October 2025

QIAstat-Dx® GI Panel 2 Mini B&V Instructions for Use

Σ 6

Version 1

IVD

For In Vitro Diagnostic Use

Rx Only For prescription use only

For use with QIAstat-Dx Analyzer 2.0

QIAGEN, GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

REF 691424

R3

Sample to Insight

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Intended Use

The QIAstat-Dx® GI Panel 2 Mini B&V is a multiplexed nucleic acid test intended for use with the QIAstat-Dx Analyzer 2.0 for the simultaneous in vitro qualitative detection and identification of nucleic acids from multiple bacteria and one virus directly from preserved stool samples (Para-Pak® C&S or FecalSwab™) obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following virus and bacteria (including several diarrheagenic *E. coli/Shigella* pathotypes) are identified with the QIAstat-Dx GI Panel 2 Mini B&V:

- Norovirus
- *Campylobacter*
- *Shigella*
- Shiga-like toxin *E. coli* (STEC)
- *Salmonella*

Concomitant culture is necessary for organism recovery and further typing of bacterial agents. The QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V. The organisms detected may not be the sole or definitive cause of the disease.

Negative QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge allows detection and differentiation of 5 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 GI Panel 2 Mini B&V are listed in Table 1.

Table 1. Pathogens detected by the QIAstat-Dx GI Panel 2 Mini B&V

Pathogen	Classification (genome type)
Norovirus	Calicivirus (RNA)
Campylobacter	Bacterium (DNA)
Shigella	Bacterium (DNA)
Salmonella	Bacterium (DNA)
Shiga-like toxin *E. coli* (STEC)	Bacterium (DNA)

Summary of detected organisms

Bacteria

Campylobacter* spp. *(Campylobacter jejuni/ Campylobacter coli/ Campylobacter upsaliensis) is a genus of gram-negative bacteria that includes more than 30 species 6. *Campylobacter jejuni* and *Campylobacter coli* are the most common *Campylobacter* species associated with diarrheal illness, with *C. jejuni* being responsible for 90% of cases (7,8). The consumption of undercooked poultry or raw milk is the most common source of *Campylobacter* infections (9,10). *Campylobacter* are highly infectious, with an infectious dose as low as 500 bacteria (11); however, person-to-person spread is uncommon(9). Systemic disease, associated with significant morbidity and mortality, may occur in individuals who are immunocompromised (9,11). Infection can result in long-term consequences such as arthritis, irritable bowel syndrome, and Guillain–Barré syndrome (9,11).

Salmonella is a gram-negative bacterium comprising more than 2600 serovars, including the distinct typhoidal serotypes, Typhi and Paratyphi A–C (12,13). Enteric (typhoid) fever is an invasive, life-threatening, systemic infection with predominantly non-gastrointestinal symptoms (12,14). 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 (12,14,15). Less common, non-typhoidal *Salmonella* serovars cause invasive disease due to bloodstream infections that are not usually associated with diarrhea (12,14). There are 100–200 million cases of non-typhoidal salmonellosis each year, resulting in approximately 85,000–155,000 deaths (14,16). The incidence of non-typhoidal *Salmonella* gastroenteritis is highest in the developing world but is also of considerable importance in developed countries (12).

Diarrheagenic *Escherichia 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* contain several pathotypes that cause a variety of

diseases (17,18). *Escherichia coli/Shigella* have a conserved core genome and a flexible gene pool containing virulence and fitness genes, which are carried on mobile genetic elements (17,18). Gene gain, via horizontal transfer, and gene loss afford the pathogenic traits to *E. coli/Shigella* that give rise to the different pathotypes (18).

Shigella is the second leading cause of diarrhea mortality, causing approximately 13% diarrhea deaths (19). The numbers of deaths are greatest in young children and the elderly (19). It is recommended that individuals with shigellosis should not take anti-diarrheal medications such as loperamide, as these can make symptoms worse (20). Enteroinvasive *E. coli* (EIEC) is an invasive strain of *E. coli* that is very closely related to *Shigella* in virulence and other pathogenic properties (21,22). EIEC is under-represented in epidemiological studies due to its less severe manifestation and potential misclassification as *Shigella* (18). EIEC infection often leads only to self-limiting, mild watery diarrhea; in rare situations, it can cause symptoms of shigellosis, but complications are uncommon (18).

Shiga-like toxin- *E. coli* (STEC) is defined by the production of Shiga toxin 1 (Stx1) or 2 (Stx2), which shows homology to Stx toxins from *Shigella dysenteriae* (18). There are >400 serotypes of STEC, of which O157:H7 is the most common (18). 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 (18,23). Approximately 5–10% of individuals diagnosed with STEC infections develop HUS, which can be a life-threatening complication (24). The impacts of STEC are often greater in infants and children, compared to other ages (23). Antibiotics should not be used to treat STEC infections as there is currently no evidence that they aid in recovery and have instead been associated with worsening of symptoms and the development of HUS (24).

Viruses

Noroviruses are small, non-enveloped, positive-stranded RNA viruses from the family Caliciviridae (25). They are responsible for >90% of viral gastroenteritis and around 50% of

all-cause gastroenteritis outbreaks globally (26), causing approximately 685 million cases every year (27). Approximately 200 million cases are in children aged <5 years, leading to 50,000 child deaths (27). 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 (27). Norovirus is infectious at very low doses and is transmitted via aerosolized droplets and by touching contaminated surfaces (27). Individuals infected with norovirus usually recover within 1–3 days. Infections in infants, older adults, and immunocompromised individuals can be severe and sometimes fatal (27). In some individuals, viral shedding can occur for many weeks or months after symptoms stop, a large contributing factor for outbreaks (28).

QIAstat-Dx GI Panel 2 Mini B&V Cartridge description

The QIAstat-Dx GI Panel 2 Mini B&V Cartridge (Figure 1) is a disposable plastic device that allows performance of fully automated molecular assays for the detection of gastrointestinal pathogens. The main features of the QIAstat-Dx GI Panel 2 Mini B&V Cartridge include compatibility with a liquid sample type, which is hermetical containment of pre-loaded reagents necessary for testing, and 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 GI Panel 2 Mini B&V Cartridge. The user does not need to come in contact with nor manipulate any reagents. The QIAstat-Dx Analyzer 2.0 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 the sample is manually loaded, the diagnostic tests within the QIAstat-Dx GI Panel 2 Mini B&V are performed on the QIAstat-Dx Analyzer 2.0. All of the sample preparation and analysis steps are performed automatically by the QIAstat-Dx Analyzer 2.0.

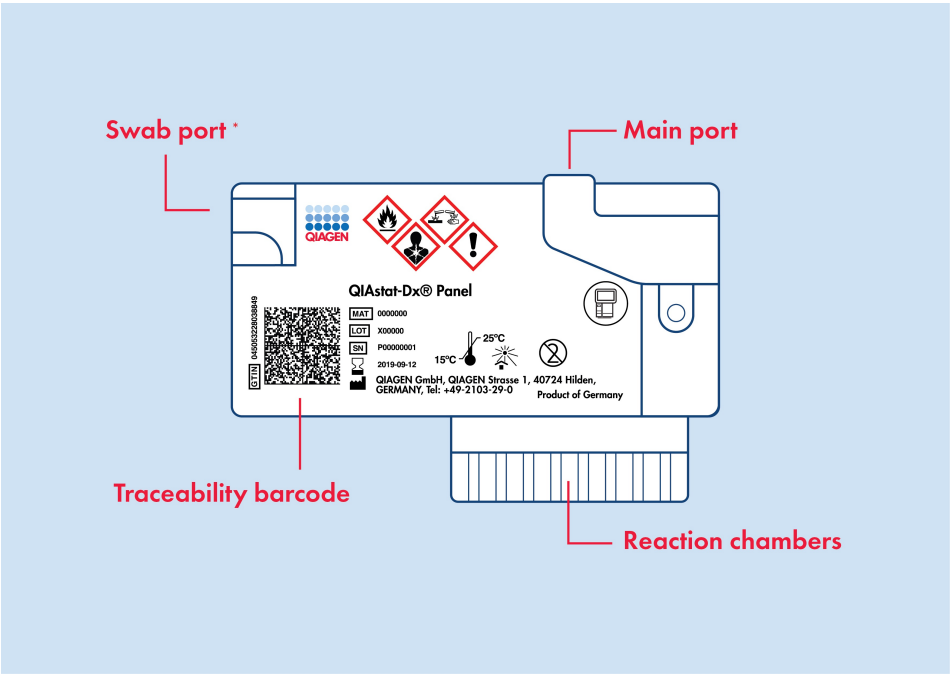

Figure 1. Layout of the QIAstat-Dx Cartridge and its features. *Note: The swab port is not used for the QIAstat-Dx GI Panel 2 Mini B&V.

Sample collection and cartridge loading

The collection of samples and their subsequent loading into the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge.
3. Liquid sample (stool resuspended in Para-Pak C&S or FecalSwab transport medium) is loaded manually into the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge barcode are scanned by the QIAstat-Dx Analyzer 2.0. If the sample barcode is not available, the sample ID is manually written using the virtual keyboard of the touchscreen.
5. The QIAstat-Dx GI Panel 2 Mini B&V Cartridge is introduced into the QIAstat-Dx Analyzer 2.0.
6. The test is started on the QIAstat-Dx Analyzer 2.0.

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 2.0.

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 GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge.
6. The mixture of sample and PCR reagents is dispensed into the QIAstat-Dx GI Panel 2 Mini B&V Cartridge PCR chambers, which contain air-dried, assay-specific primers and probes.
7. The QIAstat-Dx Analyzer 2.0 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 2.0 Software interprets the resulting data and process controls and delivers a test report.

Materials Provided

Kit contents

QIAstat-Dx GI Panel 2 Mini B&V Cartridge **Catalog number** **Number of tests**	 **691424** **6**
QIAstat-Dx GI Panel 2 Mini B&V Cartridges	6*
Transfer pipettes	6†
QIAstat-Dx GI Panel 2 Mini B&V Product information Card	1

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

† Six (6) individually packaged transfer pipettes for dispensing liquid sample into the QIAstat-Dx GI Panel 2 Mini B&V Cartridge.

