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QIAstat-Dx®
Gastrointestinal Panel 2
Instructions for Use (Handbook)

Σ 6

Version 1

IVD

For In Vitro Diagnostic Use

For use with QIAstat-Dx® Analyzer 1.0

REF 691412

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden GERMANY

R1 1131785EN

Sample to Insight

Contents

Intended Use

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

- Adenovirus F40/F41
- Astrovirus
- Norovirus (GI/GII)
- Rotavirus A
- Sapovirus (GI, GII, GIV, GV)
- *Campylobacter* (*C. jejuni*, *C. coli* and *C. upsaliensis*)
- *Clostridium difficile* (toxin A/B)
- Enteroaggregative *Escherichia coli* (EAEC)
- *Shigella*/Enteroinvasive *Escherichia coli* (EIEC)
- Enteropathogenic *Escherichia coli* (EPEC)
- Enterotoxigenic *Escherichia coli* (ETEC) lt/st
- *Plesiomonas shigelloides*
- *Salmonella* spp.
- Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2** (including specific identification of *E. coli* O157 serogroup within STEC)
- *Vibrio vulnificus*
- *Vibrio parahaemolyticus*
- *Vibrio cholerae*
- *Yersinia enterocolitica*
- *Cryptosporidium*
- *Cyclospora cayetanensis*
- *Entamoeba histolytica*
- *Giardia lamblia*

*Shiga-like toxin-producing E. coli (STEC) genes (*stx1 and stx2*) are differentiated by QIAstat-Dx Gastrointestinal Panel 2

Concomitant culture is necessary for organism recovery and further typing of bacterial agents.

The QIAstat-Dx Gastrointestinal Panel 2 is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness in conjunction with other clinical, laboratory, and epidemiological data. Confirmed positive results do not rule-out co-infection with organisms not detected by the QIAstat-Dx Gastrointestinal Panel 2. The organisms detected may not be the sole or definitive cause of the disease.

QIAstat-Dx Gastrointestinal Panel 2 is not intended to monitor or guide treatment for *C. difficile* infections.

Negative QIAstat-Dx Gastrointestinal Panel 2 results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this assay test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

The QIAstat-Dx Gastrointestinal Panel 2 also aids in the detection and identification of acute gastroenteritis in the context of outbreaks. The QIAstat-Dx Gastrointestinal Panel 2 is intended for professional use only and is not intended for self-testing. The QIAstat-Dx Gastrointestinal Panel 2 is intended for *in vitro* diagnostic use.

Intended User

This kit is intended for professional use.

The product is to be used only by personnel specifically instructed and trained in molecular biology techniques and familiar with this technology.

Summary and Explanation

QIAstat-Dx Gastrointestinal Panel 2 cartridge description

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

All reagents required for the complete execution of a test run are pre-loaded and self-contained in the QIAstat-Dx Gastrointestinal Panel 2 Cartridge. The user does not need to come in contact with and/or manipulate any reagents. The QIAstat-Dx Analyzer 1.0 and house 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.

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

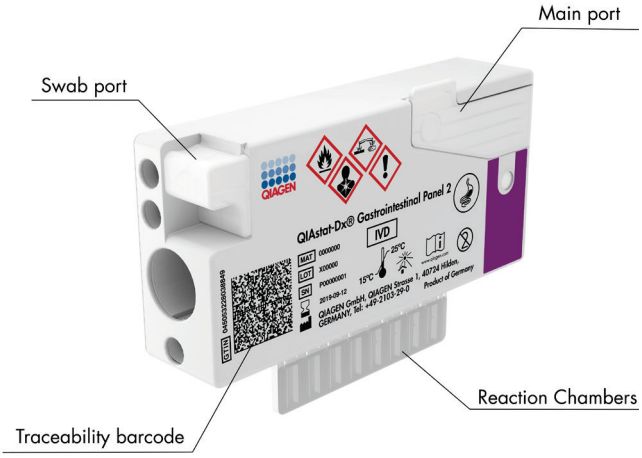

Figure 1. Layout of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge and its features.

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. The rapid and accurate determination of the presence or absence of potential causative agent(s) helps make timely decisions regarding treatment, hospital admission, infection control, and return of the patient to work and family. It may also greatly support improved antimicrobial stewardship and other important public health initiatives.

The QIAstat-Dx Gastrointestinal Panel 2 Cartridge allows detection and differentiation of 22 parasitic, viral, and bacterial pathogens that cause gastrointestinal symptoms, which includes specific identification of *E. coli* O157 serogroup within STEC, resulting in 23 targets in total. Testing requires a small sample volume and minimal hands-on time, and the results are available in approximately 78 minutes.

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

Sample collection and cartridge loading

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

The following steps are performed:

1. Fresh unpreserved stool specimen is collected and resuspended into Cary-Blair 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 Cary-Blair container.
2. The sample information is manually written on or a sample label is affixed to the top of a QIAstat-Dx Gastrointestinal Panel 2 Cartridge.

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

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

3. Liquid sample (stool resuspended in Cary-Blair transport medium) is loaded manually into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge.

