0 / 0	N/A	N/A	706 / 706	100.0	99.5-100.0
Giardia lamblia	FecalSwab	6 / 8 [u]	75.0	40.9-92.9	434 / 441 [u]	98.4	96.8-99.2
	Para-Pak C&S	1 / 1	100.0	20.7-100.0	406 / 406 [v]	100.0	99.1-100.0

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

[b] 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

[c] ten (10) FecalSwab samples positive for Norovirus GI/GII 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. The sample size for NPA is smaller for Norovirus GI/GII 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.

[d] two (2) Para-Pak C&S samples positive for Norovirus GI/GII 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) Para-Pak C&S sample negative in 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 in QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[e] 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.

[f] 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.

[g] *Campylobacter* 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.

[h] *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.

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

[j] *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

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

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

[m] *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.

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

[o] six (6) FecalSwab samples positive for ETEC in 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 in QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[p] three (3) Para-Pak C&S 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. The sample size for NPA is smaller for ETEC 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.

[q] one (1) FecalSwab 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. The sample size for NPA is smaller for STEC 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.

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

[s] *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.

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

[u] two (2) FecalSwab samples positive for *Giardia lamblia* 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. Two (2) FecalSwab samples negative in 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 in QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the prospective study.

[v] 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.

Table 5.34: Clinical Performance in the Prospective Archived study

		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 [a]	98.0	89.3 - 99.6	2 / 4 [a]	50.0	15.0-85.0
	Para-Pak C&S	26 / 28 [a,b]	92.9	77.4 - 98.0	37 / 38 [a]	97.4	86.5-99.5
Shiga-like toxin *E. coli* (STEC) *stx1/stx2*	Para-Pak C&S	12 / 13 [c,d]	92.3	66.7 - 98.6	51 / 52 [c]	98.1	89.9-99.7

[a] For Norovirus GI/GII/GII four out of the eighty-one (4/81) positive prospectively archived samples (positive by standard of care) were negative by the composite comparator and therefore included as negative samples in the NPA calculations.
[b] One (1) Para-Pak C&S sample negative in QIAstat-Dx and positive for Norovirus GI/GII with one FDA-cleared comparator with insufficient volume for complete composite comparator testing was classed as false negative in the PPA calculations.
[c] For STEC five out of the eighteen (5/18) positive prospectively archived samples (positive by standard of care) were negative by the composite comparator and therefore included as negative samples in the NPA calculations.
[d] 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.