Materials Required but Not Provided

Platform and software

The QIAstat-Dx GI Panel 2 Mini B&V is designed for use with the QIAstat-Dx Analyzer 2.0. Before beginning a test, make sure the following are available:

- QIAstat-Dx Analyzer 2.0 (at least one Operational Module PRO and one Analytical Module) with software version 1.6 or later
- *QIAstat-Dx Analyzer 2.0 User Manual* (for use with software version 1.6 or later)
- QIAstat-Dx latest Assay Definition File software for QIAstat-Dx GI Panel 2 Mini B&V installed in the Operational Module PRO

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

Warnings and Precautions

- The QIAstat-Dx GI Panel 2 Mini B&V is for in vitro diagnostic use.
- For prescription use only.
- The QIAstat-Dx GI Panel 2 Mini B&V is to be used by laboratory professionals trained in the use of QIAstat-Dx Analyzer 2.0.
- 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 GI Panel 2 Mini B&V 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 suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient PDF 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 (29).
    - 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 GI Panel 2 Mini B&V 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 2.0. Do not use a QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V.

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 (29).

- 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 GI Panel 2 Mini B&V 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 QIAstat-Dx GI Panel 2 Mini B&V are:

- *Campylobacter* spp.
- Certain *E. coli*
    - Shiga-like toxin *E. coli* (STEC)
- *Salmonella* spp.
- *Salmonella enterica* serotypes Paratyphi (A, B [tartrate negative], and C [S. Paratyphi])
- *Salmonella enterica* serotype Typhi
- *Shigella* spp.

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 in positive specimens to their state public health laboratories.

Reagent Storage and Handling

Store the QIAstat-Dx GI Panel 2 Mini B&V Cartridges in a dry, clean storage space at room temperature (15–25°C). Do not remove the QIAstat-Dx GI Panel 2 Mini B&V Cartridges or the transfer pipettes from their individual packaging until actual use. Under these conditions, QIAstat-Dx GI Panel 2 Mini B&V Cartridges can be stored until the expiration date printed on the individual packaging. The expiration date is also included in the QIAstat-Dx GI Panel 2 Mini B&V Cartridge barcode and is read by the QIAstat-Dx Analyzer 2.0 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 the 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 GI Panel 2 Mini B&V is stable until the stated expiration date on the box label.

After the cartridge package is opened, the sample should be introduced into the QIAstat-Dx GI Panel 2 Mini B&V Cartridge within 30 minutes. Sample-loaded cartridges should be loaded into the QIAstat-Dx Analyzer 2.0 within 90 minutes.

Specimen Storage and Handling

The QIAstat-Dx GI Panel 2 Mini B&V 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 points before starting

- Ensure all materials required but not provided are available.
- The QIAstat-Dx GI Panel 2 Mini B&V Cartridge (cat. no 691424) is identified by a light purple-colored ( )bar on the label and an icon indicating gastrointestinal tract (, see "Symbols" on page 98).

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 GI Panel 2 Mini B&V Cartridge

1. Open the package of a QIAstat-Dx GI Panel 2 Mini B&V Cartridge using the tear notches on the sides of the packaging (Figure 2).

   Important: After opening the package, sample should be introduced into the QIAstat-Dx GI Panel 2 Mini B&V Cartridge within 30 minutes. Sample-loaded cartridges should be loaded into the QIAstat-Dx Analyzer 2.0 within 90 minutes.

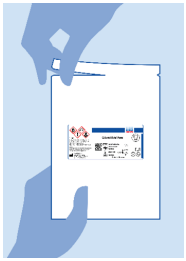

Figure 2. Opening the QIAstat-Dx GI Panel 2 Mini B&V Cartridge.

2. Remove the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge. Make sure that the label is properly positioned and does not block the lid opening (Figure 3).

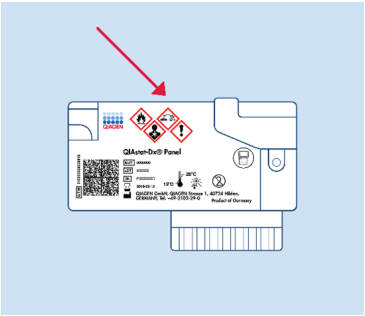

Figure 3. Sample information placement on top of QIAstat-Dx GI Panel 2 Mini B&V Cartridge.

4. Place the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge (Figure 4).

Important: Do not flip the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge if it is agitated while the lid is open.

Note: The swab port is not used for the QIAstat-Dx GI Panel 2 Mini B&V assay.

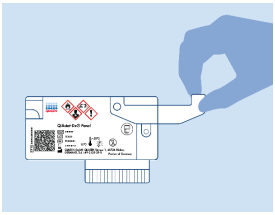

Figure 4. Opening the sample lid of main port.

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

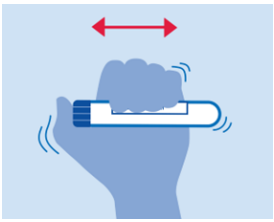

Figure 5. Mixing stool sample in transport medium.

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 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 the Troubleshooting section for further details on error codes and "Appendix C: Additional instructions for use" on page 106 for further instructions on repeating a sample with 100 µL).

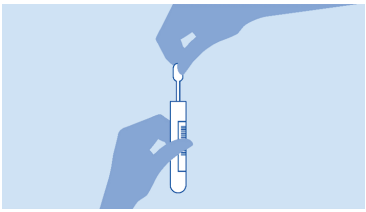

Figure 6. Drawing up sample into the supplied transfer pipette.

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

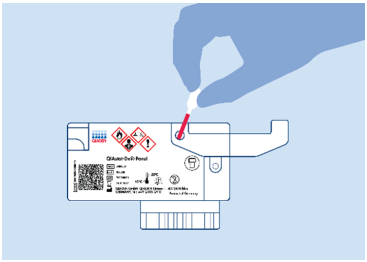

Figure 7. Transferring sample to main port of QIAstat-Dx GI Panel 2 Mini B&V Cartridge.

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

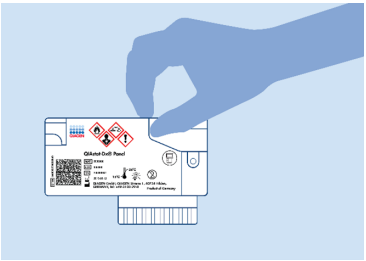

Figure 8. Closing the lid of the main port.

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

   Important: After the sample is placed inside the QIAstat-Dx GI Panel 2 Mini B&V Cartridge, the cartridge must be loaded into the QIAstat-Dx Analyzer 2.0 within 90 minutes.

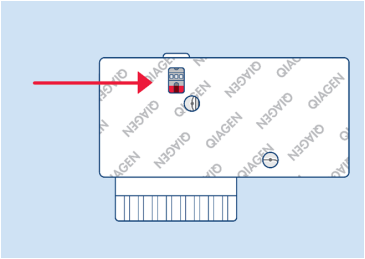

Figure 9. Sample inspection window (red arrow).

Running a test with a QIAstat-Dx Analyzer

1. Power on the QIAstat-Dx Analyzer 2.0 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 2.0 status indicators will turn blue.

2. Wait until the Main screen appears and the QIAstat-Dx Analyzer 2.0 status indicators turn green and stop blinking.
3. Enter your username and password for QIAstat-Dx Analyzer 2.0 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 2.0, follow the installation instructions prior to running the test (see "Appendix A: Installing the Assay Definition File" on page 101 for additional information).
5. Press **Run Test** in the top right corner of the touchscreen of the QIAstat-Dx Analyzer 2.0.
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 GI Panel 2 Mini B&V Cartridge (Figure 3) using the integrated front barcode reader of the QIAstat-Dx Analyzer 2.0 (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 selected system configuration, entering the patient ID may also be required at this point.

   Note: Instructions from the QIAstat-Dx Analyzer 2.0 appear in the Instructions Bar at the bottom of the touchscreen.

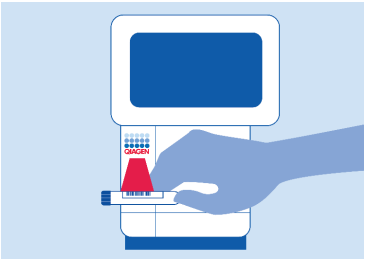

Figure 10. Scanning sample ID barcode.

7. When prompted, scan the barcode of the QIAstat-Dx GI Panel 2 Mini B&V Cartridge to be used (Figure 11). The QIAstat-Dx Analyzer 2.0 will automatically recognize the assay to be run based on the cartridge barcode.

   Note: The QIAstat-Dx Analyzer 2.0 will not accept QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge will be rejected. Refer to the *QIAstat-Dx Analyzer 2.0 User Manual* or "Appendix A: Installing the Assay Definition File" on page 101 for further details on how to install assays.

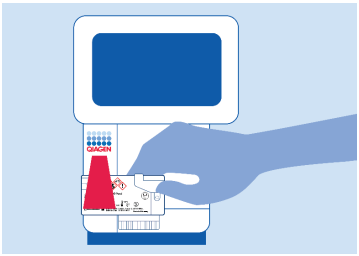

Figure 11. Scanning QIAstat-Dx GI Panel 2 Mini B&V Cartridge barcode.

8. 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.

9. 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 12).

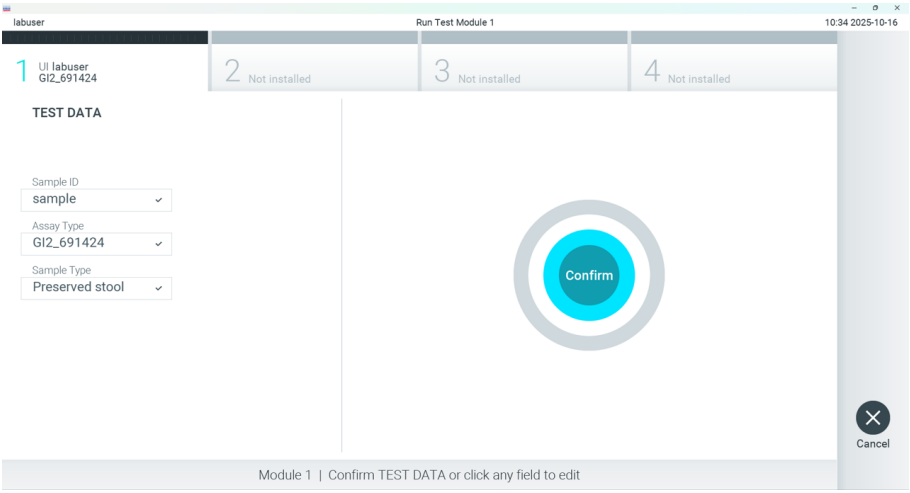

Figure 12. Confirming data entry.