   Note: Cary-Blair 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 bar code (if available) and the QIAstat-Dx Gastrointestinal Panel 2 Cartridge bar code are scanned by the QIAstat-Dx Analyzer 1.0. If sample bar code is not available, the sample ID is manually written using the virtual keyboard of the touchscreen.
5. The QIAstat-Dx Gastrointestinal Panel 2 Cartridge is introduced into the QIAstat-Dx Analyzer 1.0
6. The test is started on the QIAstat-Dx Analyzer 1.0 Sample preparation, nucleic acid amplification, and detection

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

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

Materials Provided

Kit contents

QIAstat-Dx Gastrointestinal Panel 2 Cartridge*	
Catalog number	**691412**
Number of tests	**6**
QIAstat-Dx Gastrointestinal Panel 2 Cartridges*	6
Transfer pipettes†	6

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

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

Materials Required but Not Provided

Equipment*

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

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

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

Warnings and Precautions

For in vitro diagnostic use.

The QIAstat-Dx Gastrointestinal Panel 2 is to be used by laboratory professionals trained in the use of QIAstat-Dx Analyzer 1.0

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 and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Always wear appropriate personal protective equipment, including but not limited to disposable powder-free gloves, a lab coat, and protective eyewear. Protect skin, eyes, and mucus membranes. Change gloves often when handling samples.

Handle all samples, used 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:

- OSHA®: Occupational Safety and Health Administration (United States of America)
- ACGIH®: American Conference of Government Industrial Hygienists (USA)
- COSHH: Control of Substances Hazardous to Health (United Kingdom)

Follow your institution's safety procedures for handling biological samples. Dispose of samples, QIAstat-Dx Gastrointestinal Panel 2 Cartridges, and transfer pipettes according to the appropriate regulations.

The QIAstat-Dx Gastrointestinal Panel 2 Cartridge is a closed, single-use device that contains all reagents needed for sample preparation and multiplex real-time RT-PCR within the QIAstat-Dx Analyzer 1.0. Do not use a QIAstat-Dx Gastrointestinal Panel 2 Cartridge that is past its expiration date, appears damaged, or leaks fluid. Dispose of used or damaged cartridges in accordance with all national, state, and local health and safety regulations and laws.

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 (**www.cdc.gov/od/ohs/biosfty/biosfty.htm**).

Precautions

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

Contains: ethanol; guanidine hydrochloride; guanidine thiocyanate; isopropanol; proteinase K; t-Octylphenoxypolyethoxyethanol. Danger! Highly flammable liquid and vapor. Harmful if swallowed or if inhaled. May be harmful in contact with skin. Causes severe skin burns and eye damage. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause drowsiness or dizziness. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Corrosive to the respiratory tract. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Immediately call a POISON CENTER or doctor/ physician. Remove person to fresh air and keep comfortable for breathing.

To reduce the risk of contamination when handling stool samples, it is recommended that the below guidelines are applied:

- 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).
- Prior to sample handling, the work area should be thoroughly cleaned using 10% bleach or similar disinfectant.
- QIAstat-Dx Gastrointestinal Panel 2 Cartridges and samples should be processed one at a time.
- Change gloves prior to removing cartridges from shipping boxes.
- Change gloves and clean the work area between processing each sample.
- Dispose of used cartridges in a biohazard container immediately after the run is completed and avoid excessive handling.

Reagent Storage and Handling

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

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

Specimen Handling, Storage and Preparation

The QIAstat-Dx Gastrointestinal Panel 2 is for use with QIAstat-Dx Analyzer 1.0. All samples should be treated as potentially hazardous.

Specimen collection

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

Recommended storage conditions for stool resuspended in Cary-Blair transport medium specimens are listed below:

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

Protocol: Processing Raw Stool Samples in Cary-Blair transport medium

Sample collection, transport, and storage

Collect and resuspend the stool sample in Cary-Blair transport medium according to the manufacturer's recommended procedures.

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

Note: Applicable for boththe QIAstat-Dx 1.0.

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

   IMPORTANT: After the package is opened, sample should be introduced into the QIAstat-Dx Gastrointestinal Panel 2 Cartridge and loaded into the QIAstat-Dx Analyzer 1.0 within 120 minutes.

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

2. Remove the QIAstat-Dx Gastrointestinal Panel 2 Cartridge from the packaging and position it so that the bar code on the label faces you.

3. Manually write the sample information or place a sample information label on the top of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge. Make sure that the label is properly positioned and does not block the lid opening (Figure 3).

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

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

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

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

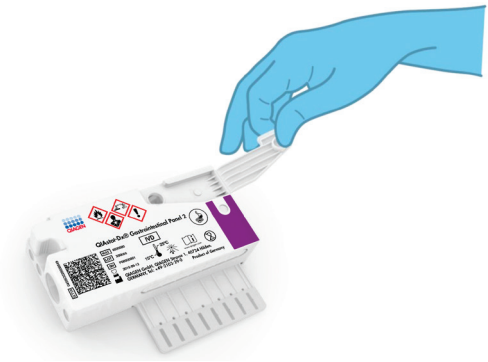

Figure 4. Opening the sample lid of main port.

5. Thoroughly mix the stool in the Cary-Blair transport medium, for example, by vigorously agitating the tube 3 times (Figure 5).

Figure 5. Mixing stool sample in Cary-Blair 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.

