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QIAstat-Dx® Gastrointestinal Panel 2 Instructions for Use

Σ 6

Version 2

IVD

For In Vitro Diagnostic Use

Rx Only

For prescription use only

For use with QIAstat-Dx Analyzer 1.0

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden

REF 691421

R1

Sample to Insight

Contents

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.

Summary and explanation

Pathogen information

Acute gastrointestinal infections can be caused by a variety of pathogens, including parasites, bacteria and viruses, and generally present with nearly indistinguishable clinical signs and symptoms (1). The rapid and accurate determination of the presence or absence of potential causative agent(s) help make timely decisions regarding treatment, hospital admission, infection control and return of the patient to work and family (2,3,4). It may also greatly support improved antimicrobial stewardship and other important public health initiatives (3,5).

The QIAstat-Dx Gastrointestinal Panel 2 Cartridge allows the detection and differentiation of 16 parasitic, viral and bacterial pathogens that cause gastrointestinal symptoms. Testing requires a small sample volume and minimal hands-on time, and the results are available in approximately 78 minutes.

Pathogens that can be detected and identified with the QIAstat-Dx Gastrointestinal Panel 2 are listed in Table 1.

Table 1. Pathogens detected by the QIAstat-Dx Gastrointestinal Panel 2

Pathogen	Classification (genome type)
Adenovirus F40/F41	Adenovirus (DNA)
Astrovirus	Astrovirus (RNA)
Norovirus GI/GII	Calicivirus (RNA)
Rotavirus A	Reovirus (RNA)
Campylobacter (*C. jejuni, C. upsaliensis, C. coli*)	Bacterium (DNA)
Enteroinvasive *E. coli* (EIEC)/ *Shigella*	Bacterium (DNA)
Enteropathogenic *E. coli* (EPEC)	Bacterium (DNA)
Enterotoxigenic *E. coli* (ETEC) lt/st	Bacterium (DNA)
Plesiomonas shigelloides	Bacterium (DNA)
Salmonella spp.	Bacterium (DNA)
Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2 (including specific identification of *E. coli* O157 serogroup within STEC)	Bacterium (DNA)
Yersinia enterocolitica	Bacterium (DNA)
Cryptosporidium	Parasite (DNA)
Cyclospora cayetanensis	Parasite (DNA)
Entamoeba histolytica	Parasite (DNA)
Giardia lamblia	Parasite (DNA)

Note: Shiga-like toxin-producing *E. coli* (STEC) stx1 and stx2 are grouped together and reported as Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2

Note: *Giardia lamblia* is also known as *Giardia intestinalis* and *Giardia duodenalis*

Summary of detected organisms

Bacteria

***Campylobacter* spp. (*C. jejuni/C. coli/C. upsaliensis*)** is a genus of gram-negative bacteria, which includes more than 30 species (6). *Campylobacter jejuni* and *Campylobacter coli* are the most common *Campylobacter* species associated with diarrheal illness, being *C. jejuni* responsible for 90% of cases (7,9). The consumption of undercooked poultry or raw milk are the most common sources of *Campylobacter* infections (10,11). *Campylobacter* are highly infectious, with an infectious dose as low as 500 bacteria (12), however, person-to-person spread is uncommon (10). Systemic disease, associated with significant morbidity and mortality, may occur in individuals who are immunocompromised (10,12). Infection can result in long-term consequences such as arthritis, irritable bowel syndrome, and Guillain-Barré syndrome (10,12).

Plesiomonas shigelloides is a facultatively anaerobic gram-negative bacterium that can cause enteric disease in humans. The prevalence of *P. shigelloides* enteritis varies considerably, with higher rates reported from Southeast Asia and Africa and lower numbers from North America and Europe. It is unknown how many people suffer from disease caused by *P. shigelloides* each year, but mortality is rare. Infection especially occurs following the consumption of raw seafood or contaminated water (13).

Salmonella is a gram-negative bacterium comprising more than 2,600 serovars, including the distinct typhoidal serotypes, Typhi and Paratyphi A–C (14,15). Enteric (typhoid) fever is an invasive, life-threatening, systemic infection with predominantly non-gastrointestinal symptoms (14,16). Non-typhoidal salmonellosis is an acute, usually self-limiting gastroenteritis that is characterized by symptoms such as watery diarrhea, fever, abdominal pain, and sometimes vomiting (14,16,17). Less commonly, non-typhoidal *Salmonella* serovars cause invasive disease due to bloodstream infections that are not usually associated with diarrhea (14,16). There are 100–200 million cases of non-typhoidal salmonellosis each year, resulting in approximately 85,000–155,000 deaths (16,18). The incidence of non-typhoidal *Salmonella*

gastroenteritis is highest in the developing world but is also of considerable importance in developed countries (14).

Yersinia enterocolitica is a gram-negative bacterium that has more than 70 serotypes (19); serotypes most commonly associated with infection are O:3, O:9, O:8, and O:5,27 (20). *Y. enterocolitica* infection have been reported frequently in northern Europe, particularly in Belgium, Norway, and the Netherlands; it is rarely observed in tropical countries (21). *Y. enterocolitica* is usually transmitted through the consumption of raw meats, unpasteurized dairy products, contaminated water, or via the fecal-oral route (22). Symptoms range from self-limiting enteritis with diarrhea, low-grade fever, and abdominal pain to severe disease such as terminal ileitis and mesenteric lymphadenitis, which also mimics appendicitis (23,24,25).

Diarrheagenic *Escherichia coli/Shigella*

E. coli/Shigella are gram-negative facultative anaerobic bacteria belonging to the Enterobacteriaceae family. In addition to being part of the normal intestinal microflora of mammals, *E. coli/Shigella* contains several pathotypes that cause a variety of diseases (26,27). There are four major pathotypes of diarrhoeagenic *E. coli/Shigella*, which each have unique features in their interaction with eukaryotic cells: Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli*/Shiga-like toxin-producing *E. coli* (EHEC/STEC), Enterotoxigenic *E. coli* (ETEC) and Enteroinvasive *E. coli* (EIEC)/*Shigella* (26,27). *E. coli/Shigella* have a conserved core genome and a flexible gene pool containing virulence and fitness genes, which are carried on mobile genetic elements (26,27). Gene gain, via horizontal transfer, and gene loss afford the pathogenic traits to *E. coli/Shigella* that give rise to the different pathotypes (27).

Enteroinvasive *E. coli* (EIEC) and *Shigella*. EIEC is an invasive strain of *E. coli* that is very closely related in virulence and other pathogenic properties to *Shigella* (28,29). Sequencing indicates that EIEC is more related to *Shigella* than to non-invasive *E. coli*; however, they are currently classified as distinct species (26,28,30). The virulence of this pathogen is primarily due a

plasmid-encoding virulence factors that allows the adhesion and invasion to the epithelial cells (31). EIEC is under-represented in epidemiological studies due its less severe manifestation and potential misclassification as *Shigella* (27). EIEC infection often leads only to self-limiting, mild watery diarrhea; in rare situations it can cause symptoms of shigellosis, but complications are uncommon (27). *Shigella* is the second leading cause of diarrhea mortality, causing approximately 13% diarrhea deaths (32). Numbers of deaths are greatest in young children and the elderly (32). It is recommended that individuals with shigellosis should not take anti-diarrheal medications such as loperamide, as these can make symptoms worse (33).

Enteropathogenic *E. coli* (EPEC) is primarily a disease of infants <2 years (27,34,35), and is commonly present in co-infections with other gastrointestinal pathogens (36). EPEC are classified into typical (tEPEC) and atypical (aEPEC) strains based on the presence of the *E. coli* adherence factor plasmid (pEAF). tEPEC is considered an important cause of infantile diarrhea in developing countries (37). Infections in adults, including travelers to developing countries, are rarely reported (27,35). aEPEC is frequently detected in both developing countries and industrialized countries and is suggested to be more prevalent that tEPEC (34). aEPEC is an important cause of both endemic diarrhea and outbreaks (34).

Enterotoxigenic *E. coli* (ETEC) is characterized by the production of heat-labile enterotoxins (LT) and heat-stable enterotoxins (ST) (38,39). ETEC is the most common diarrhea-associated *E. coli*, and although infections are usually self-limiting (39), is the eighth leading cause of diarrhea globally and accounts for >50,000 deaths every year (32). It also remains a major cause of diarrhea in travelers to low resource countries (39). ETEC is a frequent antimicrobial resistant (39).

Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2, including *E. coli* O157 is defined by the production of Shiga toxin 1 (stx1) or 2 (stx2), which show homology to stx toxins from *Shigella dysenteriae* (27). There are >400 serotypes of STEC, of which O157:H7 is the most common (27). Symptoms of STEC infection range from mild intestinal disease to hemorrhagic diarrhea and can lead to hemolytic uremic syndrome (HUS), end-stage renal disease and death (27,40). Approximately 5–10% of individuals diagnosed with STEC infections develop HUS, which can

be a life-threatening complication (41). The impacts of STEC are often greater in infants and children, compared to other ages (40). Antibiotics should not be used to treat STEC infections as there is currently no evidence they aid recovery and have instead been associated with a worsening of symptoms and the development of HUS (41).

Parasites

Cryptosporidium spp. are protozoan parasites that can infect humans and other animals, being *C. hominis* and *C. parvum* the causative strains of the majority of humans infections (42). *Cryptosporidium spp*. are found globally, but those in developing countries, particularly in sub-Saharan Africa, are at greater risk of infection due to poorer water treatment and food sanitation (32,43). It is also one of the leading causes of diarrheal mortality in children <5 years of age (32,44).

Cyclospora cayetanensis is a single-celled protozoa parasite, and the only known species of the genus *Cyclospora* to infect humans (45,46). It is endemic in tropical/subtropical areas, and in non-endemic regions, cases and outbreaks of cyclosporiasis are usually linked to international travel and consumption of contaminated produce imported from endemic regions (45,46,47). Direct fecal-oral transmission cannot occur, the unsporulated oocysts sporulate in water and food environments, enabling them to infect another host (45,46,48).

Entamoeba histolytica is an anaerobic, protozoan parasite (49). *E. histolytica* is common in developing countries, particularly those in the tropics and sub-tropics with poor sanitation (49,50,51). Only 10–20% of individuals infected with *E. histolytica* are symptomatic [1,2]. Through destruction of the intestinal walls, trophozoites can also spread systemically to the liver, lungs, and central nervous system (49,10,50,51). The liver is the most common extra-intestinal site of infection (49,50,51).

***Giardia lamblia* (also referred to as *G. duodenalis* and *G. intestinalis*)** is a unicellular, protozoan parasite that can cause disease in humans and other mammals (52,53). *G. lamblia* has a global distribution and is common in both children and adults (54,55). Prevalence of infection is higher in developing regions of the world and in children (52,54,55). The majority (50–75%) of *G. lamblia* infections are asymptomatic (56). In immunocompetent individuals, infections are usually self-limiting, although some may become chronic (52).

Viruses

Adenovirus F40/41 is a double-stranded DNA, non-enveloped virus (57,58), with many distinct serotypes described and classified into 7 species (A–G) (57). Serotypes F40/41 are the most common cause of acute gastroenteritis in young children, causing 5–20% cases. More than 80% of diagnosed infections occur in children aged <4 years (58). Adenoviruses have a worldwide distribution and infections occur throughout the year without significant seasonal variability (57). Infections are usually mild and self-limiting in immunocompetent individuals but can be fatal in individuals who are immunocompromised (57,59,60).

Astroviruses are non-enveloped, positive-sense, single-stranded RNA viruses (61). Human astroviruses are distributed all over the world and are associated with 2–9% of cases of acute, nonbacterial diarrhea in children (61,62). It is estimated that 90% of the global population aged ≥9 years have antibodies against astrovirus type 1 (61). Many infections in healthy children and adults are asymptomatic, although they can cause severe diarrhea in children, older adults and those who are immunocompromised or have comorbidities (61,62).

Noroviruses GI/GII are small, non-enveloped, positive-stranded RNA viruses from the family Caliciviridae (63). They are responsible for >90% of viral gastroenteritis and around 50% of all-cause gastroenteritis outbreaks globally (64), causing approximately 685 million cases every year (65). Approximately 200 million cases are in children aged <5 years, leading to 50,000 child deaths (65). Norovirus is commonly known as the "winter-vomiting bug"; outbreaks are more common during the winter months, but infection can occur at any time of year (65). Norovirus is infectious at very low doses and is transmitted via aerosolized droplets

and touching of contaminated surfaces (65). Individuals infected with norovirus usually recover within 1–3 days, but infections in infants, older adults and immunocompromised individuals can be severe and sometimes fatal (65). In some individuals, viral shedding can occur for many weeks/months after they have stopped experiencing symptoms, and this is a large contributing factor for outbreaks (66).

Rotavirus A is a non-enveloped, double-stranded RNA viruses of the Reoviridae family, with 10 species that cause infection in humans (A–J). However, rotavirus A is the most common species and causes >90% of all rotavirus infections (67,68). Rotavirus is a leading cause of diarrhea in children <5 years (67), with a seasonal infection pattern that differs across the world, particularly in middle–high income countries (69). Severe infection is most common in young children and infants; in adults, infections are often associated with milder symptoms (70). Two oral rotavirus vaccines are approved in the United States (71) and have been available in >100 countries since 2006 (71). These vaccines have substantially reduced the burden of rotavirus-associated illness (70).

QIAstat-Dx Gastrointestinal Panel 2 Cartridge description

The QIAstat-Dx Gastrointestinal Panel 2 Cartridge (Figure 1) is a disposable plastic device that allows performance of fully automated molecular assays for the detection of gastrointestinal pathogens. Main features of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge include compatibility with a liquid sample type, hermetical containment of the pre-loaded reagents necessary for testing and true walk-away operation. All sample preparation and assay testing steps are performed within the cartridge.

All reagents required for the complete execution of a test run are pre-loaded and self-contained in the QIAstat-Dx Gastrointestinal Panel 2 Cartridge. The user does not need to come in contact with and/or manipulate any reagents. The QIAstat-Dx Analyzer 1.0 houses air filters for both incoming and outgoing air, further safeguarding the environment. After testing, the cartridge stays hermetically closed at all times, greatly enhancing its safe disposal.

Within the cartridge, multiple steps are automatically performed in sequence using pneumatic pressure to transfer samples and fluids via the transfer chamber to their intended destinations.

Description of the process

After sample is manually loaded, the diagnostic tests with the QIAstat-Dx Gastrointestinal Panel 2 are performed on the QIAstat-Dx Analyzer 1.0. All of the sample preparation and analysis steps are performed automatically by the QIAstat-Dx Analyzer 1.0.