10. Ensure that both sample lids of the swab port and main port of the QIAstat-Dx GI Panel 2 Mini B&V Cartridge are firmly closed.

11. When the cartridge entrance port on the top of the QIAstat-Dx Analyzer 2.0 automatically opens, insert the QIAstat-Dx GI Panel 2 Mini B&V Cartridge with the barcode facing to the left and the reaction chambers facing down (Figure 13).

    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 at the bottom right corner of the touchscreen.

12. Upon detecting the QIAstat-Dx GI Panel 2 Mini B&V Cartridge, the QIAstat-Dx Analyzer 2.0 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 GI Panel 2 Mini B&V Cartridge into the QIAstat-Dx Analyzer 2.0.

    Note: The QIAstat-Dx Analyzer 2.0 will not accept a QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge is not positioned in the port. If this occurs, repeat the procedure starting with step 5.

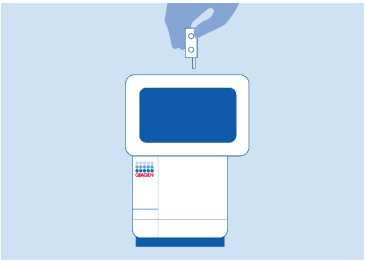

Figure 13. Inserting QIAstat-Dx GI Panel 2 Mini B&V Cartridge into the QIAstat-Dx Analyzer 2.0.

13. While the test is running, the remaining run time is displayed on the touchscreen.
14. After the test run is completed, the Eject screen will appear (Figure 14) 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 2.0 User Manual* for possible reasons and instructions on how to proceed. For additional information about specific QIAstat-Dx GI Panel 2 Mini B&V error codes and messages, please see the Troubleshooting Guide section of this document.

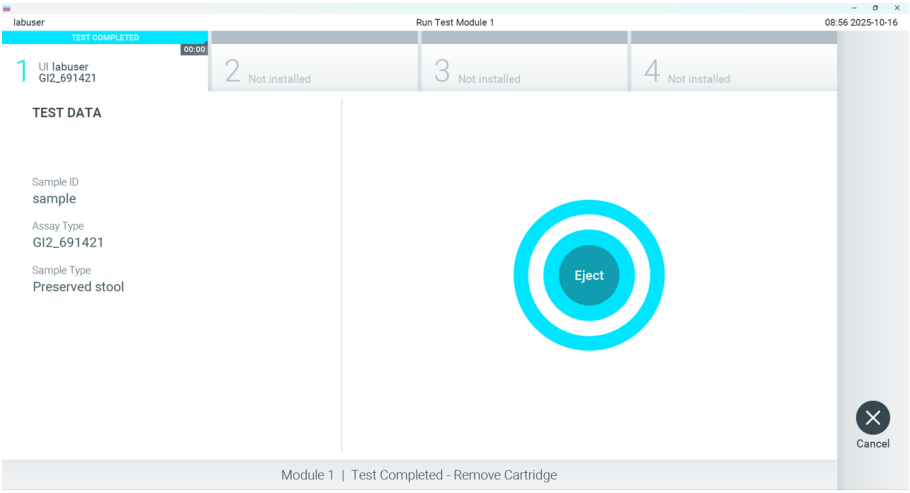

Figure 14. Eject screen display.

15. Press  **Eject** on the touchscreen to remove the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V 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 2.0 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 GI Panel 2 Mini B&V 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.

16. After the QIAstat-Dx GI Panel 2 Mini B&V Cartridge has been ejected, the results Summary screen will appear. Refer to "Interpretation of Results" on the facing page 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 2.0, refer to the *QIAstat-Dx Analyzer 2.0 User Manual*.

Interpretation of Results

Viewing results

The QIAstat-Dx Analyzer 2.0 automatically interprets and saves test results. After ejecting the QIAstat-Dx GI Panel 2 Mini B&V Cartridge, the results Summary screen is automatically displayed (Figure 15).

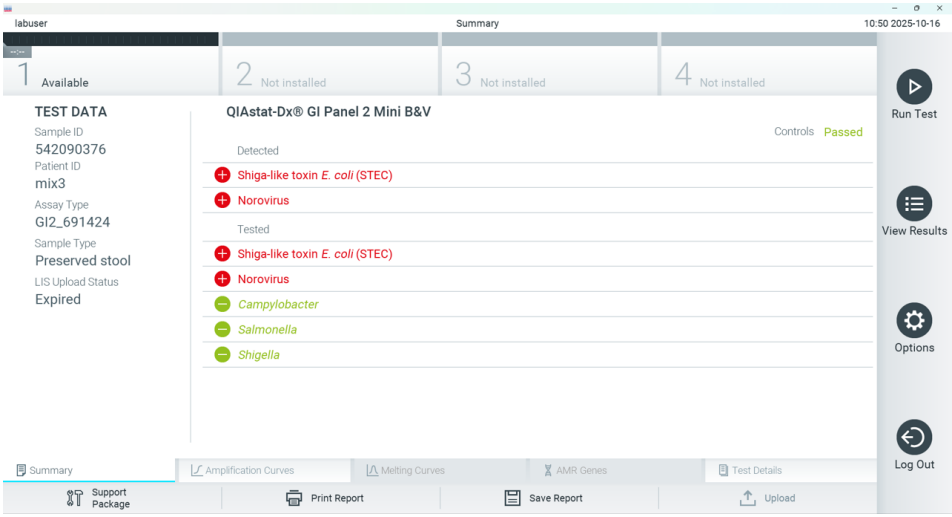

Figure 15. Results Summary screen example showing Test Data on the left panel and Test Summary in the main panel.

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 are 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 drive. Insert the USB drive into one of the USB ports of the QIAstat-Dx Analyzer 2.0 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 16).

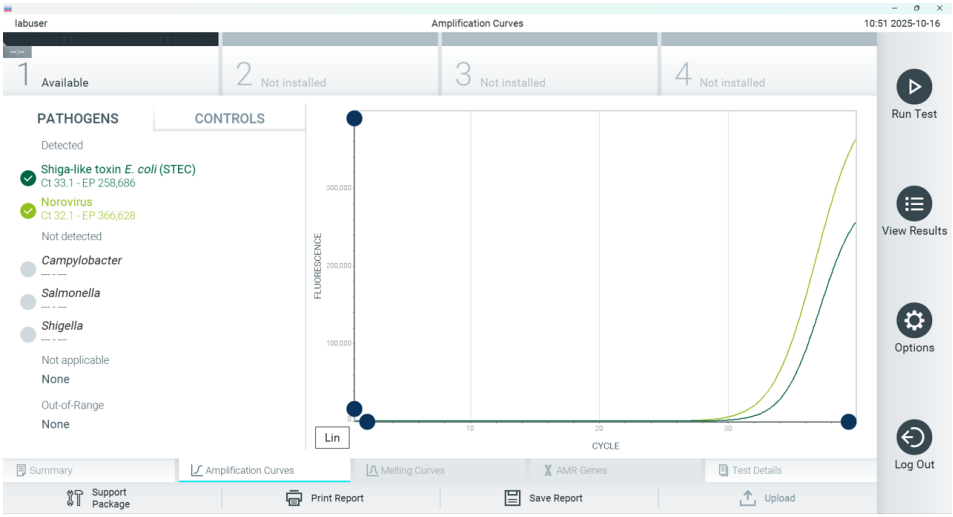

Figure 16. Amplification Curves screen (PATHOGENS tab).

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 2.0, 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 Ct 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 17).

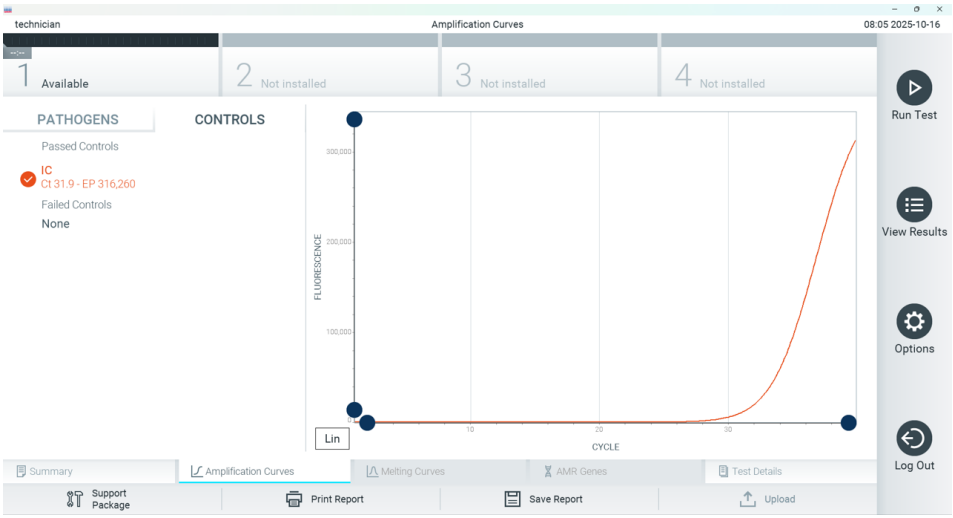

Figure 17. Amplification Curves screen (CONTROLS tab).