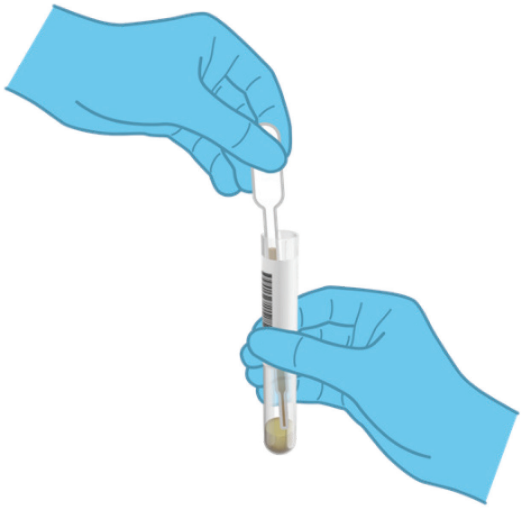

Figure 6. Drawing up sample into the supplied transfer pipette.

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

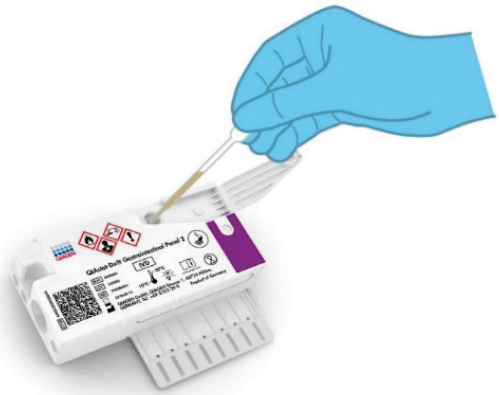

Figure 7. Transferring sample to main port of QIAstat-Dx Gastrointestinal Panel 2 Cartridge.

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

Figure 8. Closing the lid of the main port.

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

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

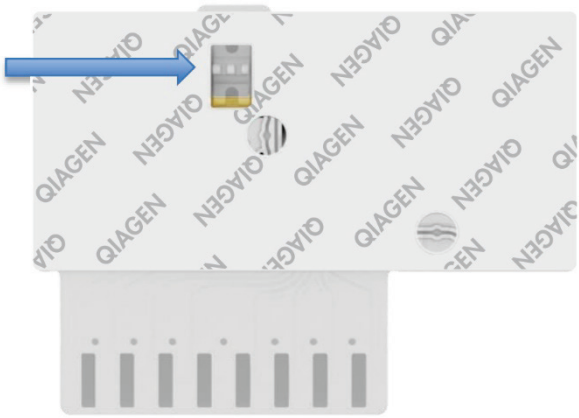

Figure 9. Sample inspection window (blue arrow).

Running a test with the QIAstat-Dx Analyzer 1.0

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

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

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

3. Log in to the QIAstat-Dx Analyzer 1.0 by entering the user name and password.

   Note: The **Login** screen will appear if **User Access Control** is activated. If the **User Access Control** is disabled, no user name/password will be required, and the **Main** screen will appear.

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

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

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

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

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

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

Figure 10. Scanning sample ID bar code.

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

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

Figure 11. Scanning QIAstat-Dx Gastrointestinal Panel 2 Cartridge bar code.

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).

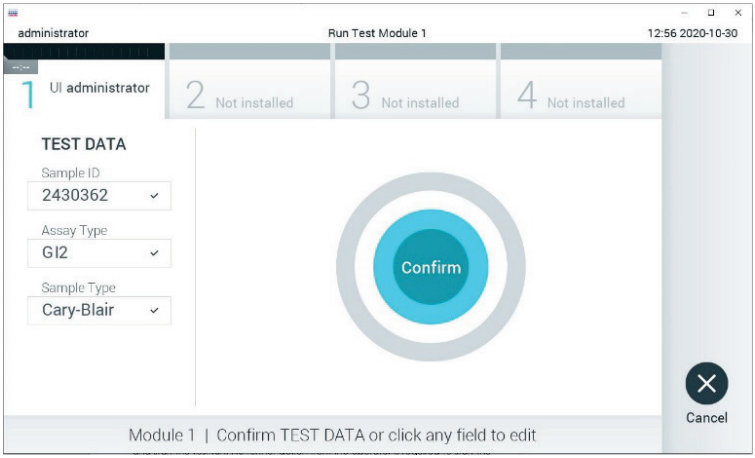

Figure 12. Confirming data entry.

10. Ensure that both sample lids of the swab port and main port of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge are firmly closed.
11. When the cartridge entrance port on the top of the QIAstat-Dx Analyzer 1.0 automatically opens, insert the QIAstat-Dx Gastrointestinal Panel 2 Cartridge with the bar code 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 in the bottom right corner of the touchscreen.
12. Upon detecting the QIAstat-Dx Gastrointestinal Panel 2 Cartridge, the QIAstat-Dx Analyzer 1.0 will automatically close the lid of the cartridge entrance port and start the test run. No further action from the operator is required to start the run.

    Note: There is no need to push the QIAstat-Dx Gastrointestinal Panel 2 Cartridge into the QIAstat-Dx Analyzer 1.0.

    Note: The QIAstat-Dx Analyzer 1.0 will not accept a QIAstat-Dx Gastrointestinal Panel 2 Cartridge other than the one used and scanned during the test setup. If a cartridge other than the one scanned is inserted, an error will be generated, and the cartridge will be automatically ejected.