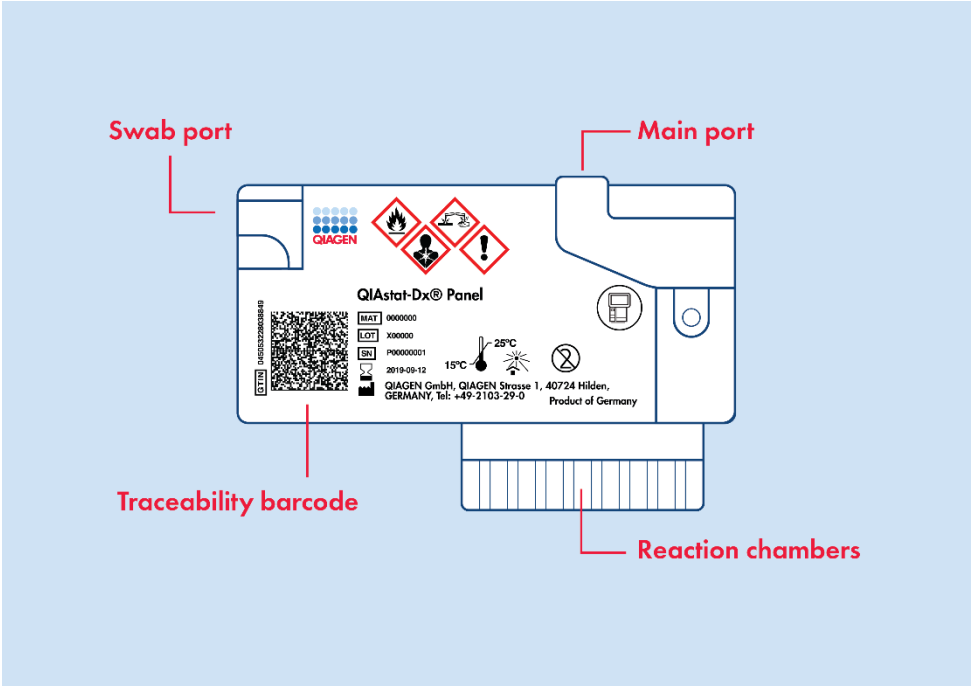

Figure 1. Layout of the QIAstat-Dx Cartridge and its features.

Note: The swab port is not used for the QIAstat-Dx Gastrointestinal Panel 2.

Sample collection and cartridge loading

The collection of samples and their subsequent loading into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge should be performed by personnel trained in safe handling of biological samples.

The following steps are performed:

1. Fresh unpreserved stool specimen is collected and resuspended in Para-Pak C&S or FecalSwab transport medium as soon as possible after collection following the manufacturer's instructions. Attention should be given not to exceed the maximum fill line of the Para-Pak C&S or FecalSwab container or overfill the FecalSwab collection device.
2. The sample information is manually written on or a sample label is affixed to the top of a QIAstat-Dx Gastrointestinal Panel 2 Cartridge.
3. Liquid sample (stool resuspended in Para-Pak C&S or FecalSwab transport medium) is loaded manually into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge.

   Note: Preserved stool specimens should present a homogenous suspension (easily vortexed).

   Note: The user must perform a visual check of the sample inspection window to confirm that the liquid sample has been loaded.
4. The sample barcode (if available) and the QIAstat-Dx Gastrointestinal Panel 2 Cartridge barcode are scanned by the QIAstat-Dx Analyzer 1.0. If sample barcode is not available, the sample ID is manually written using the virtual keyboard of the touchscreen.
5. Sample type option is selected from the list based on the sample collection device used (Para-Pak C&S or FecalSwab).
6. The QIAstat-Dx Gastrointestinal Panel 2 Cartridge is introduced into the QIAstat-Dx Analyzer 1.0.
7. The test is started on the QIAstat-Dx Analyzer 1.0.

Sample preparation, nucleic acid amplification, and detection

The extraction, amplification, and detection of nucleic acids in the sample are performed automatically by the QIAstat-Dx Analyzer 1.0.

1. The sample is pre-treated with buffer and homogenized.
2. Resuspension of Internal Control using on-cartridge buffer and mixing with the sample.
3. Cells are lysed in the lysis chamber of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge, which includes a rotor that turns at high speed and silica beads that provide effective cell disruption.
4. Nucleic acids are purified from the lysed sample via binding to a silica membrane in the purification chamber of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge in the presence of chaotropic salts and alcohol.
5. The purified nucleic acids are eluted from the membrane in the purification chamber and are mixed with the lyophilized PCR chemistry in the dried-chemistry chamber of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge.
6. The mixture of sample and PCR reagents is dispensed into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge PCR chambers, which contain air-dried, assay-specific primers and probes.
7. The QIAstat-Dx Analyzer 1.0 creates the optimal temperature profiles to carry out effective multiplex real-time RT-PCR and performs real-time fluorescence measurements to generate amplification curves.
8. The QIAstat-Dx Analyzer 1.0 Software interprets the resulting data and process controls and delivers a test report.

Materials Provided

Kit contents

QIAstat-Dx Gastrointestinal Panel 2 **Catalog number** **Number of tests**	 **691421** **6**
QIAstat-Dx Gastrointestinal Panel 2 Cartridges*	6
Transfer pipettes†	6
QIAstat-Dx Gastrointestinal Panel 2 Product information card	1

* 6 individually packaged cartridges containing all reagents needed for sample preparation and multiplex real-time RT-PCR, plus Internal Control.

† 6 individually packaged transfer pipettes for dispensing liquid sample into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge.

Materials Required But Not Provided

Platform and Software*

The QIAstat-Dx Gastrointestinal Panel 2 is designed for use with the QIAstat-Dx Analyzer 1.0. Before beginning a test, make sure the following are available:

- QIAstat-Dx Analyzer 1.0 (at least one Operational Module and one Analytical Module) with software version 1.4 or later†.
- QIAstat-Dx Analyzer 1.0 User Manual (for use with software version 1.4 or later)
- QIAstat-Dx latest Assay Definition File software for Gastrointestinal Panel 2 installed in the Operational Module.

* Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

† DiagCORE® Analyzer instruments running QIAstat-Dx software version 1.4 or later can be used as an alternative to QIAstat-Dx Analyzer 1.0 instruments.

Warnings and Precautions

- The QIAstat-Dx Gastrointestinal Panel 2 is for *in vitro* diagnostic use.
- For prescription use only
- The QIAstat-Dx Gastrointestinal Panel 2 is to be used by laboratory professionals trained in the use of QIAstat-Dx Analyzer 1.0.
- False positives and false negatives can be the result of a variety of sources and causes. A trained healthcare professional should carefully interpret the results from the QIAstat-Dx Gastrointestinal Panel 2 in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and relevant epidemiological information.
- Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and the regulatory authority in which the user and/or the patient is established.

Safety information

- When working with chemicals, always wear a lab coat, disposable gloves and protective googles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.
- Observe standard laboratory procedures for keeping the working area clean and contamination-free. Guidelines are outlined in publications such as the *Biosafety in Microbiological and Biomedical Laboratories* from the Centers for Disease Control and Prevention and the National Institutes of Health (72).
  - Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.
- Always wear appropriate personal protective equipment and follow your institution's safety procedures for handling biological samples. Handle all samples, cartridges, and transfer pipettes as if they are capable of transmitting infectious agents.

- Always observe safety precautions as outlined in relevant guidelines, such as the Clinical and Laboratory Standards Institute® (CLSI) *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline* (M29), or other appropriate documents provided by local authorities.
- The QIAstat-Dx Gastrointestinal Panel 2 Cartridge is a closed, single-use device that contains all reagents needed for sample preparation and multiplex real-time RT-PCR within the QIAstat-Dx Analyzer 1.0. Do not use a QIAstat-Dx Gastrointestinal Panel 2 Cartridge that is past its expiration date, appears damaged or leaks fluid.
- Dispose of used or damaged cartridges in accordance with all national, state and local health and safety regulations and laws.

Emergency information

CHEMTREC USA & Canada 1-800-424-9300

Precautions

The following hazard and precautionary statements apply to components of the QIAstat-Dx Gastrointestinal Panel 2.

Contains: ethanol; guanidine hydrochloride; guanidine thiocyanate; isopropanol; proteinase K; t-Octylphenoxypolyethoxyethanol. Danger! Highly flammable liquid and vapor. Harmful if swallowed or if inhaled. May be harmful in contact with skin. Causes severe skin burns and eye damage. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause drowsiness or dizziness. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Corrosive to the respiratory tract. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Immediately call a POISON CENTER or doctor. Rinse mouth. Do NOT induce vomiting. Remove person to fresh air and keep comfortable for breathing. Wash contaminated clothing before reuse. Store in a well-ventilated place. Keep container tightly closed.

To reduce the risk of contamination when handling stool samples, it is recommended that the below guidelines are applied (72).

- When handling the stool sample, a biosafety cabinet, dead air box, splash shield, or face shield should be used.
- The work area used for cartridge loading should be separate from the work area used for stool pathogen testing (i.e., stool culture, EIA) to prevent cross-contamination.
- Prior to sample handling, the work area should be thoroughly cleaned using 10% bleach or similar disinfectant.
- QIAstat-Dx Gastrointestinal Panel 2 Cartridges and samples should be processed one at a time.
- Change gloves prior to removing cartridges from shipping boxes.
- Change gloves and clean the work area between processing each sample.
- Dispose of used cartridges in a biohazard container immediately after the run is complete and avoid excessive handling.

Precautions Related to Public Health Reporting

National, state, and local public health organizations have published guidelines for the notification of reportable diseases. While the list of reportable conditions varies by state, the Council of State and Territorial Epidemiologists (CSTE) has recommended that state health departments report cases of selected diseases to CDC's National Notifiable Diseases Surveillance System (NNDSS). At the time of writing, the notifiable pathogens in the US per CDC included in the Gastrointestinal Panel 2 are:

- *Campylobacter* spp.
- Certain *E. coli*
  - O157:H7
  - Shiga toxin-producing (STEC)
- *Cryptosporidium* spp.

- *Cyclospora cayetanensis*
- *Giardia lamblia*
- *Salmonella* spp.
- *Salmonella enterica* serotypes Paratyphi A, B [tartrate negative], and C [S. Paratyphi])
- *Salmonella enterica* serotype Typhi
- *Shigella* spp./EIEC

Pathogens are notifiable due to their outbreak potential or impact on public health. Laboratories are responsible for following their state or local regulations for submission of clinical material or isolates on positive specimens to their state public health laboratories.

Reagent Storage and Handling

Store the QIAstat-Dx Gastrointestinal Panel 2 Cartridges in a dry, clean storage space at room temperature (15–25°C). Do not remove the QIAstat-Dx Gastrointestinal Panel 2 Cartridges or the transfer pipettes from their individual packaging until actual use. Under these conditions, QIAstat-Dx Gastrointestinal Panel 2 Cartridges can be stored until the expiration date printed on the individual packaging. The expiration date is also included in the QIAstat-Dx Gastrointestinal Panel 2 Cartridge barcode and is read by the QIAstat-Dx Analyzer 1.0 when the cartridge is inserted into the instrument to run a test. Once the cartridge is removed from the pouch it should be protected from sunlight.

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

In-use stability

When stored under the specified storage conditions, the QIAstat-Dx Gastrointestinal Panel 2 is stable until the stated expiration date on box label.

After the cartridge package is opened, sample should be introduced into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge within 30 minutes. Sample-loaded cartridges should be loaded into the QIAstat-Dx Analyzer 1.0 within 90 minutes.

Specimen Storage and Handling

The QIAstat-Dx Gastrointestinal panel 2 Kit is for use with stool samples resuspended in transport medium (Para-Pak C&S (Meridian Bioscience) or FecalSwab (COPAN)). All samples should be treated as potentially infectious. Discard sample and assay waste according to your local safety procedures.

Specimen collection

Stool samples should be collected and handled according to the transport medium manufacturer's recommended procedures.

Recommended storage conditions for stool resuspended in transport medium (Para-Pak C&S (Meridian Bioscience) or FecalSwab (COPAN)) specimens are listed below:

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

Procedure

Important point before starting

Ensure all materials required but not provided are available.

The QIAstat-Dx Gastrointestinal panel 2 cartridge (cat. no 691421) is identified by a purple-colored (●) bar on the label and an icon indicating gastrointestinal tract ( , see Symbols)

Protocol: stool samples in transport medium

Sample collection, transport, and storage

Collect and resuspend the stool sample in Para-Pak C&S (Meridian) or FecalSwab (COPAN) transport media according to the manufacturer's recommended procedures.

Loading a sample into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge

1. Open the package of a QIAstat-Dx Gastrointestinal Panel 2 Cartridge using the tear notches on the sides of the packaging (Figure 2).

   IMPORTANT: After the package is open, sample should be introduced into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge within 30 minutes. Sample-loaded cartridges should be loaded into the QIAstat-Dx Analyzer 1.0 within 90 minutes.

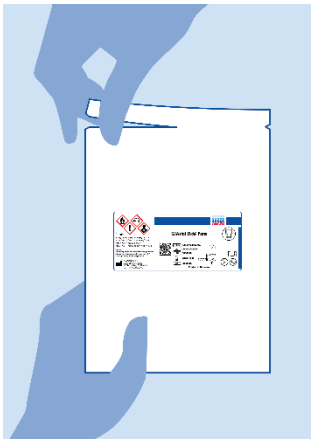

Figure 2. Opening the QIAstat-Dx Gastrointestinal Panel 2 Cartridge.

2. Remove the QIAstat-Dx Gastrointestinal Panel 2 Cartridge from the packaging and position it so that the barcode on the label faces you.
3. Manually write the sample information, or place a sample information label, on the top of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge. Make sure that the label is properly positioned and does not block the lid opening (Figure 3).

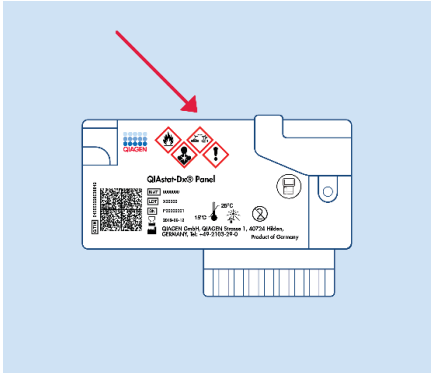

Figure 3. Sample information placement on top of QIAstat-Dx Gastrointestinal Panel 2 Cartridge.

4. Place the QIAstat-Dx Gastrointestinal Panel 2 Cartridge flat on the clean work surface so that the barcode on the label faces upwards. Open the sample lid of the main port on the front of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge (Figure 4).