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 18):

- 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 Ct and endpoint fluorescence in the event of a positive signal
- Internal Control, with Ct and endpoint fluorescence

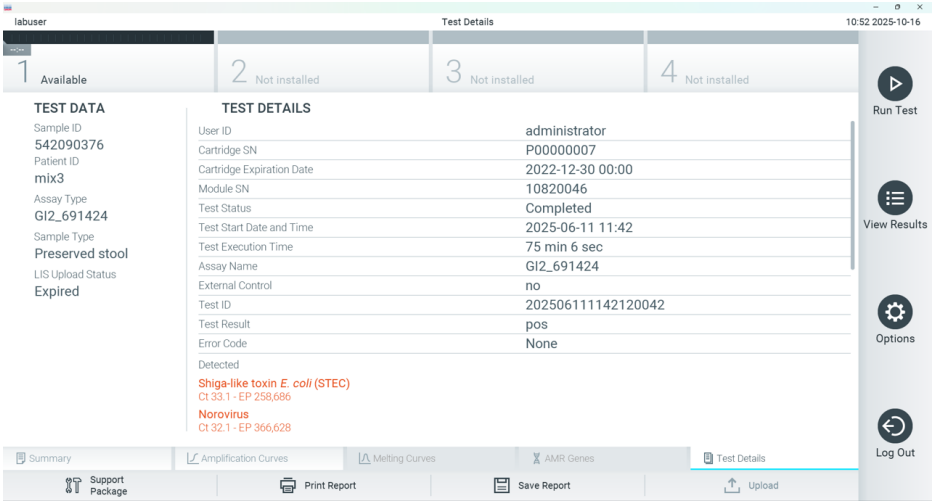

Figure 18. Example screen showing Test Data on the left panel and Test Details in the main panel.

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 19).

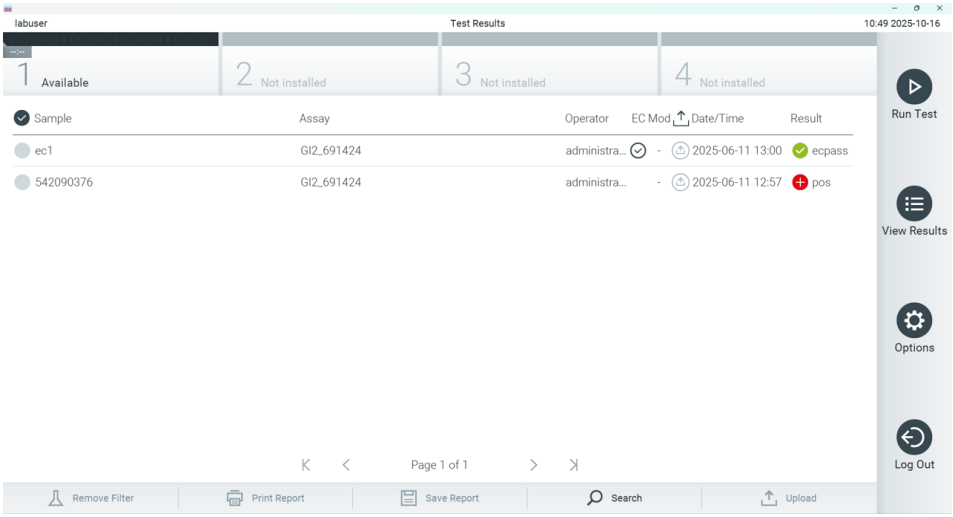

Figure 19. Example View Results screen.

The following information is available for every executed test (Figure 20):

- Sample ID
- Assay (name of test assay which is “GI2 Mini B&V” for QIAstat-Dx GI Panel 2 Mini B&V)
- 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 2.0, 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 the 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 20).

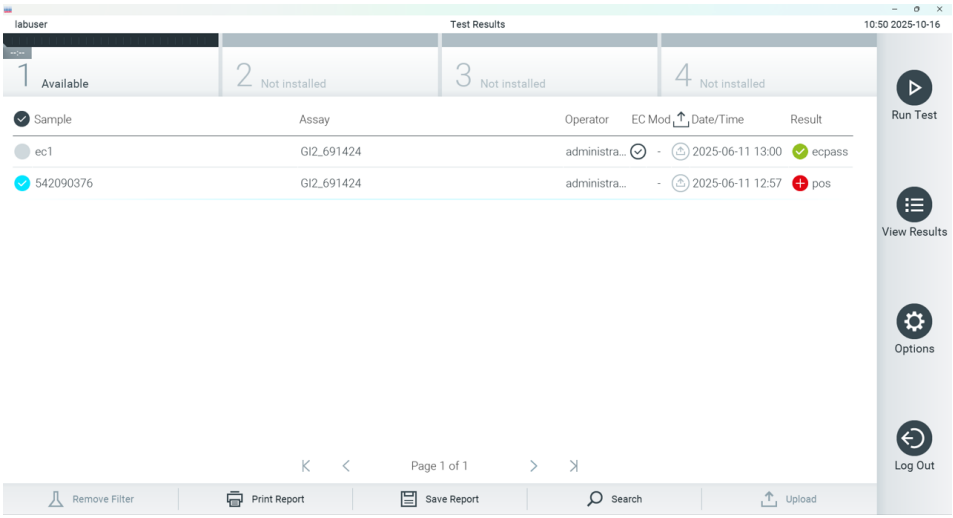

Figure 20. Example of selecting Test Results in the View Results screen.

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 the test results displayed in 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 4.
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 4.
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 4.
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 2.0, 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 to an external USB drive.

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 searched 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 box 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  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 to a USB drive. The USB port is located on the front of the QIAstat-Dx Analyzer 2.0.

Backup and regular data upload to SharePoint

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

1. Press **Options** > **System Configuration** > **System Backup** (Figure 21). Insert a USB drive into the front USB port.
2. Press **Perform Backup**. A file with the extension **.dbk** will be generated in the USB drive with a default file name.

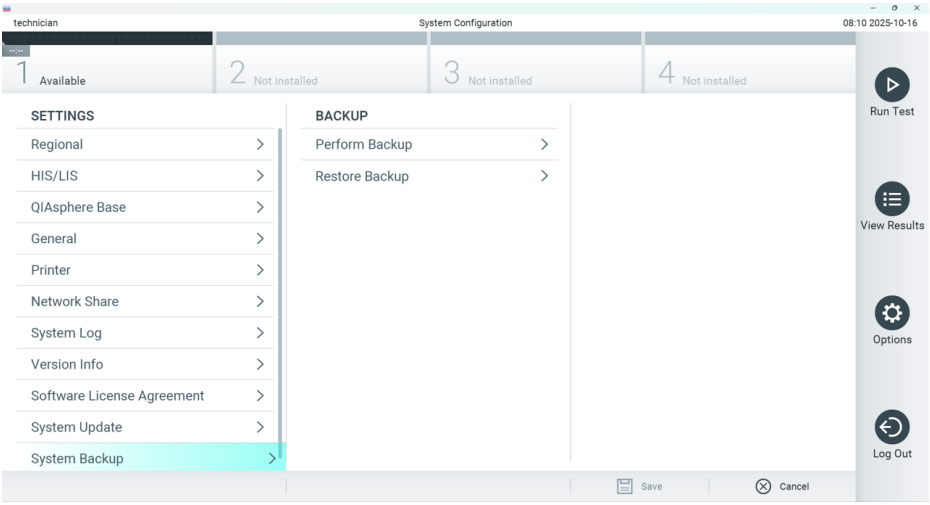

Figure 21. Perform a backup.

Printing results

Make sure a printer is connected to the QIAstat-Dx Analyzer 2.0 and the proper driver is installed. Press **Print Report** to send a copy of the PDF test results to the printer.

Sample result interpretation

Note: Due to the genetic similarity between *Shigella* and Enteroinvasive *E. coli*, the QIAstat-Dx GI Panel 2 Mini B&V cannot differentiate them (see the Limitations section). Both organisms will be detected and reported as *Shigella*.

A result for gastrointestinal organism is interpreted as "Positive" when the corresponding PCR assay is positive.

Internal control results are to be interpreted according to Table 3.

Table 3. 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 4). 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 4. 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	 !pos*	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.
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 GI Panel 2 Mini B&V Cartridge includes a full process Internal Control, which is tittered *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 GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V. 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 GI Panel 2 Mini B&V is intended for professional use only and is not intended for self-testing. The QIAstat-Dx GI Panel 2 Mini B&V is intended for in vitro diagnostic use.
- Results from the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V is not intended for testing of samples other than those described in this 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 GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V. 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 GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V can be used only with the QIAstat-Dx Analyzer 2.0.
- 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 (18). The QIAstat-Dx GI Panel 2 Mini B&V 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 (18); therefore, "Detected" results for multiple diarrheagenic *E. coli/Shigella* may be due to co-infection with multiple pathotypes.
- Due to the genetic similarity between *Shigella* and Enteroinvasive *E. coli*, the QIAstat-Dx GI Panel 2 Mini B&V cannot differentiate them and will report both as *Shigella*.
- *Shigella dysenteriae* serotype 1 possess a shiga toxin gene (*stx*) that is identical to the *stx1* gene of STEC (18). More recently, *stx* genes have been found in other *Shigella* species (e.g., *S. sonnei* and *S. flexneri*) (30,31). The detection of *Shigella* and STEC 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* (32,33,34).

- 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 three 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 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 Instructions for Use can lead to erroneous results.
- Cross-reactivity with gastrointestinal tract organisms other than those listed in the "Analytical Specificity" section of the Instructions for Use 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 Shiga-like toxin *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 instructions for use" on page 106.
- Due to the small number of positive specimens collected for certain analytes during the prospective clinical study, performance characteristics for *Shigella* and STEC were established additionally with retrospective clinical specimens.
- If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
- Virus and bacteria 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.
- The potential for competitive inhibition at high concentrations between on-panel analytes is unknown.

Performance Characteristics

The QIAstat-Dx GI Panel 2 Mini B&V (Cat. No. 691424) cartridge is a reduced version of the QIAstat-Dx Gastrointestinal Panel 2 (Cat. No. 691421) where only the following targets are reported: Norovirus, *Campylobacter*, *Shigella*, Shiga-like toxin *E. coli* (STEC) and *Salmonella*. As such, the performance characteristics of the QIAstat-Dx GI Panel 2 Mini B&V were established based on reanalyzing the previously generated analytical and the clinical study data for the QIAstat-Dx Gastrointestinal Panel 2.