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

Figure 13. Inserting QIAstat-Dx Gastrointestinal Panel 2 Cartridge into QIAstat-Dx Analyzer 1.0.

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 1.0 User Manual* for possible reasons and instructions on how to proceed. For additional information about specific QIAstat-Dx Gastrointestinal Panel 2 error codes and messages, refer to the 'Troubleshooting' section of this document.

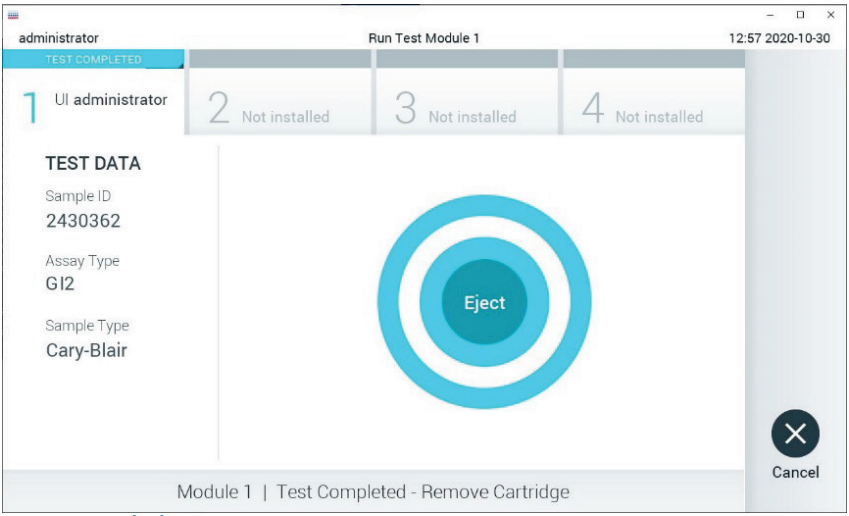

Figure 14. Eject screen display.

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

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

16. After the QIAstat-Dx Gastrointestinal Panel 2 Cartridge has been ejected, the results **Summary** screen will appear. To begin the process for running another test, press **Run Test**.

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

Viewing results with the QIAstat-Dx Analyzer 1.0

The QIAstat-Dx Analyzer 1.0 automatically interprets and saves test results. After ejecting the QIAstat-Dx Gastrointestinal Panel 2 Cartridge, the results **Summary** screen is automatically displayed (Figure 15).

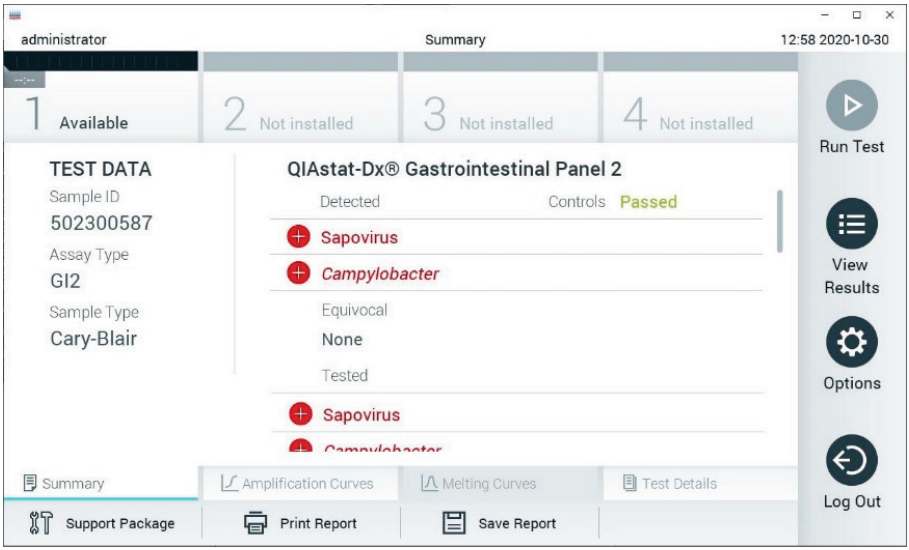

Figure 15. Results Summary screen example showing Test Data on the left panel and Test Summary in the main panel.

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 "Equivocal" is not used. "Equivocal" results are not applicable for the QIAstat-Dx Gastrointestinal Panel 2. Therefore, the "Equivocal" list will always be empty.
- The third list, under the heading "Tested", includes all pathogens tested in the sample. Pathogens detected and identified in the sample are preceded by a ⊕ sign and are

colored red. Pathogens that were tested but not detected are preceded by a ⊖ sign and are colored green. Invalid and not applicable pathogens are also displayed in this list.

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

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

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

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

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

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

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

Viewing amplification curves

To view test amplification curves of pathogens detected, press the **Amplification Curves** tab (Figure 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 1.0, the **Amplification Curves** screen is only available for operators with access rights.