   IMPORTANT: Do not flip the QIAstat-Dx Gastrointestinal Panel 2 Cartridge or agitate it while the main port lid is open. The main port contains silica beads used in the sample disruption. The silica beads could fall out of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge if it is agitated while the lid is open.

   Note: The swab port is not used for the QIAstat-Dx Gastrointestinal Panel 2 assay.

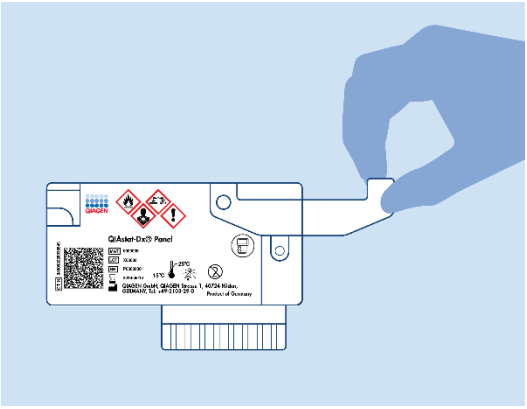

Figure 4. Opening the sample lid of main port.

5. Thoroughly mix the stool in the Para-Pak C&S or FecalSwab transport medium, for example, by vigorously agitating the tube 3 times (Figure 5).

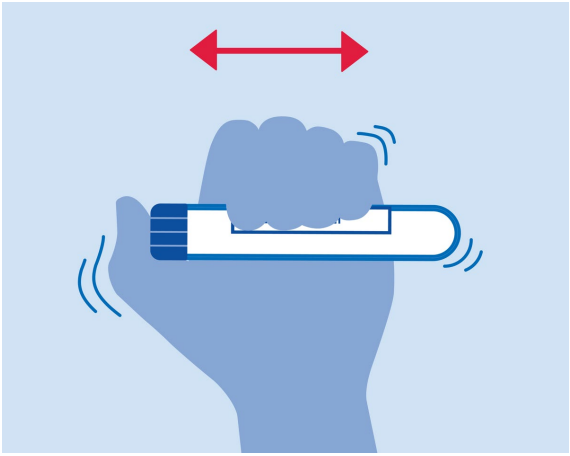

Figure 5. Mixing stool sample in transport medium.

6. Open the tube with the sample to be tested. Use the supplied transfer pipette to draw up fluid. Draw the sample to the second fill line on the pipette (i.e., 200 µL) (Figure 6).

   IMPORTANT: Do not draw air, mucus, or particles into the pipette. If air, mucus, or particles are drawn into the pipette, carefully expel the sample fluid in the pipette back into the sample tube and draw up fluid again. In the event that the supplied transfer pipette is lost please use another one from the package or any other commercially available pipette with a minimum volume of 200 µL.

   Note: In the case the test should be repeated due to previous cartridge error related to sample concentration too high, draw the sample to the first fill line on the pipette instead (100 µL) (See "Troubleshooting" section for further details on error codes and "Appendix C: Additional Instructions for use" for further instructions on repeating a sample with 100 µL).

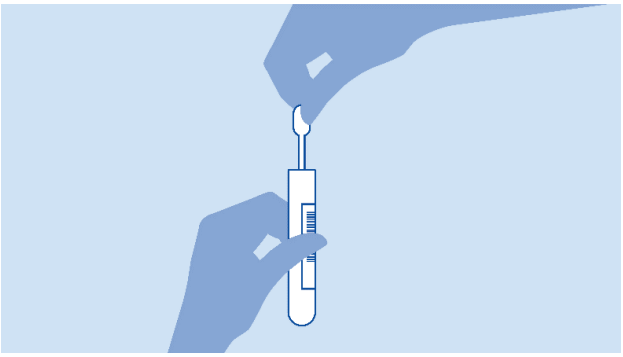

Figure 6. Drawing up sample into the supplied transfer pipette.

7. Carefully transfer the sample into the main port of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge using the supplied single-use transfer pipette (Figure 7).

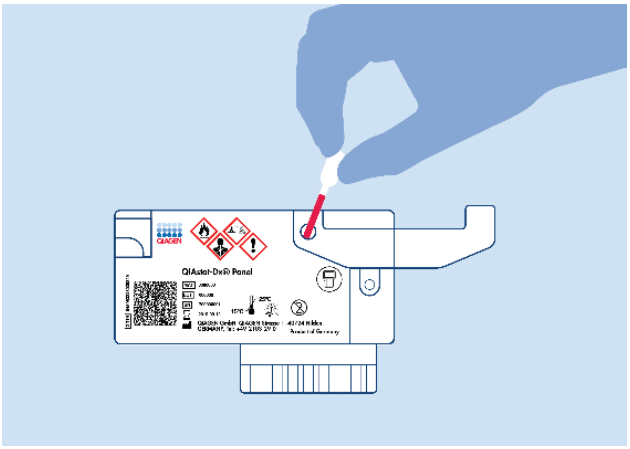

Figure 7. Transferring sample to main port of QIAstat-Dx Gastrointestinal Panel 2 Cartridge.

8. Firmly close the lid of the main port until it clicks (Figure 8).

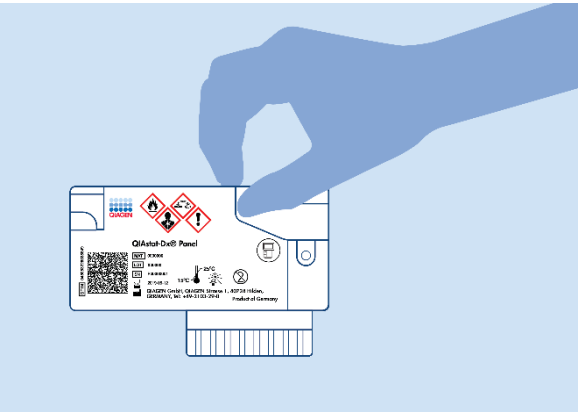

Figure 8. Closing the lid of the main port.

9. Visually confirm that the sample has been loaded by checking the sample inspection window of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge (Figure 9). A mixture of sample and silica beads should be observed.

   IMPORTANT: After the sample is placed inside the QIAstat-Dx Gastrointestinal Panel 2 Cartridge, the cartridge must be loaded into the QIAstat-Dx Analyzer 1.0 within 90 minutes.

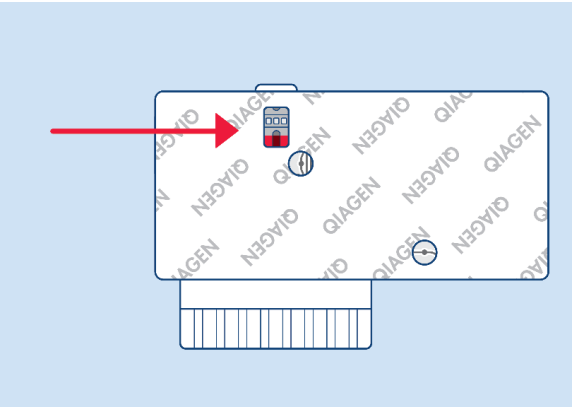

Figure 9. Sample inspection window (red arrow).

Running a test with a QIAstat-Dx analyzer

1. Power on the QIAstat-Dx Analyzer 1.0 using the **On/Off** button on the front of the instrument.

   Note: The power switch at the back of the Analytical Module must be set in the "I" position. The QIAstat-Dx Analyzer 1.0 status indicators will turn blue.

2. Wait until the Main screen appears, and the QIAstat-Dx Analyzer 1.0 status indicators turn green and stop blinking.

3. Enter your username and password for QIAstat-Dx Analyzer 1.0 to log in.

   Note: The Login screen will appear if User Access Control is activated. If the User Access Control is disabled, username/password will not be required, and the Main screen will appear.

4. If the Assay Definition File software is not installed on the QIAstat-Dx Analyzer 1.0, follow the installation instructions prior to running the test (see "Appendix A: Installing the Assay Definition File" for additional information).

5. Press **Run Test** in the top right corner of the touchscreen of the QIAstat-Dx Analyzer 1.0.

6. When prompted, scan the sample ID barcode on the resuspended sample, or scan the specimen information barcode located on the top of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge (Figure 3), using the integrated front barcode reader of the QIAstat-Dx Analyzer 1.0 (Figure 10).

   Note: It is also possible to enter the sample ID using the virtual keyboard of the touchscreen by selecting the **Sample ID** field.

   Note: Depending on the chosen system configuration, entering the patient ID may also be required at this point.

   Note: Instructions from the QIAstat-Dx Analyzer 1.0 appear in the **Instructions Bar** at the bottom of the touchscreen.

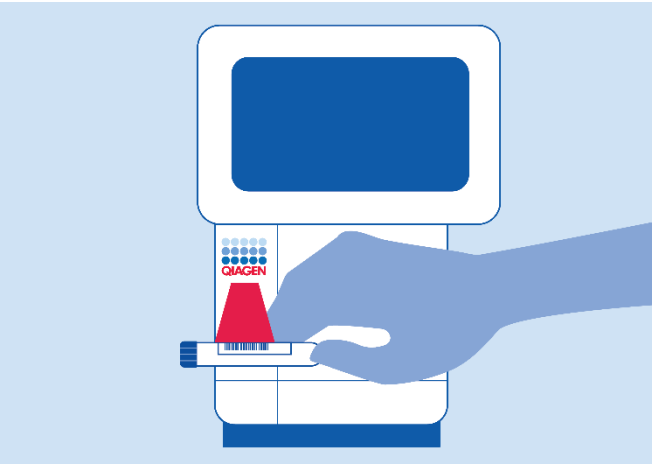

Figure 10. Scanning sample ID barcode.

7. When prompted, scan the barcode of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge to be used (Figure 11). The QIAstat-Dx Analyzer 1.0 will automatically recognize the assay to be run based on the cartridge barcode.

   Note: The QIAstat-Dx Analyzer 1.0 will not accept QIAstat-Dx Gastrointestinal Panel 2 Cartridges with lapsed expiration dates, previously used cartridges or cartridges for assays that have not been installed on the unit. An error message will be shown in these cases and the QIAstat-Dx Gastrointestinal Panel 2 Cartridge will be rejected. Refer to the *QIAstat-Dx Analyzer 1.0 User Manual* or Appendix A for further details on how to install assays.

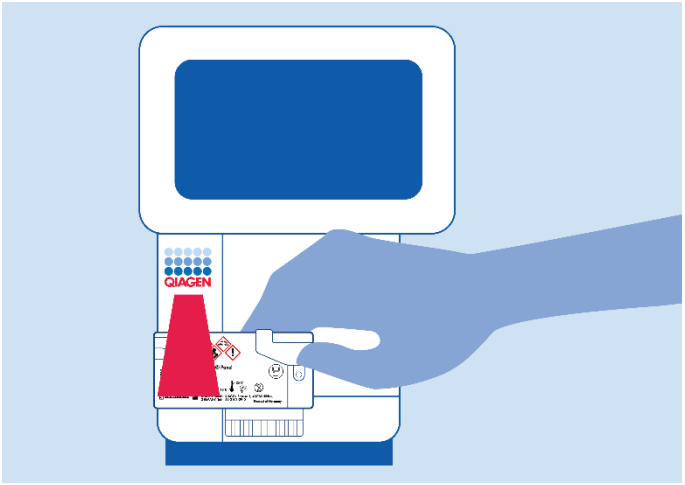

Figure 11. Scanning QIAstat-Dx Gastrointestinal Panel 2 Cartridge barcode.

8. Select sample type option from the list (Figure 12) based on the sample collection device used (Para-Pak C&S or FecalSwab).

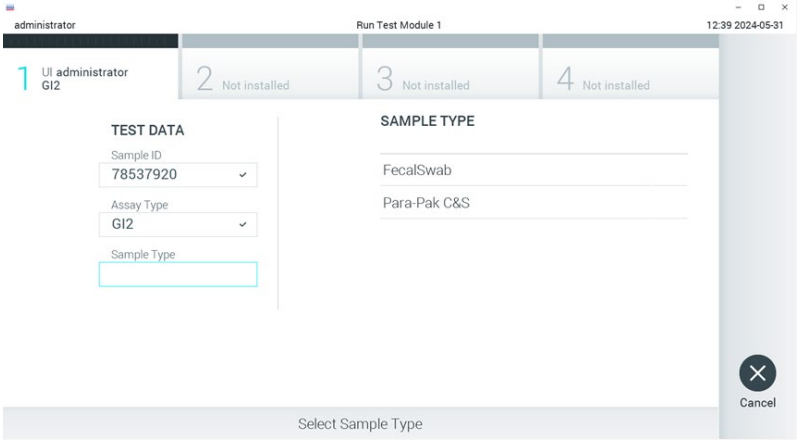

Figure 12. Selecting sample type.

9. The **Confirm** screen will appear. Review the entered data and make any necessary changes by selecting the relevant fields on the touchscreen and editing the information.
10. Press **CONFIRM** when all the displayed data are correct. If needed, select the appropriate field to edit its content, or press **Cancel** to cancel the test (Figure 13).

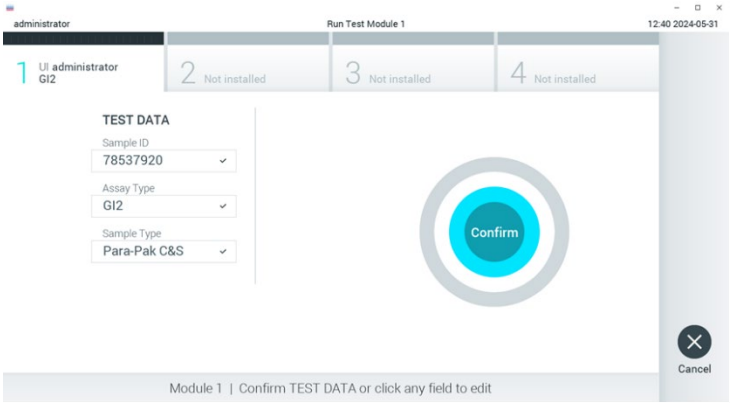

Figure 13. Confirming data entry.