The analytical and clinical performance described in this section was demonstrated using QIAstat-Dx Analyzer 1.0. The QIAstat-Dx GI Panel 2 Mini B&V is no longer commercialized for use with the QIAstat-Dx Analyzer 1.0 and it can only be used with the QIAstat-Dx Analyzer 2.0. However, the performance on QIAstat-Dx Analyzer 1.0 is applicable for QIAstat-Dx GI Panel 2 Mini B&V and remains in the Instructions for Use. The QIAstat-Dx Analyzer 2.0 uses the same Analytical Module as QIAstat-Dx Analyzer 1.0; therefore, the performance is not impacted by QIAstat-Dx Analyzer 2.0.

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 GI Panel 2 Mini B&V target pathogenic organisms was assessed, using in total 14 pathogen strains, by analyzing serial dilutions of analytical samples prepared from culture isolates from commercial suppliers (e.g., ZeptoMetrix® and ATCC®). 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 14 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.

Individual LoD values for each QIAstat-Dx GI Panel 2 Mini B&V target are shown in Table 5.

Table 5. LoD values obtained for the different gastrointestinal target strains tested with the QIAstat-Dx GI Panel 2 Mini B&V

Pathogen	Strain	Source	Concentration (molecular units)* (copies/mL)	Concentration (microbiological units)	Detection rate
Campylobacter	*Campylobacter coli* 76-GA2 [LMG 21266]	ATCC 43478	5802	1.2 CFU/mL	20/20
	Campylobacter coli CIP 7080	ATCC 33559	8941	0.6 CFU/mL	21/21
	Campylobacter jejuni Z086	ZeptoMetrix 0801650	14,491	1660 CFU/mL	20/20
	Campylobacter jejuni subsp. Jejuni RM3193	ATCC BAA-1234	7210	110 CFU/mL	19/20
	Campylobacter upsaliensis NCTC 11541	ZeptoMetrix 0801999	56,165	2259.4 CFU/mL	20/20
	Campylobacter upsaliensis RM3195	ATCC BAA-1059	7631	35 CFU/mL	19/20
Salmonella	*Salmonella enterica* Serovar choleraseus	ATCC 13312	647	91.6 CFU/mL	20/20
	Salmonella enterica Serovar Typhimurium Z005	ZeptoMetrix 0801437	1441	4518.8 CFU/mL	20/20

Table 5. LoD values obtained for the different gastrointestinal target strains tested with the QIAstat-Dx GI Panel 2 Mini B&V (continued)

Pathogen	Strain	Source	Concentration (molecular units)* (copies/mL)	Concentration (microbiological units)	Detection rate
Shigella†	*Shigella sonnei* NCDC 1120-66	ATCC 25931	488	0.2 CFU/mL	20/20
	Escherichia coli CDC EDL 1282, O29:NM†	ATCC 43892	1431	41.3 CFU/mL	20/20
Shiga-like toxin *E. coli* (STEC)	*Escherichia coli* O26:H4	ZeptoMetrix 0801748	2012	726.8 CFU/mL	20/20
	Escherichia coli O157:H7; EDL933	ZeptoMetrix 0801622	1217	2281.5 CFU/mL	19/20
Norovirus	GI.1 (recombinant)	ZeptoMetrix 0810086CF	24629	891.1 $TCID_{50}$/mL	19/20
	GII.4 (recombinant)	ZeptoMetrix 0810087CF	8998	10.5 $TCID_{50}$/mL	20/20

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

† Due to the genetic similarity between *Shigella* and Enteroinvasive *E. coli*, the QIAstat-Dx GI Panel 2 Mini B&V cannot differentiate them and will report both as *Shigella* (see Limitations).

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 GI Panel 2 Mini B&V. 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 6 and Table 7, 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 $TCID_{50}$/mL for viral, 10^5 cells/mL for fungal and 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 GI Panel 2 Mini B&V.

Table 6. List of analytical specificity On-panel pathogens tested

Type	Pathogen	
Bacteria	*Campylobacter coli*	*Salmonella enterica*
	Campylobacter jejuni	*Shigella sonnei*
	Campylobacter upsaliensis	
	Escherichia coli (STEC)	
Viruses	Norovirus GI	Norovirus GII

Table 7. List of analytical specificity Off-panel pathogens tested

Type	Pathogen (potential cross-reactant)	
Bacteria	*Abiotrophia defectiva*	*Enterobacter cloacae*
	Acinetobacter baumannii	*Enterococcus faecalis*
	Aeromonas hydrophila	*Enterococcus faecium*
	Arcobacter cryaerophilus	*Escherichia coli* (serotype O77:HN) (enteroaggregative)
	Bacillus subtilis	*Escherichia coli* (enterotoxigenic ST+; LT+)
	Bifidobacterium bifidum	*Escherichia coli* (serotype O111:NM) (enteropathogenic)
	Campylobacter fetus	*Escherichia fergusonii*
	Campylobacter gracilis	*Escherichia hermannii*
	Campylobacter helveticus (Cross-reactive for Campylobacter target)	*Escherichia vulneris*
	Campylobacter hominis	*Faecalibacterium prausnitzii*
	Campylobacter lari (Cross-reactive for Campylobacter target)	*Gardnerella vaginalis*
	Campylobacter mucosalis	*Haemophilus influenzae*
	Campylobacter rectus	*Helicobacter pylori*
	Chlamydia trachomatis	*Klebsiella pneumoniae*
	Citrobacter freundii	*Lactobacillus casei*
	Clostridium difficile non-toxigenic	*Listeria monocytogenes*
	Clostridium difficile (strain NAP1) (*tcdA* +; *tcdB* +)	*Plesiomonas shigelloides*
	Clostridium perfringens	*Proteus mirabilis*
	Clostridium septicum	*Proteus vulgaris*
	Clostridium tetani	*Pseudomonas aeruginosa*
	Corynebacterium genitalium	*Staphylococcus aureus*
	Enterobacter aerogenes	*Staphylococcus aureus* subsp. *Aureus*
		Staphylococcus epidermidis
		Streptococcus agalactiae
		Streptococcus pyogenes

Table 7. List of analytical specificity Off-panel pathogens tested (continued)

Type	Pathogen (potential cross-reactant)	
		Vibrio cholerae
		Vibrio parahaemolyticus
		Vibrio vulnificus
		Yersinia enterocolitica
Fungi	*Aspergillus fumigatus*	*Saccharomyces boulardii*
	Candida albicans	*Saccharomyces cerevisiae*
Parasites	*Babesia microti*	*Toxoplasma gondii*
	Blastocystis hominis	*Trichomonas tenax*
	Cyclospora cayetanensis	
	Entamoeba histolytica	
	Giardia lamblia	
	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
	Adenovirus F41	Rotavirus A
	Astrovirus Type 4	Sapovirus GI
	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 GI Panel 2 Mini B&V (Table 8).

Table 8. Potential cross-reactions based on in silico analysis

QIAstat-Dx GI Panel 2 Mini B&V target	Potential cross-reactive organisms
Campylobacter spp.	*Campylobacter lari* § *Campylobacter helveticus* §
Shiga-like toxin E. coli (STEC)	Shigella sonnei†§, Shigella dysenteriae†§, Acinetobacter haemolyticus†**, Citrobacter freundii†**, Enterobacter cloacae†**, Aeromonas caviae†**, Escherichia albertii†¶

† Note that these predicted cross-reactivity identified by in silico analysis reflects sequences which can be acquired between species by horizontal gene transfer (18,35)

‡ Rare or less common eae intimin carrier organisms (36).

§ On-panel target.

¶ Rare or less common *Stx* toxins producers (32,37,38,39,40,41).

** In vitro testing of *Campylobacter lari* and *Campylobacter helveticus* strains at high concentration confirmed potential cross-reactivity of these *Campylobacter* species with the QIAstat-Dx GI Panel 2 Mini B&V assay.

Inclusivity (analytical reactivity)

Inclusivity (Analytical Reactivity) 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 GI Panel 2 Mini B&V primers and probes are specific and inclusive for clinically prevalent and relevant strains for each pathogen tested.

In vitro (wet) testing

QIAstat-Dx GI Panel 2 Mini B&V is inclusive for 100% (70 out of 70) of the pathogen strains tested in vitro. Most pathogen strains evaluated in wet testing were detected at ≤3- fold (65/70, of the corresponding LoD reference strain. Less than 100% detection was observed

for one strain each of *Shigella* and two strains each of STEC and Norovirus at 3x LoD. Testing of these strains at 10x LoD generated the expected positive results for all replicates (Table 9).

Table 9. Inclusivity test results for all the pathogens tested with the QIAstat-Dx GI Panel 2 Mini B&V Assay. LoD reference strain for every pathogen is written in bold.

Table 9a. 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	804272	1x LoD
	Campylobacter coli	CIP 7080 [1407, CIP 70.80]	ATCC	33559*	3x LoD
	Campylobacter jejuni	**Z086**	**ZeptoMetrix**	**0801650***	**1x LoD**
	Campylobacter jejuni	subsp. *jejuni* RM3193	ATCC	BAA-1234*	0.1x LoD
	Campylobacter jejuni subsp. jejuni	O:19 HL7; D3180	ATCC	BAA-218	0.1x LoD
	Campylobacter jejuni subsp. jejuni	AS-83-79	ATCC	33291	0.1x LoD
	Campylobacter jejuni subsp. doylei	NCTC 11951	ATCC	49349	0.1x LoD
	Campylobacter upsaliensis	**NCTC 11541**	**ZeptoMetrix**	**0801999***	**1x LoD**
	Campylobacter upsaliensis	RM 3195 (1994)	ATCC	BAA-1059*	0.3x LoD
	Campylobacter upsaliensis	NCTC 11541 [C231]	ATCC	43954	1x LoD

* Strain tested during LoD verification study.

Table 9b. 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
	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

Table 9b. Inclusivity test results for Salmonella strains (continued)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times 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 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

Table 9b. Inclusivity test results for Salmonella strains (continued)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times 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 9c. Inclusivity test results for *Shigella* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
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.