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

Press the **CONTROLS** tab on the left side to view the controls in the amplification plot. Press the circle next to the control name to select or deselect it (Figure 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 C_T and endpoint fluorescence in the event of a positive signal
- Internal Control, with C_T and endpoint fluorescence

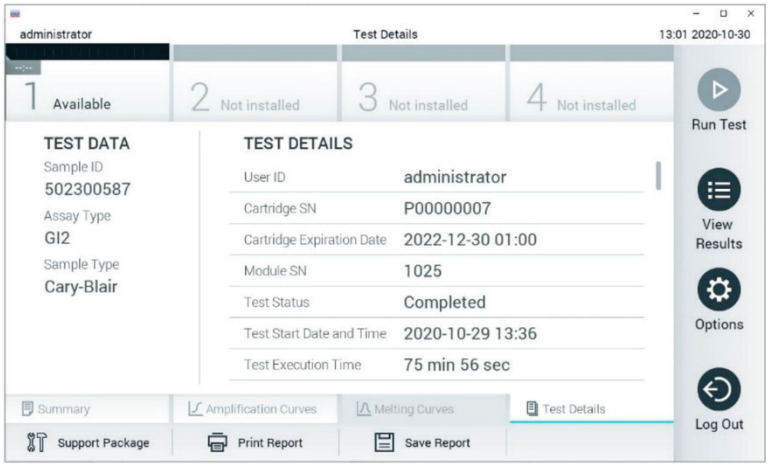

Figure 18. Example screen showing Test Data on the left panel and Test Details in the main panel.

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).

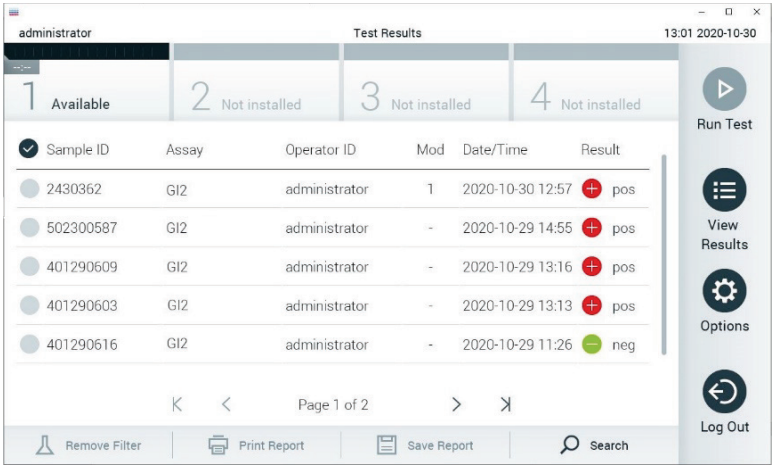

Figure 19. Example View Results screen.

The following information is available for every executed test (Figure 19):

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

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

Select one or more test results by pressing the **gray circle** to 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).

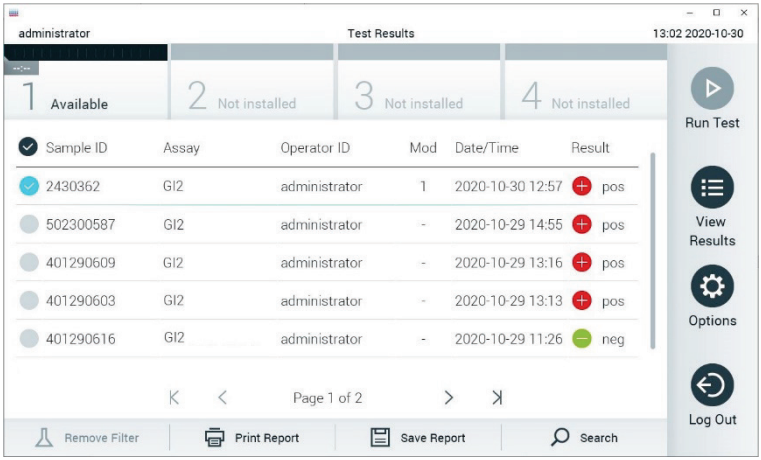

Figure 20. Example of selecting Test Results in the View Results screen.

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 test results displayed on View Results screen

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

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

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

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

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

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

For other parameters, such as **Assay**, a dialog 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 T symbol to the left of a column headline indicates that the column's filter is active.

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

Exporting results to a USB drive

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

Printing results

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

Sample result interpretation

A result for a gastrointestinal organism is interpreted as "Positive" when the corresponding PCR assay is positive, except for EPEC, STEC, and *E. coli* O157. The result interpretation for EPEC, STEC, and *E. Coli* O157 follows the rationale explained in Table 3, below.

Table 3. Interpretation of EPEC, STEC, and *E. coli* O157 results

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

***Note: Amplification curve, EP and Ct values when STEC *stx1* + *stx2* is detected correspond to the STEC *stx2* only.**

Internal control results are to be interpreted according to Table 4.