11. Ensure that both sample lids of the swab port and main port of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge are firmly closed.
12. When the cartridge entrance port on the top of the QIAstat-Dx Analyzer 1.0 automatically opens, insert the QIAstat-Dx Gastrointestinal Panel 2 Cartridge with the barcode facing to the left and the reaction chambers facing down (Figure 14).
    Note: Depending on the system configuration, the operator may be required to re-enter their user password to start the test run.
    Note: Up to this point, it is possible to cancel the test run by pressing the **Cancel** button in the bottom right corner of the touchscreen.
13. Upon detecting the QIAstat-Dx Gastrointestinal Panel 2 Cartridge, the QIAstat-Dx Analyzer 1.0 will automatically close the lid of the cartridge entrance port and start the test run. No further action from the operator is required to start the run.
    Note: There is no need to push the QIAstat-Dx Gastrointestinal Panel 2 Cartridge into the QIAstat-Dx Analyzer 1.0.
    Note: The QIAstat-Dx Analyzer 1.0 will not accept a QIAstat-Dx Gastrointestinal Panel 2 Cartridge other than the one used and scanned during the test setup. If a cartridge other

than the one scanned is inserted, an error will be generated, and the cartridge will be automatically ejected.

Note: The lid of the cartridge entrance port will close automatically after 30 seconds if a QIAstat-Dx Gastrointestinal Panel 2 Cartridge is not positioned in the port. If this occurs, repeat the procedure starting with step 5.

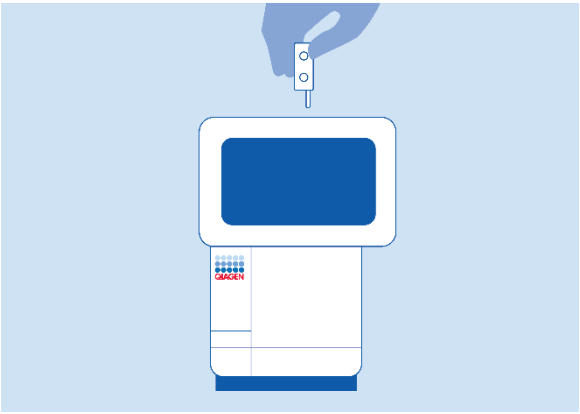

Figure 14. Inserting QIAstat-Dx Gastrointestinal Panel 2 Cartridge into QIAstat-Dx Analyzer 1.0.

14. While the test is running, the remaining run time is displayed on the touchscreen.
15. After the test run is completed, the **Eject** screen will appear (Figure 15) and the Module status bar will display the test result as one of the following options:
    - TEST COMPLETED: The test was completed successfully
    - TEST FAILED: An error occurred during the test
    - TEST CANCELED: The user canceled the test

    IMPORTANT: If the test fails, refer to the "Troubleshooting" section in the *QIAstat-Dx Analyzer 1.0 User Manual* for possible reasons and instructions on how to proceed. For additional information about specific QIAstat-Dx Gastrointestinal Panel 2 error codes and messages, please see the 'Troubleshooting' section of this document.

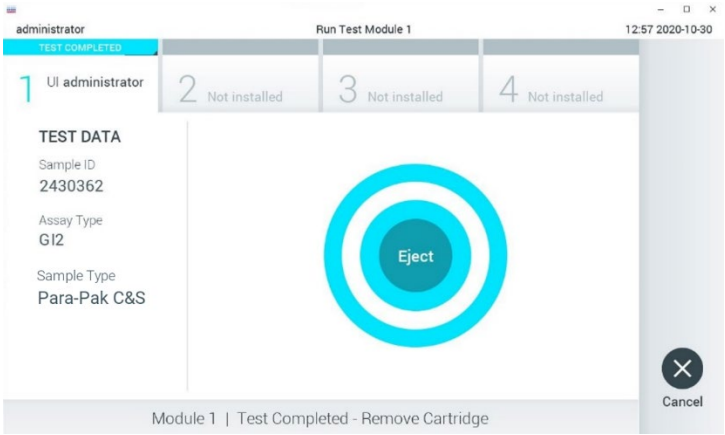

Figure 15. Eject screen display.

16. Press **Eject** on the touchscreen to remove the QIAstat-Dx Gastrointestinal Panel 2 Cartridge and dispose of it as biohazardous waste in accordance with all national, state and local health and safety regulations and laws. The QIAstat-Dx Gastrointestinal Panel 2 Cartridge should be removed when the cartridge entrance port opens and ejects the cartridge. If the cartridge is not removed after 30 seconds, it will automatically move back into the QIAstat-Dx Analyzer 1.0 and the cartridge entrance port lid will close. If this occurs, press **Eject** to open the lid of the cartridge entrance port again and then remove the cartridge.-

    IMPORTANT: Used QIAstat-Dx Gastrointestinal Panel 2 Cartridges must be discarded. It is not possible to re-use cartridges for tests for which the execution was started but then subsequently canceled by the operator, or for which an error was detected.

17. After the QIAstat-Dx Gastrointestinal Panel 2 Cartridge has been ejected, the results **Summary** screen will appear. Refer to "Interpretation of Results", page 38 for further details. To begin the process for running another test, press **Run Test**.

    Note: For further information on the use of the QIAstat-Dx Analyzer 1.0, refer to the *QIAstat-Dx Analyzer 1.0 User Manual*.

Interpretation of Results

Viewing results

The QIAstat-Dx Analyzer 1.0 automatically interprets and saves test results. After ejecting the QIAstat-Dx Gastrointestinal Panel 2 Cartridge, the results **Summary** screen is automatically displayed (Figure 16).

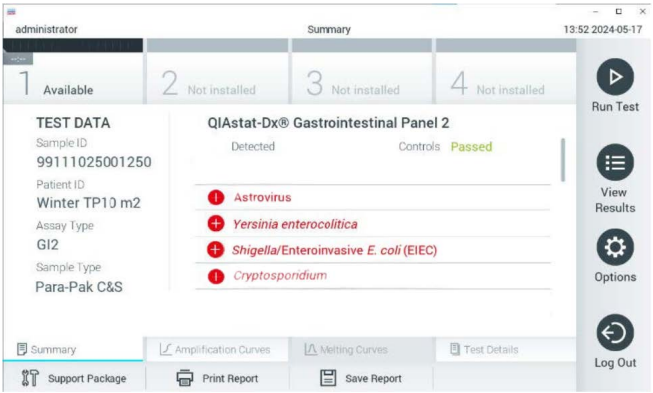

Figure 16. Results Summary screen example showing Test Data on the left panel and Test Summary in the main panel.

The main part of the screen provides the following lists and uses color-coding and symbols to indicate the results:

The first list, under the heading "Detected", includes all pathogens detected and identified in the sample, which are preceded by a ⊕ sign and are colored red.
The second list, under the heading "Tested", includes all pathogens tested in the sample. Pathogens detected and identified in the sample are preceded by a ⊕ sign and are colored red. Pathogens that were tested but not detected are preceded by a ⊖ sign and are colored green. Invalid and not applicable pathogens are also displayed in this list.

Note: Pathogens detected and identified in the sample are shown in both the "Detected" and "Tested" lists.

If the test failed to complete successfully, a message will indicate "Failed" followed by the specific Error Code.

The following Test Data is shown on the left side of the screen:

- Sample ID
- Patient ID (if available)
- Assay Type
- Sample Type

Further data about the assay is available, depending on the operator's access rights, through the tabs at the bottom of the screen (e.g., amplification plots and test details).

A report with the assay data can be exported to an external USB storage device. Insert the USB storage device into one of the USB ports of the QIAstat-Dx Analyzer 1.0 and press Save Report in the bottom bar of the screen. This report can be exported later at any time by selecting the test from the View Result List.

The report can also be sent to the printer by pressing **Print Report** in the bottom bar of the screen.

Viewing amplification curves

To view test amplification curves of pathogens detected, press the **Amplification Curves** tab (Figure 17).

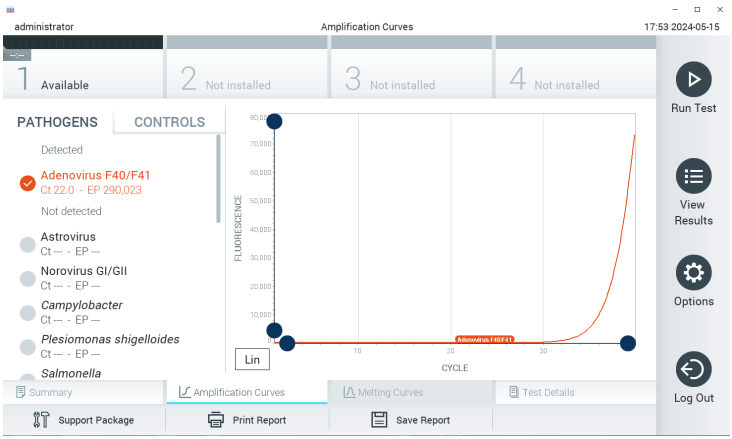

Figure 17. Amplification Curves screen (PATHOGENS tab).

Details about the tested pathogens and controls are shown on the left and the amplification curves are shown in the center.

Note: If **User Access Control** is enabled on the QIAstat-Dx Analyzer 1.0 the **Amplification Curves** screen is only available for operators with access rights.

Press the **PATHOGENS** tab on the left side to display the plots corresponding to the tested pathogens. Press on the pathogen name to select which pathogens are shown in the amplification plot. It is possible to select single, multiple or no pathogens. Each pathogen in the selected list will be assigned a color corresponding to the amplification curve associated with the pathogen. Unselected pathogens will be shown in gray.

The corresponding C_T and endpoint fluorescence (EP) values are shown below each pathogen name.

Press the **CONTROLS** tab on the left side to view the controls in the amplification plot. Press the circle next to the control name to select or deselect it (Figure 18).

Figure 18. Amplification Curves screen (CONTROLS tab).

The amplification plot displays the data curve for the selected pathogens or controls. To alternate between logarithmic or linear scale for the Y-axis, press the **Lin** or **Log** button at the bottom left corner of the plot.

The scale of the X-axis and Y-axis can be adjusted using the **blue pickers** on each axis. Press and hold a **blue picker** and then move it to the desired location on the axis. Move a **blue picker** to the axis origin to return to the default values.

Viewing test details

Press **Test Details** in the Tab Menu bar at the bottom of the touchscreen to review the results in more detail. Scroll down to see the complete report. The following Test Details are shown in the center of the screen (Figure 19):

- User ID
- Cartridge SN (serial number)
- Cartridge Expiration Date
- Module SN (serial number)

- Test Status (Completed, Failed or Canceled by operator)
- Error Code (if applicable)
- Test Start Date and Time
- Test Execution Time
- Assay Name
- Test ID
- Test Result:
    - Positive (if at least one gastrointestinal pathogen is detected/identified)
    - Positive with warning (if at least one pathogen is detected, but the Internal Control failed)
    - Negative (if no gastrointestinal pathogen is detected)
    - Failed (an error occurred, or the test was canceled by the user)
- List of analytes tested in the assay, with C_T and endpoint fluorescence in the event of a positive signal
- Internal Control, with C_T and endpoint fluorescence

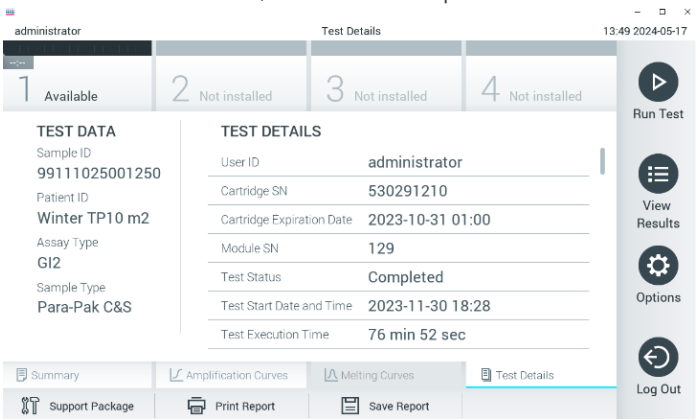

Figure 19. Example screen showing Test Data on the left panel and Test Details in the main panel.

Browsing results from previous tests

To view results from previous tests that are stored in the results repository, press **View Results** on the Main Menu bar (Figure 20).

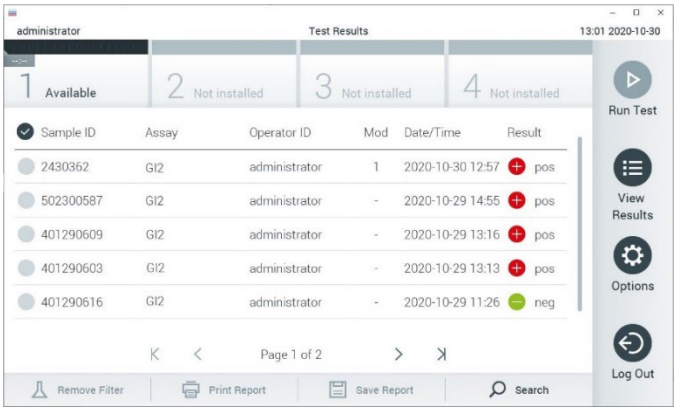

Figure 20. Example View Results screen.

The following information is available for every executed test (Figure 21):

- Sample ID
- Assay (name of test assay, which is "GI2"for Gastrointestinal Panel 2)
- Operator ID
- Mod (Analytical Module on which the test was executed)
- Date/Time (date and time when the test was finished)
- Result (outcome of the test: positive [pos], positive with warning [pos*], negative [neg], failed [fail] or successful [suc])

Note: If **User Access Control** is enabled on the QIAstat-Dx Analyzer 1.0, the data for which the user has no access rights will be hidden with asterisks.

Select one or more test results by pressing the **gray circle** to left of the sample ID. A **checkmark** will appear next to selected results. Unselect test results by pressing this **checkmark**. The entire list of results can be selected by pressing the **checkmark circle** in the top row (Figure 21).

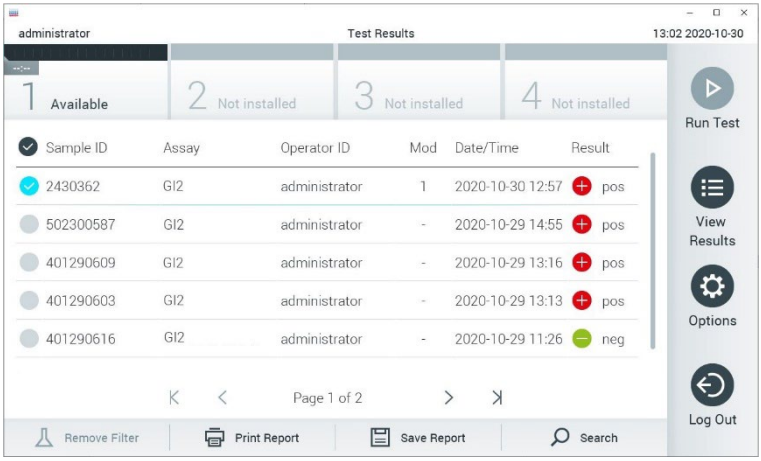

Figure 21. Example of selecting Test Results in the View Results screen.