Table 5.35: 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 [a]	88.5	71.0-96.0	203 / 203	100.0	98.2-100.0
	Para-Pak C&S	29 / 29	100.0	88.3-100.0	39 / 39	100.0	91.0-100.0
Astrovirus	FecalSwab	2 / 3 [b]	66.7	20.8-93.9	191 / 191	100.0	98.0-100.0
	Para-Pak C&S	0 / 0	N/A	N/A	14 / 14	100.0	78.5-100.0
Norovirus GI/GII	FecalSwab	28 / 32 [c]	87.5	71.9-95.0	74 / 75 [c]	98.7	92.8-100.0
	Para-Pak C&S	27 / 29	93.1	78.0-98.1	86 / 86 [d]	100.0	95.8-100.0
Rotavirus A	FecalSwab	8 / 9 [e]	88.9	56.5-98.0	185 / 185	100.0	98.0-100.0
	Para-Pak C&S	2 / 2	100.0	34.2-100.0	12 / 12	100.0	75.8-100.0
Bacteria							
Campylobacter	FecalSwab	31 / 31	100.0	89.0-100.0	161 / 163 [e]	98.8	95.6-99.7
	Para-Pak C&S	3 / 3	100.0	43.9-100.0	11 / 11	100.0	74.1-100.0
Plesiomonas shigelloides	FecalSwab	2 / 2	100.0	34.2-100.0	192 / 192	100.0	98.0-100.0
	Para-Pak C&S	33 / 36 [g]	91.7	78.2-97.1	117 / 117	100.0	96.8-100.0
Salmonella	FecalSwab	30 / 31 [h]	96.8	83.8-99.4	161 / 163 [h]	98.8	95.6-99.7
	Para-Pak C&S	1 / 1	100.0	20.7-100.0	13 / 13	100.0	77.2-100.0
Yersinia enterocolitica	FecalSwab	32 / 34 [i]	94.1	80.9-98.4	160 / 160	100.0	97.7-100.0
	Para-Pak C&S	1 / 1	100.0	20.7-100.0	14 / 14	100.0	78.5-100.0
Diarrheagenic E. coli/*Shigella*							
Enteropathogenic *E. coli* (EPEC)	Para-Pak C&S	60 / 65 [j]	92.3	83.2-96.7	42 / 42	100.0	91.6-100.0
Enterotoxigenic *E. coli* (ETEC) *lt/st*	FecalSwab	22 / 24 [k]	91.7	74.2-97.7	85 / 86 [k]	98.8	93.7-99.8
	Para-Pak C&S	23 / 24	95.8	79.8-99.3	61 / 61 [l]	100.0	94.1-100.0
Shiga-like toxin *E. coli* (STEC) *stx1/stx2*	Para-Pak C&S	60 / 64	93.8	85.0-97.5	44 / 44 [m]	100.0	92.0-100.0
***E. coli* O157**	Para-Pak C&S	39 / 42 [n]	92.9%	80.1-99.4	16 / 16	100.0	80.6-100.0
***Shigella*/Enteroinvasive *E. coli* (EIEC)**	FecalSwab	22 / 24 [o]	91.7	73.0-99.0	170 / 170	100.0	97.8-100.0
	Para-Pak C&S	0 / 0	N/A	N/A	14 / 14	100.0	78.5-100.0
Parasites							
Cryptosporidium	FecalSwab	6 / 6	100.0	54.1-100.0	186 / 188 [p]	98.9	96.2-99.9
	Para-Pak C&S	26 / 26	100.0	86.8-100.0	117 / 117	100.0	96.9-100.0
Cyclospora cayetanensis	FecalSwab	1 / 1	100.0	20.7-100.0	193 / 193	100.0	98.1-100.0
	Para-Pak C&S	1 / 1	100.0	20.7-100.0	13 / 13	100.0	77.2-100.0
Entamoeba histolytica	FecalSwab	0 / 0	N/A	N/A	194 / 194	100.0	98.1-100.0
	Para-Pak C&S	0 / 0	N/A	N/A	14 / 14	100.0	76.5-100.0
Giardia lamblia	FecalSwab	29 / 31 [q]	93.6	79.3-98.2	46 / 48 [q]	95.8	86.0-98.9
	Para-Pak C&S	27 / 28 [r]	96.4	82.3-99.4	92 / 92 [r]	100.0	96.0-100.0

[a] Adenovirus F40/41 was detected in one of the three false negatives (1/3) in FecalSwab using a different FDA-cleared test method
[b] Astrovirus was detected in the single false negative specimen (1/1) in FecalSwab using a different FDA-cleared test method.
[c] two (2) FecalSwab samples positive for Norovirus GI/GII 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. The sample size for NPA is smaller for Norovirus GI/GII 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 retrospective study.
[d] 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 in QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study
[e] Rotavirus A was detected in the single false negative specimen (1/1) in FecalSwab using a different FDA-cleared test method.

[f] *Campylobacter* was detected in one of the two false positive specimens (1/2) in FecalSwab using a different FDA-cleared test method.
[g] *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.
[h] *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.
[i] *Yersinia enterocolitica* was not detected in the two false negative specimens (0/2) in FecalSwab using a different FDA-cleared test method.
[j] 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.
[k] 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 in 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 in QIAstat-Dx and one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study.
[l] 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 in QIAstat-Dx and one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study).
[m] 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 in QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study
[n] *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.
[o] *Shigella*/ EIEC was detected in one of the two false negative specimens (1/2) in FecalSwab using a different FDA-cleared test method.
[p] *Cryptosporidium* was not detected in the two false positive specimens (0/2) in FecalSwab using PCR followed by bi-directional sequence analysis
[q] four (4) samples positive for *Giardia lamblia* 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. Two (2) FecalSwab samples negative in 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 in QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study.
[r] 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 in 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 in QIAstat-Dx and in one FDA-cleared comparator was tested with the complete composite comparator in the retrospective study.