Table 4. Interpretation of Internal Control results

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

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

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

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

Quality Control

Internal control interpretation

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

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

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

External control information

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

Limitations

- Results from the QIAstat-Dx Gastrointestinal Panel 2 are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions.
- For prescription use only.
- The performance of this test has only been validated with human stool collected in Cary-Blair transport medium, according to the media manufacturers' instructions. It has not been validated for use with other stool transport media, rectal swabs, raw stool, vomitus, or endoscopy stool aspirates.
- The QIAstat-Dx Gastrointestinal Panel 2 should not be used to test Cary-Blair vials from collection devices that have been overfilled with stool. Only stool resuspended following the collection device manufacturer's instructions should be used.
- The performance of this test has not been determined for patients without signs and symptoms of gastrointestinal illness.
- Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient. Due to high rates of asymptomatic carriage of *Clostridium difficile*, especially in very young children and hospitalized patients, the detection of toxigenic *C. difficile* should be interpreted within the context of guidelines developed by the testing facility or other experts.
- Positive results do not rule out co-infection with organisms not included in the QIAstat-Dx Gastrointestinal Panel 2. The agent detected may not be the definitive cause of the disease.
- Negative results do not preclude infection of the gastrointestinal tract. Not all agents of acute gastrointestinal infection are detected by this assay and sensitivity in some clinical settings may differ from that described in the Instructions for Use.
- A negative result with the QIAstat-Dx Gastrointestinal Panel 2 does not exclude the infectious nature of the syndrome. Negative assay results may originate from several factors and their combinations, including sample handling mistakes, variation in the nucleic acid sequences targeted by the assay, infection by organisms not included in the assay, organism levels of included organisms that are below the limit of detection for the assay and use of certain medications (e.g., calcium carbonate).

- The QIAstat-Dx Gastrointestinal Panel 2 is not intended for testing of samples other than those described in this Instructions for Use. Test performance characteristics have been established only with unpreserved stool samples resuspended in Cary-Blair transport medium.
- The QIAstat-Dx Gastrointestinal Panel 2 is intended to be used in conjunction with standard of care culture for organism recovery, serotyping and/or antimicrobial susceptibility testing where applicable.
- The results from the QIAstat-Dx Gastrointestinal Panel 2 must be interpreted by a trained healthcare professional within the context of all relevant clinical, laboratory, and epidemiological findings.
- The QIAstat-Dx Gastrointestinal Panel 2 can be used only with the QIAstat-Dx Analyzer 1.0.
- The identification of multiple diarrheagenic *E. coli* pathotypes has historically relied upon phenotypic characteristics, such as adherence patterns or toxigenicity in certain tissue culture cell lines. The QIAstat-Dx Gastrointestinal Panel 2 targets genetic determinants characteristic of most pathogenic strains of these organisms but may not detect all strains having phenotypic characteristics of a pathotype. In particular, the QIAstat-Dx Gastrointestinal Panel 2 will only detect Enteroaggregative *E. coli* (EAEC) strains carrying the *aggR* and/or *aatA* markers on the pAA (aggregative adherence) plasmid; it will not detect all strains exhibiting an aggregative adherence pattern.
- 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, therefore "Detected" results for multiple diarrheagenic *E. coli/Shigella* may be due to co-infection with multiple pathotypes or, less frequently, may be due to the presence of a single organism containing genes characteristic of multiple pathotypes. An example of the latter is the 2019 *E. coli* hybrid ETEC/STEC strains found in Sweden*.

* Bai X, Zhang J, Ambikan A, et al. Molecular Characterization and Comparative Genomics of Clinical Hybrid Shiga Toxin-Producing and Enterotoxigenic *Escherichia coli* (STEC/ETEC) Strains in Sweden. Sci Rep. 2019;9(1):5619. Published 2019 Apr 4. doi:10.1038/s41598-019-42122-z

- The QIAstat-Dx Gastrointestinal Panel 2 detects heat-stable toxin variants (ST1a and ST1b) and the heat-labile toxin (LT) of Enterotoxigenic *E. coli* (ETEC), which are associated with human disease. The variant LT-II toxin (structurally similar to LT) and the STB/ST2 toxin (structurally dissimilar to ST1) are not targeted by the ETEC oligonucleotide designs and have not been established as important in human disease.
- The QIAstat-Dx Gastrointestinal Panel 2 detects Enteropathogenic *E. coli* (EPEC) through targeting of the *eae* gene, which encodes the adhesin intimin. As some Shiga-like toxin-producing *E. coli* (STEC) also carry *eae* (in particular, strains identified as enterohemorrhagic *E. coli*; EHEC), the QIAstat-Dx Gastrointestinal Panel 2 cannot distinguish between STEC containing *eae* and a co-infection of EPEC and STEC. Therefore, the EPEC result is not applicable (N/A) and not reported for specimens in which STEC has also been detected. In rare cases, STEC may be reported as EPEC when a STEC carrying *eae* (EHEC) is present in a specimen below the LoD of the STEC oligonucleotide design(s) (*stx1/stx2*). Rare instances of other organisms carrying *eae* have been documented; e.g., *Escherichia albertii*, and *Shigella boydii*.
- *Shigella dysenteriae* serotype 1 possess a shiga toxin gene (*stx*) that is identical to the *stx1* gene of STEC. Stx genes have been more recently found in other *Shigella* species (e.g., *S. sonnei* and *S. flexneri*). The detection of both *Shigella*/Enteroinvasive *E. coli* (EIEC) and STEC *stx1/stx2* analytes in the same specimen may indicate the presence of *Shigella* species such as *S. dysenteriae*. Rare instances of the detection of Shiga-like toxin genes in other genera/species have been reported; e.g., *Acinetobacter haemolyticus*, *Enterobacter cloacae* and *Citrobacter freundii*.
- The presence of *Shigella* species *carrying the stx1* gene, such as S. *dysenteriae* in the specimen will be reported as STEC *stx1* + *Shigella*. Being the EPEC result not applicable (N/A) due to the reporting of STEC. Therefore, the QIAstat-Dx Gastrointestinal Panel will not report EPEC in the event of a co-infection with *Shigella* species carrying stx1 gene.
- *E. coli* O157 result is only reported as specific serogroup identification in association with STEC *stx1/stx2*. While non-STEC O157 strains have been detected in human stool, their role in disease has not been established. Serotype O157 EPEC has been identified and will be detected by the QIAstat-Dx Gastrointestinal Panel 2 (by the EPEC

oligonucleotides design) due to their carriage of the *eae* gene. The *E. coli* O157 result will be not applicable (N/A) due to the absence of STEC.