Press anywhere in the test row to view the result for a particular test.

Press a column headline (e.g., **Sample ID**) to sort the list in ascending or descending order according to that parameter. The list can be sorted according to only one column at a time.

The **Result** column shows the outcome of each test (Table 2):

Table 2. Descriptions of test results displayed on View Results screen.

Outcome	Result	Description	Action
Positive	pos	At least one pathogen is positive	Refer to the Summary Result Screen or Result Printout for pathogen specific results. Description of pathogen results can be found in Table 5.

Outcome	Result	Description	Action
Positive with warning	 pos*	At least one pathogen is positive, but the Internal Control failed	Refer to the Summary Result Screen or Result Printout for pathogen specific results. Description of pathogen results can be found in Table 5.
Negative	 neg	No pathogens were detected	Refer to the Summary Result Screen or Result Printout for pathogen specific results. Description of pathogen results can be found in Table 5.
Failed	 fail	The test failed because either an error occurred, the test was canceled by the user, or no pathogens were detected and the internal control failed.	Repeat the test using a new cartridge. Accept the results of the repeat testing. If the error persists, contact QIAGEN Technical Services for further instructions.
Successful	 Suc	The test is either positive or negative, but the user does not have the access rights to view the test results	Login from a user profile with rights to view the results.

Make sure a printer is connected to the QIAstat-Dx Analyzer 1.0 and the proper driver is installed. Press **Print Report** to print the report(s) for the selected result(s).

Press **Save Report** to save the report(s) for the selected result(s) in PDF format to an external USB storage device.

Select the report type: List of Tests or Test Reports.

Press **Search** to search the test results by Sample ID, Assay and Operator ID. Enter the search string using the virtual keyboard and press **Enter** to start the search. Only the records containing the search text will be displayed in the search results.

If the results list has been filtered, the search will only apply to the filtered list.

Press and hold a column headline to apply a filter based on that parameter. For some parameters, such as **Sample ID**, the virtual keyboard will appear so the search string for the filter can be entered.

For other parameters, such as **Assay**, a dialog will open with a list of assays stored in the repository. Select one or more assays to filter only the tests that were performed with the selected assays.

The T symbol to the left of a column headline indicates that the column's filter is active.

A filter can be removed by pressing **Remove Filter** in the Submenu bar.

Exporting results to a USB drive

From any tab of the **View Results** screen, select **Save Report** to export and save a copy of the test results in PDF format to a USB drive. The USB port is located on the front of the QIAstat-Dx Analyzer 1.0.

Backup and regular data upload to SharePoint

The results can be exported from the instrument following these steps:

1. Press the **Options** button, then the System Configuration button and then System Backup (Figure 22). Insert a USB storage device into the front USB port.
2. Press the **Perform Backup** button. A file with the extension .dbk will be generated in the USB with a default file name.

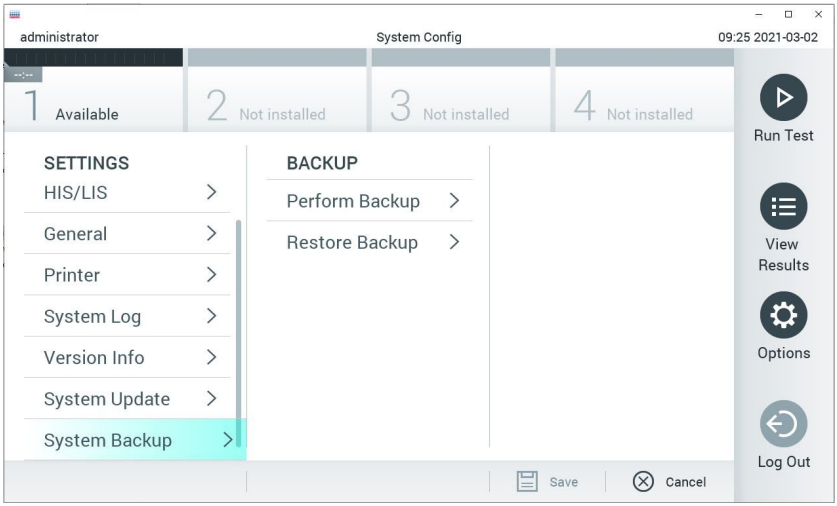

Figure 22. Perform a backup.

Printing results

Make sure a printer is connected to the QIAstat-Dx Analyzer 1.0 and the proper driver is installed. Press **Print Report** to send a copy of the test results to the printer.

Sample result interpretation

Note: STEC, *E. coli* O157, and EPEC results are not reported for stool specimens collected in FecalSwab transport media.

For samples in FecalSwab collection device, a result for a gastrointestinal organism is interpreted as "Positive" when the corresponding PCR assay is positive. EPEC, STEC, and *E. coli* O157 targets are not applicable and will not appear in the results section.

For samples in Para-Pak C&S collection device, a result for a gastrointestinal organism is interpreted as "Positive" when the corresponding PCR assay is positive except for EPEC, STEC

and *E. coli* O157. The result interpretation for EPEC, STEC and *E. coli* O157 follows the rationale explained in Table 3, below.

Table 3. Interpretation of EPEC, STEC, and *E. coli* O157 results only applicable for Para-Pak C&S samples.

EPEC Result	STEC stx1/stx2 Result	E. coli O157 Result	Description
Negative	Negative	N/A	Enteropathogenic *E. coli* (EPEC) was not detected and Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* is negative as both *stx1* and *stx2* have not been detected. *E. coli* O157 result is not applicable (N/A) when STEC *stx1/stx2* is not detected due to *E. coli* O157 being a specific serotype of STEC.
Positive	Negative	N/A	EPEC was detected and STEC *stx1/stx2* is negative as both *stx1* and *stx2* have not been detected. *E. coli* O157 result is not applicable (N/A) when STEC *stx1/stx2* is not detected due to *E. coli* O157 being a specific serotype of STEC.
N/A	Positive	Negative	EPEC result is not applicable because EPEC detection cannot be differentiated when STEC *stx1* or *stx2* is detected. *E. coli* O157 was not detected.
N/A	Positive	Positive	EPEC result is not applicable because EPEC detection cannot be differentiated when STEC *stx1* or *stx2* is detected. *E. coli* O157 was detected.

For both sample collection devices, internal control results are to be interpreted according to Table 4.

Table 4. Interpretation of Internal Control results.

Control Result	Explanation	Action
Passed	The Internal Control amplified successfully	The run was completed with success. All results are validated and can be reported. Detected pathogens are reported as "positive" and undetected pathogens are reported as "negative".
Failed	The Internal Control failed	Positively detected pathogen(s) are reported, but all negative results (tested but not detected pathogen[s]) are invalid. Repeat the testing using a new Cartridge. Accept the results of the repeat testing. If the invalid result persists, contact QIAGEN Technical Services for further instruction.

The software provides an overall test result (Table 2) as well as a result for individual pathogens. Possible results for each organism include Detected/Positive, Not Detected/Negative, N/A, and Invalid (Table 5). If the internal control has failed and no positive signal was detected or if there is an instrument error, there will be no pathogen results provided.

Table 5. Description of Pathogen results as displayed on Summary Result Screen and the Result Printout.

Result	Symbol	Explanation	Action
Positive/ Detected		A positive signal was detected for this pathogen. Result of the Internal Control is passed.	None. Report results.
Positive/ Detected with Warning		A positive signal was detected for this pathogen, but the result of the internal control has failed.	Report positive analyte. Repeat the test using a new cartridge. Accept the results of the repeat testing. If the invalid result persists, contact QIAGEN Technical Services for further instructions.
Negative/ Not Detected		No signal was detected for this pathogen. The Internal Control passed.	None. Report results.
N/A (applies to *E. coli* O157 and EPEC only)		The run was successfully completed and the Internal Control passed. For *E. coli* O157 N/A: Shiga-like toxin-producing *E. coli* (STEC) was not detected. For EPEC N/A: Shiga-like toxin producing *E. coli* (STEC) was detected.	None. Report results.
Invalid		No signal was detected for this pathogen and the Internal Control failed (but other pathogens have been detected).	Repeat the test using a new cartridge. Accept the results of the repeat testing. If the invalid result persists, contact QIAGEN Technical Services for further instructions.

Quality Control

Internal control interpretation

The QIAstat-Dx Gastrointestinal Panel Cartridge includes a full process Internal Control, which is titered *Schizosaccharomyces pombe*. *Schizosaccharomyces pombe* is a yeast (fungi) that is included in the cartridge in dried form and is rehydrated upon sample loading. This Internal Control material verifies all steps of the analysis process, including sample homogenization, lysis of viral and cellular structures (by means of chemical and mechanical disruption), nucleic acid purification, reverse transcription, and real-time PCR.

A passed result for the Internal Control indicates that all processing steps performed by the QIAstat-Dx Gastrointestinal Panel Cartridge were successful.

A failed result of the Internal Control does not negate any positive results for detected and identified targets, but it does invalidate all negative results in the analysis. Therefore, the test should be repeated if the Internal Control signal is negative.

External control information

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

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

Limitations

- The QIAstat-Dx Gastrointestinal Panel 2 is intended for professional use only and is not intended for self-testing. The QIAstat-Dx Gastrointestinal Panel 2 is intended for *in vitro* diagnostic use.
- Results from the QIAstat-Dx Gastrointestinal Panel 2 are not intended to be used as the sole basis for diagnosis, treatment or other patient management decisions.
- All assay results should be used and interpreted by a trained healthcare professional in the context of a full clinical evaluation, laboratory and epidemiological findings, as an aid in the diagnosis of gastrointestinal infection.
- The performance of this test has not been determined for patients without signs and symptoms of gastrointestinal illness.
- The QIAstat-Dx Gastrointestinal Panel 2 is not intended for testing of samples other than those described in these Instructions for Use (IFU). The performance of this test has only been validated with human stool preserved in transport medium (Para-Pak C&S or FecalSwab), according to the media manufacturers' instructions. It has not been validated for use with other stool transport media, rectal swabs, raw stool, vomitus, or endoscopy stool aspirates. The QIAstat-Dx Gastrointestinal Panel 2 should not be used to test Para-Pak C&S or FecalSwab collection devices that have been overfilled with stool. Only stool resuspended following the collection device manufacturer's instructions should be used. The overfilling of Para-Pak C&S or FecalSwab collection devices can result in a failed test with an error indicating 'Sample concentration too high'.
- The detection of viral, bacterial, or parasitic sequences is dependent upon proper specimen collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
- Positive results do not rule out co-infection with organisms not included in the QIAstat-Dx Gastrointestinal Panel 2. The agent detected may not be the definitive cause of the disease.
- Not all agents of acute gastrointestinal infection are detected by this assay.

- The QIAstat-Dx Gastrointestinal Panel 2 is intended to be used in conjunction with standard of care culture for organism recovery, serotyping and/or antimicrobial susceptibility testing where applicable.
- The QIAstat-Dx Gastrointestinal Panel 2 can be used only with the QIAstat-Dx Analyzer 1.0.
- The identification of multiple diarrheagenic *E. coli* pathotypes has historically relied upon phenotypic characteristics, such as adherence patterns or toxigenicity in certain tissue culture cell lines (27). The QIAstat-Dx Gastrointestinal Panel 2 targets genetic determinants characteristic of most pathogenic strains of these organisms but may not detect all strains having phenotypic characteristics of a pathotype.
- Genetic virulence markers associated with diarrheagenic *E. coli/ Shigella* pathotypes are often carried on mobile genetic elements (MGEs) that can be transferred horizontally between different strains (27), therefore "Detected" results for multiple diarrheagenic *E. coli/ Shigella* may be due to co-infection with multiple pathotypes or, less frequently, may be due to the presence of a single organism containing genes characteristic of multiple pathotypes. An example of the latter is the 2019 *E. coli* hybrid ETEC/STEC strains found in Sweden (73).
- The QIAstat-Dx Gastrointestinal Panel 2 detects Enteropathogenic *E. coli* (EPEC) in Para-Pak C&S collected samples through targeting of the *eae* gene, which encodes the adhesin intimin. As some Shiga-like toxin-producing *E. coli* (STEC) also carry *eae* (in particular, strains identified as enterohemorrhagic *E. coli*; EHEC) (27), the QIAstat-Dx Gastrointestinal Panel 2 cannot distinguish between STEC containing *eae* and a co-infection of EPEC and STEC. Therefore, the EPEC result is not applicable (N/A) and not reported for specimens in which STEC has also been detected. In rare cases, STEC may be reported as EPEC when an STEC carrying *eae* (EHEC) is present in a specimen below the LoD of the STEC oligonucleotide design(s). Rare instances of other organisms carrying *eae* have been documented; e.g., *Escherichia albertii*, and *Shigella boydii* (74).
- *Shigella dysenteriae* serotype 1 possess a shiga toxin gene (*stx*) that is identical to the *stx1* gene of STEC (27) . Stx genes have been more recently found in other *Shigella* species (e.g., *S. sonnei* and *S. flexneri*) (75,76) . The detection of both *Shigella*/Enteroinvasive *E.*

coli (EIEC) and STEC *stx1/stx2* analytes in the same specimen may indicate the presence of *Shigella* species such as *S. dysenteriae*. Rare instances of the detection of Shiga-like toxin genes in other genera/species have been reported, e.g., *Acinetobacter haemolyticus*, *Enterobacter cloacae* and *Citrobacter freundii* (77,78,79) .

- Results for STEC, *E. coli* O157 and EPEC are not reported for stool specimens preserved in FecalSwab transport media.
- *E. coli* O157 result is only reported as specific serogroup identification in association with STEC *stx1/stx2* in Para-Pak C&S collected samples. While non-STEC O157 strains have been detected in human stool (80), their role in disease has not been established (81). Serotype O157 EPEC have been identified and will be detected by the QIAstat-Dx Gastrointestinal Panel 2 (by the EPEC oligonucleotides design) due to their carriage of the *eae* gene.
- The QIAstat-Dx Gastrointestinal Panel 2 cannot distinguish between infections with a single toxigenic STEC O157 or rare co-infections of STEC (non-O157) with a *stx1/stx2*-negative *E. coli* O157 in Para-Pak C&S samples.
- This test only detects *Campylobacter jejuni*, *C. coli* and *C. upsaliensis* and does not differentiate between these three species of *Campylobacter*. Additional testing is required to differentiate between these species and to detect other *Campylobacter* species that may be present in stool specimens. In particular, the *Campylobacter upsaliensis* oligonucleotides design may cross-react with the *Campylobacter* species *C. lari* and *C. helveticus* organisms.
- Negative results do not exclude the possibility of gastrointestinal infection. Negative test results may occur from sequence variants in the region targeted by the assay, the presence of inhibitors, technical errors, sample mix-ups, or an infection caused by an organism not detected by the panel. Test results may also be affected by use of certain medications (e.g., calcium carbonate), concurrent antimicrobial therapy or levels of organism in the sample that are below the limit of detection for the test. Sensitivity in some clinical settings may differ from that described in the Instructions for Use. Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions.