The proportion of failed runs on initial attempt, and following repeats are summarized in **Table 5.36.** The error breakdown due to instrument, invalid results, 'sample too concentrated' failures and other run failures are summarized in **Table 5.37.**

Table 5.36 Failure Rates Summary

Transport Media	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
	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 5.37 Failure Types Breakdown

Transport Media	Study	Failure Reason	Initial Runs		Final Runs	
			N / Total	%	N / Total	%
FecalSwab	Prospective	Instrument	0 / 1227	0.0	0 / 1227	0.0
		Invalid [a]	0 / 1227	0.0	0 / 1227	0.0
		Sample too Concentrated [b]	5 / 1227	0.4	0 / 1227	0.0
		Other [c]	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 Archived Frozen	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] 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 detailed in Appendix C.
[c] Run failures related to workflow checkpoints.

Co-infections

The QIAstat-Dx Gastrointestinal Panel 2 reported multiple organism detections (i.e., co-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 infections are shown in Table 5.38 and Table 5.39 below.

Table 5.38: **Most Prevalent Multiple Detection Combinations (≥2 instances) as Determined by the QIAstat-Dx Gastrointestinal Panel 2 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 5.39: Most Prevalent Multiple Detection Combinations (≥2 instances) as Determined by the QIAstat-Dx Gastrointestinal Panel 2 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 the FecalSwab specimens were *Campylobacter* (9), Norovirus GI/GII (7), Rotavirus (4) and ETEC (3) as shown in Table 5.4040 while the analytes most commonly found in mixed infections in the 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 5.4141 and Table 5.4242.

Table 5.4040: 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 5.4141: 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
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

Contrived Specimens Testing

Several analytes, such as *Entamoeba histolytica* are so rare that both prospective and archived testing efforts were insufficient to demonstrate system performance. To

supplement the prospective and archived specimens' test results, an evaluation of contrived specimens was performed. Contrived specimens were prepared using negative residual specimens that had previously tested negative by QIAstat-Dx Gastrointestinal Panel 2 and comparator methods. At least, 50% of these specimens were spiked at concentrations at concentrations slightly above the Limit of Detection (2x LoD) and the rest at 5x and 10x LoD, using quantified strains for each pathogen. A minimum of 50 contrived specimens were tested for each evaluated analyte. The analyte status of each contrived specimen was blinded to the users analyzing the specimens. Results are summarized in Table 5.4242 Table 5.42**.**

Table 5.4242: Test Results Summary for Contrived Specimens

QIAstat-Dx GI2 Target	Medium Brand	Fraction	Percentage	95% CI
Astrovirus	FecalSwab	33 / 34	97.1	85.1-99.5
	Para-Pak C&S	34 / 34	100.0	89.8-100.0
Rotavirus A	FecalSwab	35 / 35	100.0	90.1-100.0
	Para-Pak C&S	34 / 35	97.1	85.5-99.5
Plesiomonas shigelloides	FecalSwab	33 / 33	100.0	89.6-100.0
	Para-Pak C&S	34 / 35	97.1	85.5-99.5
Yersinia enterocolitica	FecalSwab	34 / 34	100.0	89.8-100.0
	Para-Pak C&S	34 / 35	97.1	85.5-99.5
***Shigella*/EIEC**	FecalSwab	35 / 35	100.0	90.1-100.0
	Para-Pak C&S	34 / 34	100.0	89.8-100.0
Cryptosporidium	FecalSwab	27 / 27	100.0	87.5-100.0
	Para-Pak C&S	31 / 31	100.0	89.0-100.0
Cyclospora cayetanensis	FecalSwab	26 / 26	100.0	87.1-100.0
	Para-Pak C&S	30 / 30	100.0	88.6-100.0
Entamoeba histolytica	FecalSwab	35 / 35	100.0	90.1-100.0
	Para-Pak C&S	34 / 35	97.1	85.5-99.5

Conclusions

The QIAstat-Dx Gastrointestinal Panel 2 is substantially equivalent to the legally marketed: BioFire Diagnostics LLC FilmArray Gastrointestinal (GI) Panel 510(k) # K140407.