- The QIAstat-Dx Gastrointestinal Panel 2 cannot distinguish between infections with a single toxigenic STEC O157 or rare co-infections of STEC (non-O157) with a stx-negative E. coli O157, that will also be detected as STEC O157.
- This test only detects *Campylobacter jejuni*, *C. coli* and *C. upsaliensis* and does not differentiate between these three species of *Campylobacter*. Additional testing is required to differentiate between these species and to detect other *Campylobacter* species that may be present in stool specimens. In particular the *Campylobacter upsaliensis* oligonucleotides design may cross-react with the *Campylobacter* species *C. lari* and *C. helveticus* organisms.
- A negative QIAstat-Dx Gastrointestinal Panel 2 result does 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 error, sample mix-up, or an infection caused by an organism not detected by the panel. Test results may also be affected by concurrent antimicrobial therapy or levels of organism in the sample that are below the limit of detection for the test. 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.
- The performance of the QIAstat-Dx Gastrointestinal Panel 2 has not been established in individuals who received Rotavirus A vaccine. Recent oral administration of a Rotavirus A vaccine may cause positive results for Rotavirus A if the virus is passed in the stool.
- Based on the available sequences, a few *Cryptosporidium* species, or certain variants of species, including *C. wrari*, may not be efficiently detected by the *Cryptosporidium* design. These species are rarely detected in human samples.
- 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.
- State and local public health authorities have published guidelines for notification of reportable diseases in their jurisdictions including *Salmonella*, *Shigella*, *V. cholerae*, *E. coli* O157, Enterotoxigenic *E. coli* (ETEC) *lt/st*, and Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* to determine necessary measures for verification of results to identify and trace outbreaks. Laboratories are responsible for following their state or local regulations for submission of clinical material or isolates on positive specimens to their state public health laboratories.
- There is a risk of false-positive values resulting from cross-contamination by target organisms, their nucleic acids, or the amplified product.
- All assay results should be used and interpreted in the context of a full clinical evaluation as an aid in the diagnosis of gastrointestinal infection.
- There is a risk of false-positive values resulting from non-specific signals in the assay.
- Analyte targets (virus, bacteria, or parasite nucleic acid sequences) may persist *in vivo*, independent of virus, bacteria, or parasite viability. Detection of analyte target(s) does not guarantee that the corresponding live organism(s) is present, or that the corresponding organism(s) is the causative agent for clinical symptoms.
- 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.
- Underlying polymorphisms in primer-binding regions can affect the targets being detected and subsequently the test results returned.
- There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
- There is a risk of false negative values due to the presence of strain/species sequence variability in the targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.

- The performance of this test has not been established for monitoring treatment of infection with any of the targeted microorganisms.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is low.
- The effect of interfering substances has only been evaluated for those listed in the labeling at its indicated amount or concentration. Interference by substances other than those described in the "Interfering Substances" section of the Instruction for Use can lead to erroneous results.
- Cross-reactivity with gastrointestinal tract organisms other than those listed in the "Analytical Specificity" section of the package insert may lead to erroneous results.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- The assay sensitivity to detect *Cyclospora cayetanensis*, Adenovirus F41, *Entamoeba histolytica* and the Shiga-like toxin- producing *Escherichia coli* (STEC) might be reduced up to 3.16-fold when using half-input sample volume (100 µL) workflow detailed in Appendix C.

Performance Characteristics

Analytical performance

Sensitivity (Limit of Detection)

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

The LoD for each of the QIAstat-Dx Gastrointestinal Panel 2 target pathogenic organisms was assessed, using in total 48 pathogen strains, by analyzing serial dilutions of analytical samples prepared from culture isolates from commercial suppliers (e.g., ZeptoMetrix® and ATCC®), confirmed clinical isolates, or artificial samples for target analytes commercially unavailable. Each sample tested was prepared in human stool matrix, which consists of a pool of previously tested negative clinical stool specimens resuspended in Cary-Blair transport medium.

Each of the 48 strains was tested in human stool matrix prepared following the manufacturer's instructions for the Para-Pak C&S® collection device.

Individual LoD values for each QIAstat-Dx Gastrointestinal Panel 2 target is shown in Table 6.