- Organism and amplicon contamination may produce erroneous results for this test. Particular attention should be given to the Laboratory Precautions noted under the Laboratory Precautions section.
- There is a risk of false-positive values resulting from cross-contamination by target organisms, their nucleic acids or the amplified product, or from non-specific signals in the assay.
- There is a risk of false negative results due to the presence of strains with sequence variability in the target regions of the oligonucleotides design. Refer to the inclusivity testing section of this document for additional information.
- Not all *Salmonella* serotypes were tested in validation studies; however, representatives of the 20 most prevalent serotypes recently circulating in the US (CDC National *Salmonella* Surveillance Annual Summary 2016) were evaluated during analytical reactivity studies. *In silico* sequence analysis supports detection of all subspecies and serotypes of *Salmonella*.
- The performance of the QIAstat-Dx Gastrointestinal Panel 2 has not been established in individuals who received Rotavirus A vaccine. Recent oral administration of a Rotavirus A vaccine may cause positive results for Rotavirus A if the virus is passed in the stool.
- The performance of this test has not been evaluated for immunocompromised individuals.
- Underlying polymorphisms in primer-binding regions can affect the targets being detected and subsequently the test results returned.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is low.
- The effect of interfering substances has only been evaluated for those listed in the labeling at its indicated amount or concentration. Interference by substances other than those described in the "Interfering Substances" section of the Instruction for Use can lead to erroneous results.
- Cross-reactivity with gastrointestinal tract organisms other than those listed in the "Analytical Specificity" section of the package insert may lead to erroneous results.

- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- The assay sensitivity to detect *Cyclospora cayetanensis*, Adenovirus F40/F41, *Entamoeba histolytica* and the Shiga-like toxin- producing *Escherichia coli* (STEC) might be reduced up to 3.16-fold when using half-input sample volume (100 µL) workflow detailed in Appendix C: Additional Instruction for use.
- Due to the small number of positive specimens collected for certain analytes during the prospective clinical study, performance characteristics for Adenovirus 40/41, ETEC, *Plesiomonas shigelloides*, *Shigella*/EIEC, STEC, *E. coli* 0157, *Yersinia enterocolitica*, *Cryptosporidium*, and *Giardia lamblia* were established additionally with retrospective clinical specimens. Performance characteristics for Astrovirus and *Entamoeba histolytica* were established primarily with contrived clinical specimens.
- If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
- Virus, bacteria, and parasite nucleic acid may persist *in vivo* independently of organism viability. Additionally, some organisms may be carried asymptomatically. Detection of organism targets does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
- For ETEC, 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 Human Astrovirus types MLB1-3 and VA1-5.
- The potential for competitive inhibition at high concentrations between on-panel analytes was evaluated for a limited number of pathogens (See Table 14). The potential for competitive inhibition between other on-panel analytes is unknown.

Performance Characteristics

Analytical performance

Limit of Detection

The 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 pathogen strains, by analyzing serial dilutions of analytical samples prepared from culture isolates from commercial suppliers (e.g., ZeptoMetrix® and ATCC®), confirmed clinical isolates, or artificial samples 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 transport medium.

Each of the 36 strains was tested in human stool matrix prepared following the manufacturer's instructions for the Para-Pak C&S collection device. The confirmed LoD was established by testing 20 replicates at the concentration determined from the preliminary LoD for each strain. The LoD for each strain was confirmed if ≥ 95% of the replicates were positive. To further confirm the LoD, at least one dilution below the LoD was tested for each strain and was also tested in 20 replicates and was required to result in less than 95% positivity. A transport media 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 target are shown in Table 6.

Table 6. LoD values obtained for the different gastrointestinal target strains tested with the QIAstat-Dx Gastrointestinal Panel 2.

Pathogen	Strain	Source	Concentration (molecular units)*	Concentration (microbiological units)	Detection rate
Campylobacter	*Campylobacter coli* 76-GA2 [LMG 21266]	ATCC 43478	5802 copies/mL	1.2 CFU/mL	20/20
	Campylobacter coli CIP 7080	ATCC 33559	8941 copies/mL	0.6 CFU/mL	20/20
	Campylobacter jejuni Z086	ZeptoMetrix 801650	14491 copies/mL	1660 CFU/mL	20/20
	Campylobacter jejuni subsp. Jejuni RM3193	ATCC BAA-1234	7210 copies/mL	110 CFU/mL	19/20
	Campylobacter upsaliensis NCTC 11541	ZeptoMetrix 0801999	56165 copies/mL	2259.4 CFU/mL	20/20
	Campylobacter upsaliensis RM3195	ATCC BAA-1059	7631 copies/mL	35 CFU/mL	19/20
Plesiomonas shigelloides	Z130	ZeptoMetrix 801899	481 copies/mL	2291 CFU/mL	20/20
	Bader	ATCC 14029	116 copies/mL	2.7 CFU/mL	19/20
Salmonella	*Salmonella enterica* Serovar choleraseus	ATCC 13312	647 copies/mL	91.6 CFU/mL	20/20
	Salmonella enterica Serovar Typhimurium Z005	ZeptoMetrix 801437	1441 copies/mL	4518.8 CFU/mL	20/20
Yersinia enterocolitica	Z036	ZeptoMetrix 0801734	719 copies/mL	2070 CFU/mL	20/20
	subsp. *enterocolitica* NTCC 11175, Biotype 4, serotype 3	ATCC 700822	2496 copies/mL	120.1 CFU/mL	20/20
Enteroinvasive *E. coli* (EIEC)/ *Shigella*	*Shigella sonnei* NCDC 1120-66	ATCC 25931	488 copies/mL	0.2 CFU/mL	20/20
	Escherichia coli CDC EDL 1282, O29:NM	ATCC 43892	1431 copies/mL	41.3 CFU/mL	20/20
Enteropathogenic *E. coli* (EPEC)*	*Escherichia coli* O111:NM (EPEC)	ZeptoMetrix 0801747	1817 copies/mL	2581.7 CFU/mL	20/20
	Escherichia coli 7.1493; EPEC; O84:H28	Zeptometrix 801938	29021 copies/mL	1190 CFU/mL	20/20
Enterotoxigenic *E. coli* (ETEC) *lt/st*	*Escherichia coli* H10407, O78:H11	ATCC 35401	367 copies/mL	10.1 CFU/mL	19/20
	Escherichia coli ETEC; ST+, LT+	ZeptoMetrix 801624	855 copies/mL	567 CFU/mL	20/20

(continued on next table)

Table 6 (continued from previous table)

Pathogen	Strain	Source	Concentration (molecular units)*	Concentration (microbiological units)	Detection rate
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2**	*Escherichia coli* O26:H4	ZeptoMetrix 801748	2012 copies/mL	726.8 CFU/mL	20/20
Shiga-like toxin-producing *E. coli* (STEC) *E. coli* O157*	*Escherichia coli* O157:H7; EDL933	ZeptoMetrix 801622	1217 copies/mL	2281.5 CFU/mL	STEC stx 1/stx2 19/20 O157: 19/20
Cryptosporidium	*Cryptosporidium hominis*	Public Health Wales UKM 84	357 copies/mL	N/A	20/20
	Cryptosporidium parvum – Iowa isolate	Waterborne® P102C	661 copies/mL	N/A	20/20
Cyclospora cayetanensis	N/A	LACNY-Clinical sample LAC2825	53 copies/mL	N/A	19/20
	N/A	LACNY Clinical sample LAC2827	137 copies/mL	N/A	20/20
Entamoeba histolytica	HM-1:IMSS (Mexico City 1967)	ATCC 30459	7 copies/mL	0.2 cells/mL	20/20
	HK-9 (Korea)	ATCC 30015	1 copy/mL	0.13 cells/mL	19/20
Giardia lamblia	WB (Bethesda)	ATCC 30957	11850 copies/mL	790 cells/mL	19/20
	Portland-1	ATCC 30888	14500 copies/mL	635 cells/mL	20/20
Adenovirus F40/F41	Type 40 (Dugan)	ZeptoMetrix 0810084CF	11726 copies/mL	0.1 TCID50/mL	20/20
	Type 41 (Tak)	ZeptoMetrix 0810085CF	979 copies/mL	0.05 TCID50/mL	19/20
Astrovirus	ERE IID 2371 (type 8)	Zeptometrix 0810277CF	11586371 copies/mL	11.7 TCID50/mL	20/20
	ERE IID 2868 (type 4)	Zeptometrix 0810276CF	52184 copies/mL	1.3 TCID50/mL	19/20
Norovirus GI/GII	GI.1 (recombinant)	ZeptoMetrix 0810086CF	24629 copies/mL	891.1 TCID50/mL	19/20
	GII.4 (recombinant)	ZeptoMetrix 0810087CF	8998 copies/mL	10.5 TCID50/mL	20/20
Rotavirus A	69M	ZeptoMetrix 0810280CF	5787 copies/mL	436.1 TCID50/mL	19/20
	Wa	ZeptoMetrix 0810041CF	5201 copies/mL	14.1 TCID50/mL	19/20

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

† Only applicable for samples with Para-Pak C&S collection device

Exclusivity (Analytical Specificity)

The analytical specificity study was carried out by *in vitro* testing and *in silico* analysis to assess the potential cross-reactivity and exclusivity 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 7 and Table 8, respectively.

Samples were prepared by single spiking organisms into negative stool resuspended in Para-Pak C&S media at the highest concentration possible based on the organism stock, preferably at 10^5 TCID50/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 7. List of Analytical Specificity on-panel pathogens tested.

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

*Only applicable for samples with Para-Pak C&S collection device

Table 8. List of Analytical Specificity off-panel pathogens tested

Type	Pathogen (potential cross-reactant)	
Bacteria	*Abiotrophia defectiva*	*Enterobacter aerogenes*
	Acinetobacter baumannii	*Enterobacter cloacae*
	Aeromonas hydrophila	*Enterococcus faecalis*
	Arcobacter cryaerophilus	*Enterococcus faecium*
	Bacillus subtilis	*Escherichia fergusonii*
	Bifidobacterium bifidum	*Escherichia hermannii*
	Campylobacter fetus	*Escherichia vulneris*
	Campylobacter gracilis	*Faecalibacterium prausnitzii*
	Campylobacter helveticus (Cross-reactive for Campylobacter target)	*Gardnerella vaginalis*
	Campylobacter hominis	*Haemophilus influenzae*
	Campylobacter lari (Cross-reactive for Campylobacter target)	*Helicobacter pylori*
	Campylobacter mucosalis	*Klebsiella pneumoniae*
	Campylobacter rectus	*Lactobacillus casei*
	Chamydia trachomatis	*Listeria monocytogenes*
	Citrobacter freundii	*Proteus mirabilis*
	Clostridium difficile non-toxigenic	*Proteus vulgaris*
	Clostridium perfringens	*Pseudomonas aeruginosa*
	Clostridium septicum	*Staphylococcus aureus*
	Clostridium tetani	*Staphylococcus aureus* subsp. *Aureus*
	Corynebacterium genitalium	*Staphylococcus epidermidis*
		Streptococcus agalactiae
		Streptococcus pyogenes
Fungi	*Aspergillus fumigatus*	*Saccharomyces boulardii*
	Candida albicans	*Saccharomyces cerevisiae*
Parasites	*Babesia microti*	*Toxoplasma gondii*
	Blastocystis hominis	*Trichomonas tenax*
	Giardia muris	
Viruses	Adenovirus C:2	Coronavirus 229E
	Adenovirus B:34	Coxsackievirus B3
	Adenovirus B3	Cytomegalovirus
	Adenovirus E:4a	Enterovirus 6 (*Echovirus*)
	Adenovirus serotype 1	Enterovirus 68
	Adenovirus serotype 5	Herpes Simplex Virus Type 2
	Adenovirus serotype 8	Rhinovirus 1A
	Bocavirus Type 1	

In silico predictions of potential cross-reactions showed that the following cross-reactions may occur when testing stool samples with the QIAstat-Dx Gastrointestinal Panel 2 (Table 9).

Table 9. Potential cross-reactions based on *in silico* analysis.

QIAstat-Dx Gastrointestinal Panel 2 Target	Potential cross-reactive organisms
Enteropathogenic *E. coli* (EPEC)*	*Shigella boydii* †‡§, *Escherichia albertii* †‡
Campylobacter spp.	*Campylobacter lari* §, *Campylobacter helveticus* §
Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2*	*Shigella sonnei* †§, *Shigella dysenteriae*†§, *Acinetobacter haemolyticus*†**, *Citrobacter freundii*†**, *Enterobacter cloacae*†**, *Aeromonas caviae*†**, *Escherichia albertii* †¶
E. coli O157*	Non-STEC *E.coli* O157 strains††

* Only applicable for samples with Para-Pak C&S collection device.

† Note that these predicted cross-reactivity identified by *in silico* analysis reflects sequences which can be acquired between species by horizontal gene transfer(27,82)

‡ Rare or less common *eae* intimin carrier organisms (74).

§ On-panel target.

¶ Rare or less common Stx toxins producers (77,83,84,85,86,87)

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

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

Inclusivity (Analytical Reactivity)

Analytical Reactivity (Inclusivity) was evaluated with gastrointestinal pathogen isolates/strains that were selected based on clinical relevance and genetic, temporal and geographical diversity. Samples were prepared by spiking organisms into negative stool matrix resuspended in Para-Pak C&S transport media. 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 (Wet) 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 wet testing were detected at ≤ 3-fold

(104/114), of the corresponding LoD reference strain. Less than 100% detection was observed for one strain each of ETEC, EIEC/*Shigella* and Rotavirus and two strains each of STEC (one STEC O157), Adenovirus and Norovirus at 3x LoD. Testing of these strains at 10x LoD generated the expected positive resulted for all replicates (Table 10).

Table 10. Inclusivity test results for all the pathogens tested with the QIAstat-Dx Gastrointestinal Panel 2 Assay. LoD reference strain for every pathogen is written in bold.

Table 10a. Inclusivity test results for *Campylobacter* strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Campylobacter	***Campylobacter coli***	**76-GA2 [LMG 21266]**	**ATCC**	**43478**	**1x LoD**
	Campylobacter coli	Z293	ZeptoMetrix	0804272	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.