† Due to the genetic similarity between *Shigella* and Enteroinvasive *E. coli*, the QIAstat-Dx GI Panel 2 Mini B&V cannot differentiate them and will report both as Shigella (see Limitations).

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

Table 9d. Inclusivity test results for Shiga-like toxin *E. coli* (STEC)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Shiga-like toxin producing *E. coli* (STEC	**Shiga-like toxin *E. coli* (STEC)**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622***	**1x LoD**
	Shiga-like toxin *E. coli* (STEC)	O26:H4,*stx1* (+)	ZeptoMetrix	0801748*	1x LoD
	Shiga-like toxin *E. coli* (STEC)	Reference ATCC® 35150 (EDL 931),O157:H7,*stx1* (+), (+)	Microbiologics	617	3x LoD
	Shiga-like toxin *E. coli* (STEC)	Reference CDC 00-3039,O45:H2,unknown	Microbiologics	1098	1x LoD
	Shiga-like toxin *E. coli* (STEC)	O103:H2,*stx1* (+)	SSI Diagnostica	82170	3x LoD
	Shiga-like toxin *E. coli* (STEC)	O22:H8,*stx1c*	SSI Diagnostica	91350	1x LoD
	Shiga-like toxin *E. coli* (STEC)	O92,O107:K+:H48,*stx2d* (+)	SSI Diagnostica	91352	10x LoD
	Shiga-like toxin *E. coli* (STEC)	O101:K32:H-,*stx2e* (+)	SSI Diagnostica	91354	0.3x LoD
	Shiga-like toxin *E. coli* (STEC)	O128ac:H-,*stx2f* (+)	SSI Diagnostica	91355	10x LoD
	Shiga-like toxin *E. coli* (STEC)	O26:H11,*stx2a* (+)	SSI Diagnostica	95211	1x LoD
	Shiga-like toxin *E. coli* (STEC)	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 9e. Inclusivity test results for Norovirus strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Norovirus	**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

Table 9e. Inclusivity test results for Norovirus strains (continued)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times 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%.

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 GI Panel 2 Mini B&V (Table 10).

Table 10. Organisms with predicted reactivity based on in silico analysis

QIAstat-Dx GI Panel 2 Mini B&V target	Organisms with predicted reactivity
Bacteria	
Campylobacter	*Campylobacter coli**, *Campylobacter jejuni*, *Campylobacter jejuni* subsp. *jejuni*, *Campylobacter jejuni* subsp. doylei, *Campylobacter upsaliensis*

Table 10. Organisms with predicted reactivity based on in silico analysis (continued)

QIAstat-Dx GI Panel 2 Mini B&V target	Organisms with predicted reactivity
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*)
Shigella	Enteroinvasive *E. coli* (EIEC)§, *Escherichia coli* sp., *Shigella flexneri*, *Shigella dysenteriae*, *Shigella boydii*, *Shigella sonnei*.
Shiga-like toxin-*E. coli* (STEC)	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*

Table 10. Organisms with predicted reactivity based on in silico analysis (continued)

QIAstat-Dx GI Panel 2 Mini B&V target	Organisms with predicted reactivity
Norovirus	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, GI.2, GI.3*, GI.4*, GI.5, GI.6*, GI.7*, GI.8, GI.9.

*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.

§ Due to the genetic similarity between *Shigella* and Enteroinvasive *E. coli*, the QIAstat-Dx GI Panel 2 Mini B&V cannot differentiate them and will report both as *Shigella* (see Limitations).

Interfering substances

The effect of potentially interfering substances on the detectability of the QIAstat-Dx GI Panel 2 Mini B&V 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 exception of calcium carbonate that demonstrated interference at high concentration.

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

Results from the 34 interfering substances that could be present or introduced in a stool specimen are provided in Table 11.

Table 11. 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	No Interference
Triglycerides	5% w/v	No Interference
Exogenous substances		
Bacitracin	250 U/mL	No Interference
Bisacodyl	0.3% w/v	No Interference
Bismuth subsalicylate	0.35% w/v	No Interference
Calcium carbonate (TUMS® Extra Strength 750)	5% w/v 0.5% w/v	Interference No Interference
Docusate sodium	2.5% w/v	No Interference
Doxycycline hydrochloride	0.05% w/v	No Interference

Table 11. Final highest concentration without observable inhibitory effect (continued)

Substance tested	Concentration tested	Result
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	No Interference
Nystatin	10,000 USP units/mL	No Interference
Phenylephrine hydrochloride	0.075% w/v	No Interference
Sodium phosphate	5% w/v	No Interference
Vaccine components		
Rotavirus reassortant WC3:2-5, R574 (9) - VR 2195	8.89×10^{-3} $TCID_{50}$/mL	No Interference
Rotavirus reassortant WI79-4,9 - VR 2415	1.10×10^{2} pfu/mL	No Interference
Technique-specific Substances, Transport Media		
Bleach	0.5% v/v	No Interference
Ethanol	0.2% v/v	No Interference

Table 11. Final highest concentration without observable inhibitory effect (continued)

Substance tested	Concentration tested	Result
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/2 mL Cary-Blair	No Interference

* Performance not established for this transport media.

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 GI Panel 2 Mini B&V targets. Clinically relevant and challenging concentrations of non-target organisms (1×10^6 CFU/mL for bacteria, 1×10^5 cells/mL for yeast, and 1×10^5 $TCID_{50}$/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 GI Panel 2 Mini B&V targets. See Table 12 for a list of the non-target organisms tested and the result summary.

Table 12. 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

Table 12. Final highest concentration without observable inhibitory effect (continued)

Substance tested	Concentration tested	Result
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
Rotavirus*	2.9 x 10^5 copies/mL	No Interference

* Tested against Norovirus at 3x LoD.

Carryover

A carryover study was performed to evaluate the potential occurrence of cross-contamination between consecutive runs when using the QIAstat-Dx GI Panel 2 Mini B&V 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 negative samples, were tested on two QIAstat-Dx Analyzer 1.0 instruments.

No carryover between samples was observed in the QIAstat-Dx GI Panel 2 Mini B&V, 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 variations 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 13 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 variations introduced by sites, days, replicates, cartridge lots, operators, and QIAstat-Dx analyzers were 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 13. 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)**
	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98–100.00%)
Campylobacter ZeptoMetrix 801650	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98–100.00%)
	None	Not Detected	31/31 100%	31/31 100%	31/31 100%	93/93 100% (96.11–100.00%)

Table 13. Detection rate per target and concentration for each site of the Reproducibility study and exact 2-sided 95% Confidence Interval by target and concentration (continued)

			% Agreement with expected result			
Pathogen tested	**Concentration tested**	**Expected result**	**Site A**	**Site B**	**Site C**	**All sites (95% Confidence Interval)**
Norovirus GII ZeptoMetrix 0810087CF	3x LoD	Detected	30/30 100%	31/31 100%	30/30 100%	91/91 100% (96.03–100.00%)
	1x LoD	Detected	29/30 96.67%	30/30 100%	30/30 100%	89/90 98.89% (93.96–99.97%)
	None	Not Detected	31/31 100%	31/31 100%	31/31 100%	93/93 100% (96.11–100.00%)
Shiga-like toxin *E. coli* (STEC) 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	31/31 100%	31/31 100%	31/31 100%	93/93 100% (96.11–100.00%)

Table 13. Detection rate per target and concentration for each site of the Reproducibility study and exact 2-sided 95% Confidence Interval by target and concentration (continued)

			% Agreement with expected result			
Pathogen tested	**Concentration tested**	**Expected result**	**Site A**	**Site B**	**Site C**	**All sites (95% Confidence Interval)**
Salmonella enterica ZeptoMetrix 0801437	3x LoD	Detected	30/30 100%	31/31 100%	30/30 100%	91/91 100% (96.03–100.00%)
	1x LoD	Detected	30/30 100%	29/30 96.67%	29/30 96.67%	88/90 97.78% (92.20–99.73%)
	None	Not Detected	31/31 100%	31/31 100%	31/31 100%	93/93 100% (96.11–100.00%)

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 GI Panel 2 Mini B&V detected pathogens included in the positive samples were *Campylobacter*, Norovirus, *Salmonella*, and STEC. 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 GI Panel 2 Mini B&V in the prospective clinical evaluation, stratified by age group, are presented in Table 14. Overall, the QIAstat-Dx GI Panel 2 Mini B&V 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 14. Expected values summary by age group for the prospective clinical study as determined by the QIAstat-Dx GI Panel 2 Mini B&V

Pathogen	Medium brand	Overall	0-5 years	6-21 years	22-49 years	50+ years	Not reported
			Viruses				
Norovirus	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%)
			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%)
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%)
			Diarrheagenic *E. coli*/*Shigella*				
Shiga-like toxin *E. coli* (STEC)	FecalSwab	15 (1.2%)	9 (4.9%)	1 (0.8%)	2 (0.7%)	3 (0.5%)	0 (0.0%)
	Para-Pak C&S	9 (1.3%)	0 (0.0%)	0 (0.0%)	6 (2.8%)	3 (0.7%)	0 (0.0%)

Table 14. Expected values summary by age group for the prospective clinical study as determined by the QIAstat-Dx GI Panel 2 Mini B&V (continued)

Pathogen	Medium brand	Overall	0-5 years	6-21 years	22-49 years	50+ years	Not reported
Shigella	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%)

Clinical performance

The clinical performance described in this section was demonstrated using QIAstat-Dx Analyzer 1.0. The QIAstat-Dx Analyzer 2.0 uses the same Analytical Modules as QIAstat-Dx Analyzer 1.0; therefore, performance is not impacted by the QIAstat-Dx Analyzer 2.0.

The clinical performance of QIAstat-Dx GI Panel 2 Mini B&V was established during a multi-center international prospective study conducted at thirteen clinical settings representatives of different geographical areas within US and Europe (9 sites in US 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 15 provides a summary of prospective specimen distribution across all study sites.

Table 15. 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

Table 15. Prospective specimens distribution across the study sites (continued)

Site/Country	FecalSwab	Para-Pak C&S	Total
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 1939 specimens evaluated in the prospective study is summarized in Table 16.

Table 16. Demographic data for prospective evaluated specimens

	FecalSwab		Para-Pak C&S	
Demographic data	**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. of 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 GI Panel 2 Mini B&V was evaluated for each panel test result using one FDA-cleared test as comparator for *Campylobacter*, *Salmonella*, and *Shigella*, and a composite comparator consisting of three independent FDA-cleared test methods for Norovirus and STEC (Table 17).

Table 17. Comparator methods for the clinical evaluation of QIAstat-Dx GI Panel 2 Mini B&V

QIAstat-Dx GI Panel 2 Mini B&V test result	Comparator method
Campylobacter	One cleared FDA method
Salmonella	
Shigella	
Norovirus	Composite of three FDA-cleared test methods
Shiga-like toxin *E. coli* (STEC)	Composite of three FDA-cleared test methods

Additional prospective archived samples were collected for Norovirus (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. A second collection of 75 prospective archived samples positive for STEC preserved in FecalSwab from three different collection sites in the US and 17 negative specimens were analyzed.

In addition, to supplement the results of the prospective clinical studies, a total of 587 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 17 were used as confirmatory testing for the presence of the nucleic acids from the expected analytes. In total, 2527 specimens (1939 prospective, 211 prospective archived, and 377 retrospective) were

evaluated in the clinical study. These specimens were collected using Para-Pak C&S (1064) or FecalSwab (1463).

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 GI Panel 2 Mini B&V and comparator method showed a positive result for this specific target, and false negative (FN) indicates that the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V and the comparator method showed negative results, and a false positive (FP) indicates that the QIAstat-Dx GI Panel 2 Mini B&V 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 17), 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 Table 18, Table 19, and Table 20, respectively.

Discrepancies between the QIAstat-Dx GI Panel 2 Mini B&V and the comparator methods were investigated for the analytes that the QIAstat-Dx GI Panel 2 Mini B&V test result was compared to one FDA-cleared method. Discrepancies analyses are footnoted on each clinical performance summary (Table 18 and Table 20).

Table 18. 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**
				Virus			
Norovirus	FecalSwab	31/33 [a]	93.9	80.4–98.3	493/495 [a]	99.6	98.6–100.0
	Para-Pak C&S	14/18 [b]	77.8	54.8–91.0	399/399 [b]	100.0	99.1–100.0
				Bacteria			
Campylobacter	FecalSwab	65/67 [c]	97.0	89.8–99.2	1151/1155 [c]	99.7	99.1–99.9
	Para-Pak C&S	30/31 [d]	96.8	83.8–99.4	675/677 [d]	99.7	98.9–99.9
Salmonella	FecalSwab	14/16 [e]	87.5	64.0–96.5	1206/1206	100.0	99.7–100.0
	Para-Pak C&S	19/20 [g]	95.0	76.4–99.1	688/688	100.0	99.4–100.0
Yersinia entero-colitica	FecalSwab	15 / 16 [e]	93.8	71.7–99.0	1199 / 1206 [e]	99.4	98.8–99.7
	Para-Pak C&S	3 / 3	100.0	43.9–100.0	698 / 703 [f]	99.3	98.4–99.7
				Diarrheagenic *E. coli*/*Shigella*			
Shiga-like toxin *E. coli*(STEC)	FecalSwab	3 / 5 [h]	60.0	23.1–88.2	434 / 438 [h]	99.1	97.7–99.6
	Para-Pak C&S	5/6 [i]	83.3	43.7–97.0	397/400 [i]	99.3	97.8–99.7

Table 18. Clinical performance in the prospective study (continued)

		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Medium brand**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Shigella	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 [i]	99.9	99.2–100.0

[a] Ten (10) FecalSwab samples positive for Norovirus 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 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.

[b] Two (2) Para-Pak C&S samples positive for Norovirus 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 classified 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.

[c] *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.

[d] *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.

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

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

Table 18. Clinical performance in the prospective study (continued)

		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Medium brand**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**

[g] Eight (8) FecalSwab sample positive for STEC 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 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.

[h] 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.

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

Table 19. Clinical performance in the prospective archived study

Analyte	Medium brand	Positive Percent Agreement			Negative Percent Agreement		
		TP/TP+FN	%	95% CI	TN/TN+FP	%	95% CI
Norovirus	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)	FecalSwab	24 / 24 **	100.0	86.2–100.0	67 / 68**	98.5	92.1–99.7
	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 fifty-one out of the seventy-five (51/75) prospectively archived FecalSwab 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.

§ 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.

Table 20. Clinical performance in the Retrospective study

Analyte	Medium brand	Positive Percent Agreement			Negative Percent Agreement		
		TP/TP+FN	%	95% CI	TN/TN+FP	%	95% CI
Virus							
Norovirus	FecalSwab	28/32 [a]	87.5	71.9–95.0	74/75 [a]	98.7	92.8–99.8
	Para-Pak C&S	27/29	93.1	78.0–98.1	86/86 [b]	100.0	95.7–100.0
Bacteria							
Campylobacter	FecalSwab	31/31	100.0	89.0–100.0	161/163 [c]	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
Salmonella	FecalSwab	30/31 [d]	96.8	83.8-99.4	161/163[d]	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
Diarrheagenic *E. coli*/*Shigella*							
Shiga-like toxin *E. coli* (STEC)	FecalSwab	2 / 3 [e]	66.7	20.8–93.9	62 / 63 [e]	98.4	91.5–99.7
	Para-Pak C&S	60/64	93.8	85.0–97.5	44/44 [e]	100.0	92.0–100.0
Shigella	FecalSwab	22/24 [f]	91.7	74.2–97.7	170/170	100.0	97.8–100.0

Table 20. Clinical performance in the Retrospective study (continued)

Analyte	Medium brand	Positive Percent Agreement			Negative Percent Agreement		
		TP/TP+FN	%	95% CI	TN/TN+FP	%	95% CI
	Para-Pak C&S	0/0	N/A	N/A	14/14	100.0	78.5–100.0

[a] Two (2) FecalSwab samples positive for Norovirus 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 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.

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

[b] The sample size for NPA is smaller for Norovirus 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.

[d] *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.

[e] Fifteen (15) FecalSwab sample positive for STEC 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 STEC in FecalSwab 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.

[f] 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.

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

The proportion of failed runs on initial attempt, and following repeats are summarized in Table 21. The error breakdown due to instrument, invalid results, "sample too concentrated" failures, and other run failures are summarized in Table 22.

Table 21. Failure rates summary

		Initial runs			Final runs		
Transport media	**Study**	**N/Total**	**%**	**95% CI**	**N/Total**	**%**	**95% CI**
FecalSwab	**Prospective**	15/1227	1.2	0.7 – 2.0	2/1227	0.2	0.0 – 0.6
	Prospective Archived	0/145	0.0	0.0 – 2.6	0/145	0.0	0.0 – 2.6
	Retrospective	5/366	1.4	0.6 – 3.2	2/366	0.5	0.2 – 2.0
	Total	20/1738	1.2	0.8 – 1.8	4/1738	0.2	0.1 – 0.6
Para-Pak C&S	**Prospective**	73/740	9.9	7.9 – 12.2	26/740	3.5	2.4 – 5.1
	Prospective Archived	3/66	4.5	1.6 – 12.5	0/66	0.0	0.0 – 5.5
	Retrospective	32/454	7.0	5.0 – 9.8	13/454	2.9	1.5 – 4.8
	Total	108/1260	8.6	7.2 – 10.3	39/1260	3.1	2.2 – 4.2

Table 22. 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‡	10/1227	0.8	2/1227	0.2
	Prospective Archived	Instrument	0/145	0.0	0/145	0.0
		Invalid	0/145	0.0	0/145	0.0
		Sample too Concentrated	0/145	0.0	0/145	0.0
		Other	0/145	0.0	0/145	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	3/366	0.8	2/366	0.5

Table 22. Failure types breakdown (continued)

Transport media	Study	Failure reason	Initial runs		Final runs	
			N/Total	%	N/Total	%
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	24/740	3.2	12/740	1.6
	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	2/454	0.4	3/454	0.7
		Sample too Concentrated	10/454	2.2	2/454	0.4
		Other	19/454	4.2	8/454	1.8

* 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: Additional instructions for use" on page 1.

‡ Run failures related to workflow checkpoints.

Co-infections

The QIAstat-Dx GI Panel 2 Mini B&V reported multiple organism detections (i.e., co-infections) for a total of 4 prospective specimens in FecalSwab and 4 prospective specimens in Para-Pak

C&S. This represents 3.0% of positive specimens (4/132) in FecalSwab and 5.3% of positive specimens (4/75) in Para-Pak C&S. All multiple detections in FecalSwab and Para-Pak C&S specimens (4/4; 100.0%) contained two organisms. The multiple infections for FecalSwab and Para-Pak C&S are listed in Table 23 and Table 24, respectively. The analytes found in mixed infections in the FecalSwab specimens were *Campylobacter* (3), Norovirus (5), *Shigella* (2), and STEC (2),while the analytes found in mixed infections in the Para-Pak C&S specimens were *Campylobacter* (2), Norovirus (2), *Shigella* (2), and STEC (2).

Table 23. Multiple detection combinations as determined by the QIAstat-Dx GI Panel 2 Mini B&V in the prospective clinical study in FecalSwab specimens

Multiple detection combination	Number of FecalSwab specimens
Campylobacter + *Shigella*	1
Norovirus + *Shigella*	1
Campylobacter + Norovirus	2
Norovirus + STEC	2

Table 24. Multiple detection combinations as determined by the QIAstat-Dx GI Panel 2 Mini B&V in the prospective clinical study in Para-Pak C&S specimens

Multiple detection combination	Number of Para-Pak C&S specimens
Campylobacter + Shiga-like toxin *E. coli* (STEC)	1
Campylobacter + *Shigella*	1
Norovirus + Shiga-like toxin *E. coli* (STEC)	1
Norovirus + *Shigella*	1

Contrived specimens testing

An evaluation of contrived specimens was performed to supplement the prospective and retrospective specimens' test results for *Shigella*. Contrived specimens were prepared using

negative residual specimens that had previously tested negative by QIAstat-Dx GI Panel 2 Mini B&V 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 to spike a minimum of 50 specimens across FecalSwab and Para-Pak C&S. The analyte status of each contrived specimen was blinded to the users analyzing the specimens. Results are summarized in Table 25.