Table10b. Inclusivity test results for *Plesiomonas shigelloides* strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
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 10c. Inclusivity test results for *Salmonella* strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
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, serovar Saintpaul	ATCC	9712	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Berta	NCTC	NC05770	0.1x LoD

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Table 10c (continued from previous page)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
	Salmonella enterica	Subsp. Salame, II NCTC 10310 [JT945, SS140/61]	ATCC	700151	0.1x LoD
	Salmonella enterica	Subsp. diarizonae IIIb, 62	ATCC	29934	0.1x LoD
	Salmonella enterica	Subsp. houtenae IV, CIP 82.32 [264.66]	ATCC	43974	0.1x LoD
	Salmonella enterica	Subsp. Indica VI, CIP 102501 [F. Kauffmann 1240]	ATCC	43976	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Agona, CDC 873 [CDC 1111-61]	ATCC	51957	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Muenchen, 54	ATCC	8388	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Oranienburg, E1093	ATCC	9239	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Paratyphi B var. Java, CDC 5	ATCC	51962	0.1x LoD
Salmonella	*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, serovar Mississippi, CDC 2012K-0487	ATCC	BAA-2739	0.3x LoD

* Strain tested during LoD verification study.

Table 10d. Inclusivity test results for *Yersinia enterocolitica* strains.

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

* Strain tested during LoD verification study.

Table 10e. Inclusivity test results for Enteropathogenic *E. coli* (EPEC) strains. Only applicable for Para-Pak C&S samples.

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

* Strain tested during LoD verification study.

Table 10f. Inclusivity test results for Enterotoxigenic *E. coli* (ETEC) strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Enterotoxigenic *E. coli* (ETEC) lt/st	**Enterotoxigenic *E. coli* (ETEC) lt/st**	**ST+, LT+**	**ZeptoMetrix**	**0801624***	**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 10g. Inclusivity test results for Enteroinvasive *E. coli* (EIEC)//*Shigella* strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Enteroinvasive *E. coli (EIEC)/ Shigella*	**Enteroinvasive *E. coli* (EIEC)**	**CDC EDL 1282, O29:NM**	**ATCC**	**43892***	**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 10h. 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 target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Shiga-like toxin producing *E. coli* (STEC) - stx1/2	**Shiga-like toxin producing *E. coli* (STEC) - stx1/stx2**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622***	**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,stx1 (+), stx2 (+)	Microbiologics	617	3x LoD

(continued on the next page)

Table 10h (continued from the previous page)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	Reference CDC 00-3039,O45:H2,unknown	Microbiologics	1098	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	O103:H2,stx1 (+)	SSI Diagnostica	82170	3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1/stx2	O22:H8,stx1c (+), stx2b (+)	SSI Diagnostica	91350	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O92,O107:K+:H48, 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 10i. Inclusivity test results for Shiga-like toxin producing *E. coli* (STEC) *stx1/stx2* O157 strains. Only applicable for Para-Pak C&S samples.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalogue ID	Times LoD
Shiga-like toxin producing *E. coli* (STEC) O157	**Shiga-like toxin producing *E. coli* (STEC) - O157**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622***	**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 an FDA-cleared test .

‡ Testing at a lower concentration resulted in a detection rate of <100%.

Table 10j. Inclusivity test results for *Cryptosporidium* strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
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 10k. Inclusivity test results for *Cyclospora cayetanensis* strains.

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

* Strain tested during LoD verification study.

Table 10l. Inclusivity test results for Entamoeba histolytica strains.

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

* Strain tested during LoD verification study.

Table 10m. Inclusivity test results for Giardia lamblia strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalogue ID	Times LoD
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 10n. Inclusivity test results for Adenovirus F40/F41 targets.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
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 Type 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 10o. Inclusivity test results for Astrovirus strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
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 10p. Inclusivity test results for Norovirus GI/GII strains.

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

* Strain tested during LoD verification study.

Testing at a lower concentration resulted in a detection rate of <100%.

Table 10q. Inclusivity test results for Rotavirus A strains.

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

* 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 11).

Table 11. 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)

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Table 11. (continued from previous page)

Bacteria	
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*/stx2β	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: 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) O157β	*Escherichia coli* O157 including: STEC O157:H7 strains (e.g. EDL933) and *E. coli* O157: non-H7 groups including non-Shiga-toxigenic *E. coli* O157 bacteria (e.g. serotype O157:H45)
Parasites	
Cryptosporidium‡	Common *Cryptosporidium* species involved in human disease: *C. parvum, C. hominis.* Less common *Cryptosporidium* species involved in human infections: *C. meleagridis, C. felis , C. bovis, C. viatorum, C. ubiquitum,C. tyzzeri, C. cuniculus, Cryptosporidium sp. Chipmunk genotype I, C. canis*.* Rare or non-human species: *Cryptosporidium wrairi*
Cyclospora cayetanensis	*Cyclospora cayetanensis* (including strains LG, CY9, NP20 and NP21) *
Entamoeba histolytica	*Entamoeba histolytica* (e.g. strains HM-1: IMSS, EHMfas1, HK-9)*
Giardia lamblia	*Giardia lamblia* (aka *Giardia duodenalis*, *Giardia intestinalis*)*
Viruses	
Adenovirus	Human Adenovirus F40/41
Astrovirus§	Human Astrovirus (including types 1, 2, 3, 4, 5, 6, 7, 8)
Norovirus GI/GII	Norovirus genogroup II genotypes: GII.1, GII.2, GII.3*, GII.4*, GII.5, GII.6, GII.7, GII.8, GII.9, GII.10, GII.12, GII.13, GII.14, GII.16, GII.17, GII.20, GII.21, GII.22, GII.23, GII.24*, GII.25, GII.26, GII.27, GII.NA1 and GII.NA2* Norovirus genogroup I genotypes: GI.1, GI2, GI.3*, GI.4*, GI.5, GI.6*, GI.7*, GI.8, GI.9.
Rotavirus	Rotavirus A including genotypes: G1P[8]*, G2P[4]*, G3P[8]*, G4P[8]*, G9P[6], G9P[8]*, G12P[6]* and G12P[8]*

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

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

‡The assay is not predicted to detect other *Cryptosporidium spp.* less involved in human disease: *C. andersoni* and *C. muris.* (88)

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

¶ Only applicable for samples with Para-Pak C&S collection device

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. 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.

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

For the vast majority of substances tested, no interference was observed, with the exceptions of mucin, calcium carbonate, nonoxynol-9 and Rotavirus reassortants, that demonstrated interference 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 at concentrations above 0.5% w/v was found to generate false negative results for all the QIAstat-Dx Gastrointestinal Panel 2 targets and the internal control.

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

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 $8.89x10^{-5}$ TCID50/mL and 1.10 PFU/mL, respectively (Table 12) for other concentrations tested.

Results from the 34 interfering substances that could be present or introduced in a stool specimen are provided in (Table 12).

Table 12. Final highest concentration without observable inhibitory effect.

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
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 2.5% w/v	Interference† No Interference
Triglycerides	5% w/v	No Interference
Exogenous substances		
Bacitracin	250U/mL	No Interference
Bisacodyl	0.3% w/v	No Interference
Bismuth subsalicylate	0.35% w/v	No Interference
Calcium carbonate (TUMS® Extra Strength 750)	5%w/v 0.5% w/v	Interference No Interference

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Table 12 (continued from previous page)

Substance tested	Concentration tested	Result
Exogenous substances		
Docusate sodium	2.5% w/v	No Interference
Doxycycline hydrochloride	0.05% w/v	No Interference
Glycerin	50% v/v	No Interference
Hydrocortisone	0.5% w/v	No Interference
Loperamide hydrochloride	0.078% w/v	No Interference
Magnesium hydroxide	0.1% w/v	No Interference
Metronidazole	1.5% w/v	No Interference
Mineral oil	50% v/v	No Interference
Naproxen sodium	0.7% w/v	No Interference
Nonoxynol-9	1.2% v/v 0.6% v/v 0.3% v/v 0.15% v/v 0.075% v/v 0.02% v/v	Interference Interference Interference Interference Interference No Interference
Nystatin	10000 USP units/mL	No Interference
Phenylephrine hydrochloride	0.075% w/v	No Interference
Sodium phosphate	5% w/v	No Interference
Vaccine components		
Rotavirus reassortant WC3:2-5, R574(9) - VR 2195	8.89×10^{-3} TCID50/mL 8.89×10^{-4} TCID50/mL 8.89×10^{-5} TCID50/mL	Interference Interference No Interference
Rotavirus reassortant WI79-4,9 - VR 2415	1.10×10^{2} pfu/mL 1.10×10^{1} pfu/mL 1.10 pfu/mL	Interference Interference No Interference
Technique-specific Substances, Transport Media		
Bleach	0.5% v/v	No Interference
Ethanol	0.2% v/v	No Interference
Puritan Fecal Opti-Swab Collection &Transport System with Cary-Blair Medium*	100%	No Interference
Puritan PurSafe® DNA/RNA Preservative*	100%	No Interference
Sigma Fecal Transwab*	1 swab/2mL Cary Blair	No Interference

Substance tested	Concentration tested	Result
Exogenous substances		
Docusate sodium	2.5% w/v	No Interference
Doxycycline hydrochloride	0.05% w/v	No Interference
Glycerin	50% v/v	No Interference
Hydrocortisone	0.5% w/v	No Interference
Loperamide hydrochloride	0.078% w/v	No Interference
Magnesium hydroxide	0.1% w/v	No Interference
Metronidazole	1.5% w/v	No Interference
Mineral oil	50% v/v	No Interference
Naproxen sodium	0.7% w/v	No Interference
Nonoxynol-9	1.2% v/v 0.6% v/v 0.3% v/v 0.15% v/v 0.075% v/v 0.02% v/v	Interference Interference Interference Interference Interference No Interference
Nystatin	10000 USP units/mL	No Interference
Phenylephrine hydrochloride	0.075% w/v	No Interference
Sodium phosphate	5% w/v	No Interference

* Performance not established for this transport media

† This substance was tested by another FDA-cleared test 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 (1 x 10^6 CFU/mL for bacteria, 1 x 10^5 cells/mL for yeast and 1 x 10^5 $TCID_{50}$/mL for viruses) 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 13 for a list of the non-target organisms tested and the result summary.

Table 13. 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 Species 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 14.

Table 14. QIAstat-Dx Gastrointestinal Panel 2 results for competitive interference.

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

*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 in Para-Pak C&S transport media, with alternating high-positive (10^6 CFU/mL for bacteria-, 10^5 $TCID_{50}$ or organism/mL for viruses and parasites) and negative samples, were conducted on two QIAstat-Dx Analyzer 1.0 instruments.

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.

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.

Table 15 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. 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 15. Detection rate per target and concentration for each site of the Reproducibility study and exact 2-sided 95% Confidence Interval by target and concentration.

			% Agreement with Expected Result			
Pathogen Tested	**Concentration Tested**	**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* 96.7% (90.98 – 98.9%)

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Table 15 (Continued from the previous page)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Campylobacter ZeptoMetrix 801650	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	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%)
Escherichia coli **EPEC** [§] ZeptoMetrix 801747	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 [‡] 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%)

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Table 15 (Continued from the previous page)

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‡ 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**§ 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%)
***Escherichia coli* (STEC)**§ stx1/stx2 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%)

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Table 15 (continued from previous page)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Salmonella enterica ZeptoMetrix 0801437	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%)
	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Yersinia enterocolitica Zeptometrix 0801734	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%)

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

† 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.

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

§ Only applicable for Para-Pak C&S samples

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*, *Entamoeba histolytica*,

Giardia lamblia, Norovirus GII, Rotavirus, *Salmonella enterica*, and *Yersinia enterocolitica, and additionally* Enteropathogenic *E. coli* (EPEC), STEC stx1/stx2, and *E. coli* O157 which are only applicable for Para-Pak C&S samples. 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.

Expected Values

The number and percentage of positive results as determined by the QIAstat-Dx Gastrointestinal Panel 2 in the prospective clinical evaluation, stratified by age group, are presented in Table 16. Overall, the QIAstat-Dx Gastrointestinal Panel 2 detected at least 1 organism 17.4% (213/1222) and 23.8% (171/717) of the prospectively collected stool specimens in FecalSwab and Para-Pak C&S, respectively.

Table 16. 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%)

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Table 16 (continued from previous page)

Pathogen	Medium Brand	Overall	0-5 years	6-21 years	22-49 years	50+ years	Not Reported
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 representatives 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. A total of 1939 prospectively collected stool specimens (stool preserved in Para-Pak C&S (Meridian Bioscience) or FecalSwab (COPAN)) were obtained from patients with clinical indications of diarrhea caused by gastrointestinal infection. Table 17 provides a summary of prospective specimen's distribution across all study sites.

Table 17. Prospective Specimens Distribution Across the study sites.

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

* The specimens from this site were excluded from the analysis because they were collected with another device different to Para-Pak C&S or FecalSwab.

The demographic information for the 1,939 specimens evaluated in the prospective study is summarized in Table 18 below.

Table 18. Demographic data for prospective evaluated specimens.

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

The performance of the QIAstat-Dx Gastrointestinal Panel 2 was evaluated for each panel test result using one FDA-cleared test as comparator for the most analytes. A composite comparator consisting of either three independent FDA-cleared test methods or two independent FDA-cleared tests methods and two validated PCR assays followed by bi-directional sequencing was used for Norovirus GI/GII, ETEC, STEC and *Giardia lamblia* (Table 19).

Table 19. Comparator Methods for the Clinical Evaluation of QIAstat-Dx Gastrointestinal Panel 2.

QIAstat-Dx GI Panel 2 Test Result	Comparator Method
Adenovirus F40/F41 Astrovirus Rotavirus A *Campylobacter* *Plesiomonas shigelloides* *Salmonella* *Yersinia enterocolitica* *E. coli* O157 * Enteropathogenic *E. coli* (EPEC)* *Shigella*/Enteroinvasive *E. coli* (EIEC) *Cryptosporidium* *Cyclospora cayetanensis* *Entamoeba histolytica*	One FDA-cleared test method
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**	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†

* Targets evaluated in Para-Pak C&S specimens only.
† 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 19 were used as confirmatory testing for the presence of the nucleic acids from the expected analytes. 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 exact binomial two-sided 95% confidence intervals for PPA and NPA were calculated.

Where a composite comparator was used (Table 19), 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 20, 21 and 22, 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 (Tables 20 and 22).