Table 25. Test results summary for contrived specimens

		Positive Percent Agreement (PPA)		
QIAstat-Dx GI Panel 2 Mini B&V target	**Medium brand**	**Fraction**	**Percentage**	**95% CI**
Shigella	FecalSwab	35/35	100.0	90.1–100.0
	Para-Pak C&S	34/34	100.0	89.8–100.0

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 GI Panel 2 Mini B&V error codes and messages can be found in Table 26:

Table 26. Information about specific QIAstat-Dx GI Panel 2 Mini B&V error codes and messages

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

When the sample concentration is too high and the test must be repeated by loading 100 µL, follow the workflow detailed in "Appendix C: Additional instructions for use" on page 106 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 labeling:

Symbol	Symbol definition
	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)
	Global Trade Item Number
	Gastrointestinal application

Symbol	Symbol definition
Rn	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Caution
SN	Serial number
	Do not use if package is damaged
	Flammable, risk of fire

Symbol	Symbol definition
	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 GI Panel 2 Mini B&V must be installed on the QIAstat-Dx Analyzer 2.0 prior to testing with QIAstat-Dx GI Panel 2 Mini B&V Cartridges.

Note: Whenever a new version of the QIAstat-Dx GI Panel 2 Mini B&V assay is released, the new QIAstat-Dx GI Panel 2 Mini B&V 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 2.0. This USB drive must be formatted with a FAT32 file system.

To import an ADF from the USB drive to the QIAstat-Dx Analyzer 2.0, proceed with the following steps:

1. Insert the USB drive containing the Assay Definition File into one of the USB ports on the QIAstat-Dx Analyzer 2.0.
2. Press **Options** > **Assay Management**.

   The Assay Management screen appears in the Content area of the display (Figure 22).

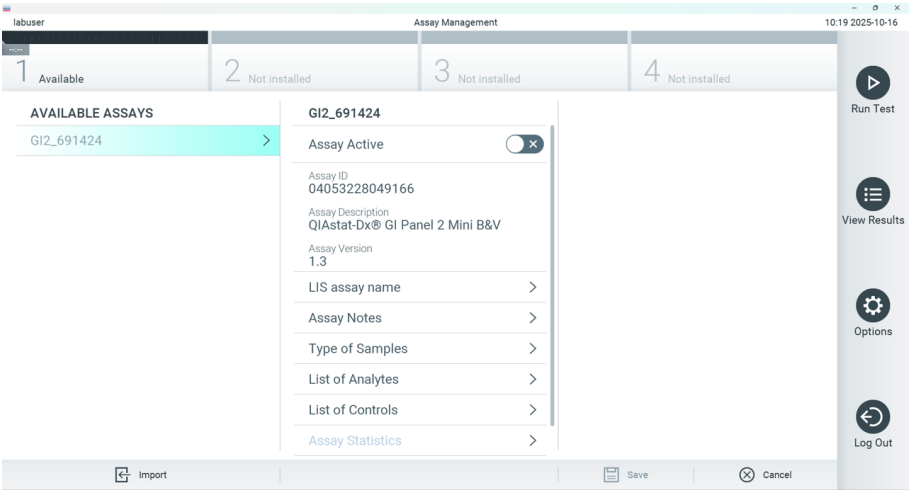

Figure 22. Assay Management screen.

3. Press **Import** located in the bottom left of the screen (Figure 22).
4. Select the file corresponding to the assay to be imported from the USB drive.

   A dialog box will appear to confirm the upload of the file.

   In case the ADF is upgraded, a dialog box may appear to override the current version with a new one. Press **Yes** to override.
5. To activate the assay, enable the **Assay Active** option (Figure 23).

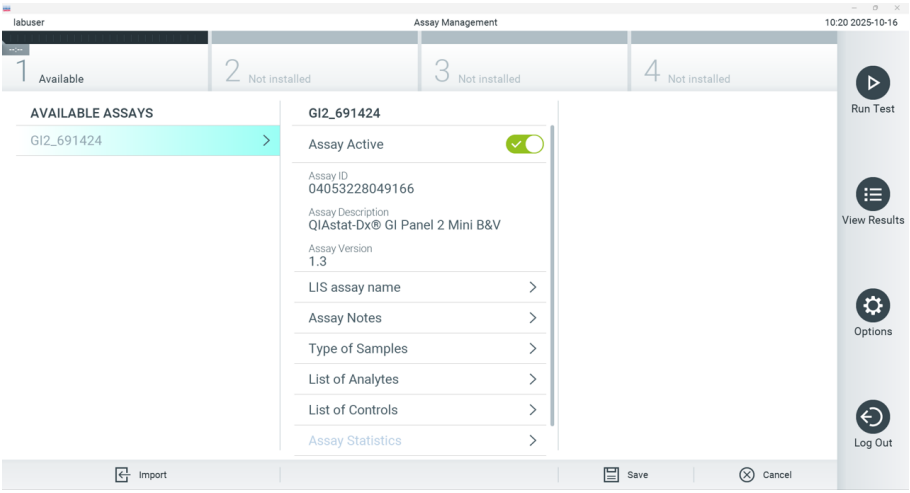

Figure 23. Activating the assay.

6. To assign the active assay to a user, perform these steps:
   a. Go to **Options** > **User Management**.
   b. 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.

   c. Select **Assign Assays** from the User Options tab.
   d. Enable the assay and press **Save** (Figure 24).

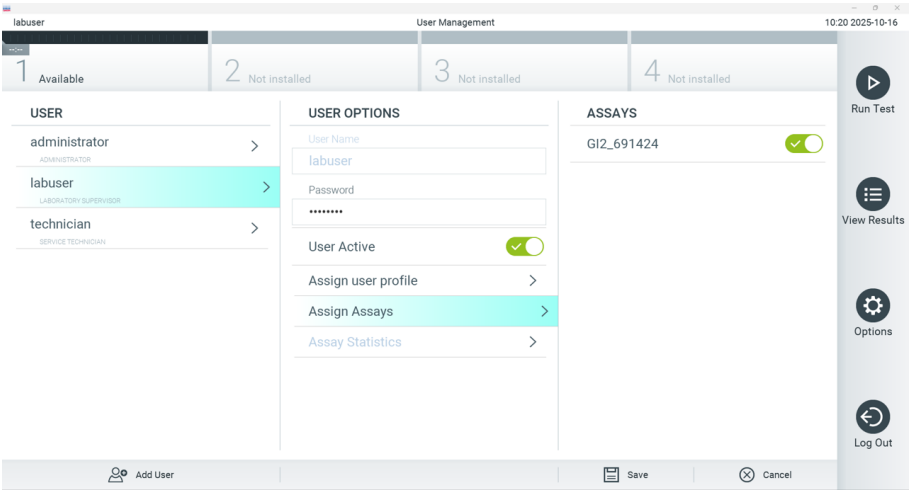

Figure 24. Assigning the active assay.

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 or the QIAstat-Dx Analyzer 2.0 hardware module, in charge of executing tests on QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V 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).

Operational Module PRO (OM PRO): The dedicated QIAstat-Dx Analyzer 2.0 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 Analyzer 2.0: The QIAstat-Dx Analyzer 2.0 consists of an Operational Module PRO and Analytical Module. The Operational Module PRO includes elements that provide connectivity to the Analytical Module and enables user interaction with the QIAstat-Dx Analyzer 2.0. The Analytical Module contains the hardware and software for sample testing and analysis.

QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge, inlet for dry swabs. The swab port is not used for the QIAstat-Dx GI Panel 2 Mini B&V assay.

User: A person who operates the QIAstat-Dx Analyzer 1.0 / QIAstat-Dx Analyzer 2.0 / QIAstat-Dx GI Panel 2 Mini B&V 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 2.0 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 of the IFU adapted to 100 µL sample input volume:

1. Open the package of a new QIAstat-Dx GI Panel 2 Mini B&V Cartridge using the tear notches on the sides of the packaging.
2. Remove the QIAstat-Dx GI Panel 2 Mini B&V Cartridge from the packaging.
3. Manually write the sample information, or place a sample information label, on the top of the QIAstat-Dx GI Panel 2 Mini B&V Cartridge. Make sure that the label is properly positioned and does not block the lid opening.
4. Place the QIAstat-Dx GI Panel 2 Mini B&V Cartridge flat on the clean work surface so that the bar code on the label faces upwards. Open the sample lid of the main port on the front of the QIAstat-Dx GI Panel 2 Mini B&V 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 GI Panel 2 Mini B&V Cartridge using the supplied single-use transfer pipette.
8. Firmly close the lid of the main port until it clicks.
9. From this point, proceed following the instructions described in the IFU.

Ordering Information

Product	Contents	Cat. no.
QIAstat-Dx GI Panel 2 Mini B&V	For 6 tests: 6 individually packaged QIAstat-Dx GI Panel 2 Mini B&V Cartridges and 6 individually packaged transfer pipettes	691424
Related Products		
QIAstat-Dx Analyzer 2.0	1 QIAstat-Dx Analytical Module, 1 QIAstat-Dx Operational Module PRO, and related hardware and software to run molecular diagnostic QIAstat-Dx assay cartridges	9002828

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit Instructions for Use or User Manual. QIAGEN kit Instructions for Use 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
R1, January 2025	Initial release
R2, April 2025	Analyzer 2.0 incorporation
R3, October 2025	Removal of Analyzer 1.0 in the document Removal of sample type selection for Para-Pak C&S and FecalSwab (sample type will be defined as "Preserved Stool", independently of the sample collection method) and removal of limitation of reporting of Enteropathogenic *E. coli* (EPEC), STEC stx1/stx2 and STEC O157 for FecalSwab Addition of clinical performance data in FecalSwab specimens for STEC Minor editorial/grammatical changes/corrections/updates throughout the document

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