Table 20. 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	1216/1216	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

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Table 20 (continued from the previous page)

		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Medium Brand**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
				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 [i]	83.3	43.7-97.0	698/700 [i]	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	43.7-97.0	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
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
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
[i] *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) 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. 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 21. 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*	98.0	89.3 - 99.6	2/4*	50.0	15.0-85.0
	Para-Pak C&S	26/28*†	92.9	77.4 - 98.0	37/38*	97.4	86.5-99.5
Shiga-like toxin *E. coli* (STEC) *stx1/ stx2*	Para-Pak C&S	12/13‡§	92.3	66.7 - 98.6	51/52‡	98.1	89.9-99.7

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

† 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.

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

§ 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 22. 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	98.1-100.0
	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	92.8-99.8
	Para-Pak C&S	27/29	93.1	78.0-98.1	86/86 [d]	100.0	95.7-100.0
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

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Table 22 (continued from the previous page)

		Positive Percent Agreement			Negative Percent Agreement		
	Medium Brand	TP/TP+FN	%	95% CI	TN/TN+FP	%	95% CI
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	74.2-97.7	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	61-100.0	186/188 [p]	98.9	96.2-99.7
	Para-Pak C&S	26/26	100.0	87.1-100.0	117/117	100.0	96.8-100.0
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*/Enteroinvasive *E. coli* (EIEC) was detected in one of the two false negative specimens (1/2) in FecalSwab using a different FDA-cleared test method.

[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 23. The error breakdown due to instrument, invalid results, 'sample too concentrated' failures and other run failures are summarized in Table 24.

Table 23. 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 C&S	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 24. 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*	0/1227	0.0	0/1227	0.0
		Sample too Concentrated†	5/1227	0.4	0/1227	0.0
		Other‡	11/1227	0.9	3/1227	0.2
	Prospective Archived	Instrument	0/53	0.0	0/53	0.0
		Invalid	0/53	0.0	0/53	0.0
		Sample too Concentrated	0/53	0.0	0/53	0.0
		Other	0/53	0.0	0/53	0.0
	Retrospective	Instrument	1/366	0.3	0/366	0.0
		Invalid	1/366	0.3	0/366	0.0
		Sample too Concentrated	0/366	0.0	0/366	0.0
		Other	9/366	2.5	5/366	1.4
Para-Pak C&S	Prospective	Instrument	9/740	1.2	2/740	0.3
		Invalid	5/740	0.7	5/740	0.7
		Sample too Concentrated	35/740	4.7	7/740	0.9
		Other	17/740	2.3	7/740	0.9
	Prospective Archived	Instrument	0/66	0.0	0/66	0.0
		Invalid	1/66	1.5	0/66	0.0
		Sample too Concentrated	1/66	1.5	0/66	0.0
		Other	1/66	1.5	0/66	0.0
	Retrospective	Instrument	1/454	0.2	0/454	0.0
		Invalid	10/454	2.2	6/454	1.3
		Sample too Concentrated	10/454	2.2	2/454	0.4
		Other	25/454	5.5	17/454	3.7

* Internal Control failures with at least one analyte detected and the other analytes reported as 'invalid'

† Run failures related to 'sample concentration too high'. These specimens were repeated with 100 microliters as detailed in Appendix 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 25 and Table 26 below.

Table 25. Most Prevalent Multiple Detection Combinations (≥2 instances) as Determined by the QIAstat-Dx Gastrointestinal Panel 2 in the Prospective Clinical study in FecalSwab specimens.

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

Table 26. Most Prevalent Multiple Detection Combinations (≥2 instances) as Determined by the QIAstat-Dx Gastrointestinal Panel 2 in the Prospective Clinical study in Para-Pak C&S specimens.

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

The analytes most commonly found in mixed infections in the FecalSwab specimens were *Campylobacter* (9), Norovirus GI/GII (7), Rotavirus (4) and ETEC (3) as shown in Table 27, 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 28.

Table 27. 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 28. 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 retrospective testing efforts were insufficient to demonstrate system performance. To supplement the prospective and retrospective 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 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 29 below.

Table 29. Test Results Summary for Contrived Specimens.

QIAstat-Dx GI2 Target		Positive Percent Agreement (PPA)		
	Medium Brand	Fraction	Percentage	95% CI
Astrovirus	FecalSwab	33/34	97.1	85.1-99.5
	Para-Pak C&S	34/34	100.0	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

Troubleshooting

This troubleshooting guide may be helpful in solving any problems that may arise. For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/support** (for contact information, visit **www.qiagen.com**).

Additional information about specific QIAstat-Dx Gastrointestinal Panel 2 error codes and messages can be found in Table 30:

Table 30. Information about specific QIAstat-Dx Gastrointestinal Panel 2 error codes and messages

Error Code	Error message displayed
0x02C9 **0x032D** **0x0459** **0x045A** **0x04BF** **0x0524** **0x058B** **0x05E9** **0x0778** **0x077D** **0x14023**	Cartridge execution failure: Sample concentration too high. Please repeat by loading 100 microliters of the sample in a new cartridge (as per Appendix C explanation)

When the sample concentration is too high and the test must be repeated by loading 100 µL, follow the workflow detailed in the Appendix C of this document.

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 800-426-8157, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Symbols

The following symbols may appear in the instructions for use or on the packaging and labelling:

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
	For in vitro diagnostic use
	Prescription Use Only
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Gastrointestinal application
Rn	R is for revision of the Instruction for use and n is the revision number
	Temperature limitation
	Consult instructions for use
	Caution
	Serial number
	Do not reuse
	Keep away from sunlight
	Do not use if package is damaged

GTIN	Global Trade Item Number
	Flammable, risk of fire
	Corrosive, risk of chemical burn
	Health hazard, risk of sensitization, carcinogenicity
	Risk of harm

Appendices

Appendix A: Installing the Assay Definition File

The Assay Definition File (ADF) of the QIAstat-Dx Gastrointestinal Panel 2 must be installed on the QIAstat-Dx Analyzer 1.0 prior to testing with QIAstat-Dx Gastrointestinal Panel 2 Cartridges.

Note: Whenever a new version of the QIAstat-Dx Gastrointestinal Panel 2 assay is released, the new QIAstat-Dx Gastrointestinal Panel 2 Assay Definition File must be installed prior to testing.

The Assay Definition File (**.asy** file type) is available at **www.qiagen.com**. The Assay Definition file (.asy file type) must be saved onto a USB Drive prior to installation on the QIAstat-Dx Analyzer 1.0. This USB Drive must be formatted with a FAT32 file system.

To import an ADF from the USB to the QIAstat-Dx Analyzer 1.0, proceed with the following steps:

1. Insert the USB stick containing the Assay Definition File into one of the USB ports on the QIAstat-Dx Analyzer 1.0.
2. Press the **Options** button and then select **Assay Management**. The Assay Management screen appears in the Content area of the display (Figure 32).

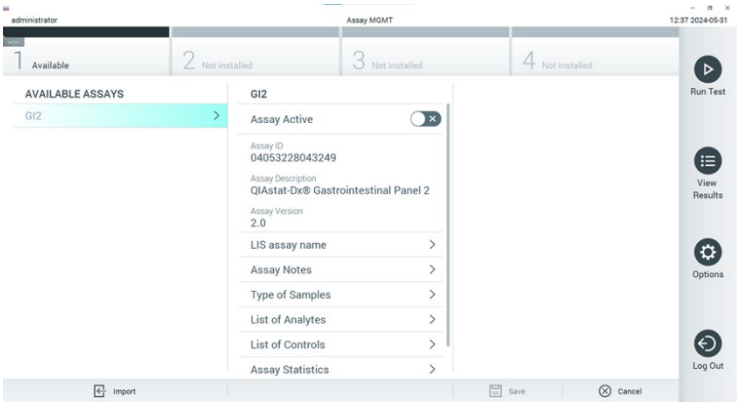

Figure 32. Assay Management screen.

3. Press the **Import** icon in the bottom left of the screen (Figure 32).
4. Select the file corresponding to the assay to be imported from the USB drive.
   A dialog will appear to confirm upload of the file.
5. A dialog may appear to override the current version by a new one. Press yes to override (Figure 33).

Figure 33. Dialog that appears when upgrading the ADF version.

6. The assay becomes active by selecting **Assay Active** (Figure 34).

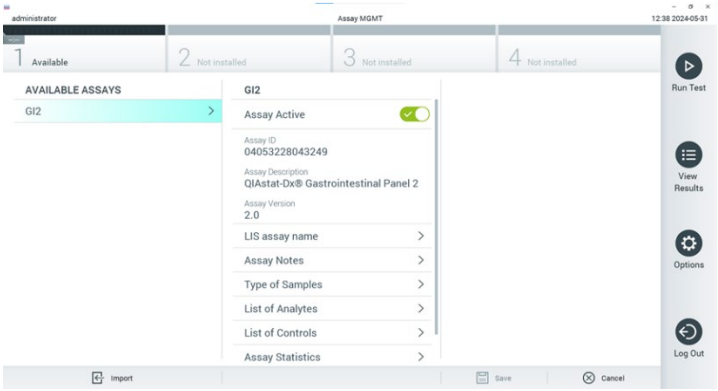

Figure 34. Activating the assay.

7. To assign the active assay to a user, perform these steps:

   7a. Go to **Options** > **User Management**.

   7b. Select the user who should be allowed to run the assay.

   Note: If needed, this step can be repeated for every user created in the system.

   7c. Select **Assign Assays** from the User Options tab.

   7d. Enable the assay, then press **Save** (Figure 35).

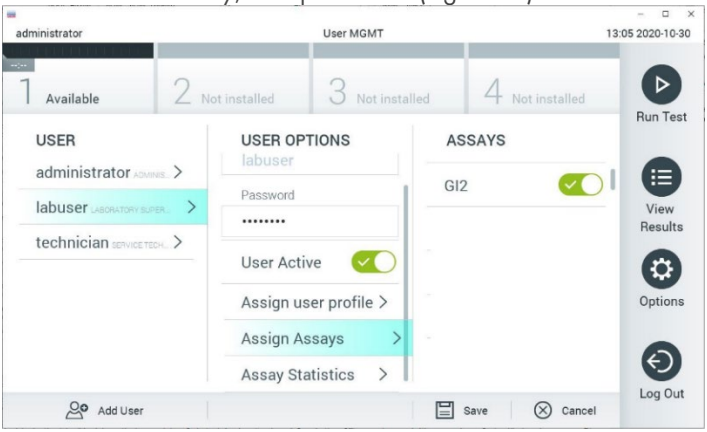

Figure 35. Assigning the active assay.

Appendix B: Glossary

Amplification curve: Graphical representation of the multiplex real-time RT-PCR amplification data.

Analytical Module (AM): The main QIAstat-Dx Analyzer 1.0 hardware module, in charge of executing tests on QIAstat-Dx Gastrointestinal Panel 2 Cartridges. It is controlled by the Operational Module. Several Analytical Modules can be connected to one Operational Module.

IFU: Instructions For Use.

Main port: In the QIAstat-Dx Gastrointestinal Panel 2 Cartridge, inlet for transport medium liquid samples.

Nucleic acids: Biopolymers, or small biomolecules composed of nucleotides, which are monomers made of three components: a 5-carbon sugar, a phosphate group and a nitrogenous base.

Operational Module (OM): The dedicated QIAstat-Dx Analyzer 1.0 hardware that provides the user interface for 1–4 Analytical Modules (AM).

PCR: Polymerase Chain Reaction.

QIAstat-Dx Analyzer 1.0: The QIAstat-Dx Analyzer 1.0 consists of an Operational Module and an Analytical Module. The Operational Module includes elements that provide connectivity to the Analytical Module and enables user interaction with the QIAstat-Dx Analyzer 1.0. The Analytical Module contains the hardware and software for sample testing and analysis.

QIAstat-Dx Gastrointestinal Panel 2 Cartridge: A self-contained disposable plastic device with all pre-loaded reagents required for the complete execution of fully automated molecular assays for the detection of gastrointestinal pathogens.

RT: Reverse Transcription.

Swab port: In the QIAstat-Dx Gastrointestinal Panel 2 Cartridge, inlet for dry swabs. The swab port is not used for the QIAstat-Dx Gastrointestinal Panel 2 assay.

User: A person who operates the QIAstat-Dx Analyzer 1.0/QIAstat-Dx Gastrointestinal Panel 2 Cartridge in the intended way.

Appendix C: Additional Instructions for use

In case cartridge execution failures corresponding to error codes (0x02C9, 0x032D, 0x0459, 0x045A, 0x04BF, 0x0524, 0x058B, 0x05E9, 0x0778, 0x077D, 0x14023) occur during the testing, the following error message will be displayed in the QIAstat-Dx Analyzer 1.0 screen after the run has finalized:

Cartridge execution failure: Sample concentration too high. Please repeat by loading 100 microliters of the sample in a new cartridge (as per IFU explanation)'.

In this case the test should be repeated using 100 µL of the same sample following equivalent testing procedures detailed in the "Procedure" Section in the IFU adapted to 100 µL sample input volume:

1. Open the package of a new QIAstat-Dx Gastrointestinal Panel 2 Cartridge using the tear notches on the sides of the packaging.
2. Remove the QIAstat-Dx Gastrointestinal Panel 2 Cartridge from the packaging.
3. Manually write the sample information, or place a sample information label, on the top of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge. Make sure that the label is properly positioned and does not block the lid opening.
4. Place the QIAstat-Dx Gastrointestinal Panel 2 Cartridge flat on the clean work surface so that the barcode on the label faces upwards. Open the sample lid of the main port on the front of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge.
5. Thoroughly mix the stool in the transport medium, for example, by vigorously agitating the tube 3 times.
6. Open the tube with the sample to be tested. Use the supplied transfer pipette to draw up fluid. Draw the sample to the first fill line on the pipette (i.e., 100 µL).
   IMPORTANT: Do not draw air, mucus, or particles into the pipette. If air, mucus, or particles are drawn into the pipette, carefully expel the sample fluid in the pipette back into the sample tube and draw up fluid again.
7. Carefully transfer the sample into the main port of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge using the supplied single-use transfer pipette (Figure 7).
8. Firmly close the lid of the main port until it clicks (Figure 8).

From this point proceed following the instructions described in the IFU.

Ordering Information

Product	Contents	Cat. no.
QIAstat-Dx Gastrointestinal Panel 2	For 6 tests: 6 individually packaged QIAstat-Dx Gastrointestinal Panel 2 Cartridges and 6 individually packaged transfer pipettes	691421
Relative Products		
QIAstat-Dx Analyzer 1.0	1 QIAstat-Dx Analytical Module, 1 QIAstat-Dx Operational Module and related hardware and software to run molecular diagnostic QIAstat-Dx assay cartridges	9002824

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit IFU or user manual. QIAGEN kit IFU and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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Document Revision History

Revision	Description
Revision 1 06/2024	Initial release

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