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

Pathogen	Strain	Source	Concentration (molecular units: 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	20/20
	Campylobacter jejuni Z086	ZeptoMetrix 801650	14491	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	56165	2259.4 CFU/ml	20/20
	Campylobacter upsaliensis RM3195	ATCC BAA-1059	7631	35 CFU/vial	19/20
Clostridium difficile toxin A/B	(NAP1A) Toxinotype III A+ B+	ZeptoMetrix 801619	11083	515 CFU/ml	19/20
	Toxinotype 0 A+ B+	ATCC 9689	101843	853.2 CFU/ml	20/20
Plesiomonas shigelloides	Z130	ZeptoMetrix 801899	481	2291 CFU/ml	20/20
	Bader	ATCC 14029	116	2.7 CFU/vial	19/20
Salmonella	*Salmonella enterica* Serovar choleraseus	ATCC 13312	647	91.6 CFU/ml	20/20
	Salmonella enterica Serovar Typhimurium Z005	ZeptoMetrix 801437	1441	4518.8 CFU/ml	20/20
Vibrio cholerae	Z132; toxigenic	ZeptoMetrix 801901	28298	13600 CFU/ml	20/20
	Z133; non-toxigenic	ZeptoMetrix 801902	79749	54668 CFU/ml	20/20
Vibrio parahaemolyticus	EB 101	ATCC 17802	12862	1600 CFU/ml	20/20
	Z134	ZeptoMetrix 801903	8904	143 CFU/ml	20/20

(continued on the next page)

Table 6. LoD values obtained for the different gastrointestinal target strains tested with the QIAstat-Dx Gastrointestinal Panel 2 (continued from previous page)

Pathogen	Strain	Source	Concentration (molecular units: copies/ml)	Concentration (microbiological units)	Detection rate
Vibrio vulnificus	329 [CDC B3547]	ATCC 33817	109131	260 CFU/ml	20/20
	324 [CDC B629]	ATCC 27562	2983	1305.1 CFU/ml	20/20
Yersinia enterocolitica	Z036	ZeptoMetrix 0801734	719	2070 CFU/ml	20/20
	subsp. *enterocolitica* NTCC 11175, Biotype 4, serotype 3	ATCC 700822	2496	120.1 CFU/ml	20/20
Enteroaggregative *E. coli* (EAEC)	*Escherichia coli* 92.0147, O77:HN	ZeptoMetrix 0801919	1075	634 CFU/ml	20/20
	Escherichia coli CDC3250-76, O111a, 111b: K58:H21	ATCC 29552	842	87 CFU/ml	19/20
Enteroinvasive *E. coli* (EIEC)/ *Shigella*	*Shigella sonnei* Z004	ZeptoMetrix 25931	488	0.2 CFU/ml	20/20
	Escherichia coli CDC EDL 1282, O29:NM	ATCC 43892	1431	41.3 CFU/ml	20/20
Enteropathogenic *E. coli* (EPEC)	*Escherichia coli* O111:NM (EPEC)	ZeptoMetrix 0801747	1817	2581.7 CFU/ml	20/20
	Escherichia coli 7.1493; EPEC; O84:H28	Zeptometrix 801938	29021	1190 CFU/ml	20/20
Enterotoxigenic *E. coli* (ETEC) *lt/st*	*Escherichia coli* H10407, O78:H11	ATCC 35401	367	10.1 CFU/ml	19/20
	Escherichia coli ETEC; ST+, LT+	ZeptoMetrix 801624	855	567 CFU/ml	20/20
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	*Escherichia coli* O26:H4	ZeptoMetrix 801748	2012	726.8 CFU/ml	20/20
Shiga-like toxin-producing *E. coli* (STEC) *E. coli* O157	*Escherichia coli* O157:H7; EDL933	ZeptoMetrix 801622	1217	2281.5 CFU/ml	STEC stx 1: 19/20 STEC stx2: 19/20 O157: 19/20

(continued on the next page)

Table 6. LoD values obtained for the different gastrointestinal target strains tested with the QIAstat-Dx Gastrointestinal Panel 2 (continued from previous page)

Pathogen	Strain	Source	Concentration (molecular units: copies/ml)	Concentration (microbiological units)	Detection rate
Cryptosporidium	*Cryptosporidium hominis*	Public Health Wales UKM 84	357	N/A	20/20
	Cryptosporidium parvum – Iowa isolate	Waterborne® P102C	661	N/A	20/20
Cyclospora cayetanensis	N/A	LACNY-Clinical sample LAC2825	53	N/A	19/20
	N/A	LACNY Clinical sample LAC2827	137	N/A	20/20
Entamoeba histolytica	HM-1:IMSS (Mexico City 1967)	ATCC 30459	7	0.2 cells/ml	20/20
	HK-9 (Korea)	ATCC 30015	1	0.01 cells/ml	19/20
Giardia lamblia	WB (Bethesda)	ATCC 30957	11850	632 cells/ml	19/20
	Portland-1	ATCC 30888	14500	635 cells/ml	20/20
Adenovirus F40/F41	Type 40 (Dugan)	ZeptoMetrix 0810084CF	11726	0.1 $TCID_{50}$/ml	20/20
	Type 41 (Tak)	ZeptoMetrix 0810085CF	979	0.5 $TCID_{50}$/ml	19/20
Astrovirus	ERE IID 2371 (type 8)	Zeptometrix 0810277CF	11586371	11.7 $TCID_{50}$/ml	20/20
	ERE IID 2868 (type 4)	Zeptometrix 0810276CF	52184	1.3 $TCID_{50}$/ml	19/20
Norovirus GI	GI.1 (recombinant)	ZeptoMetrix 0810086CF	24629	891.1 $TCID_{50}$/ml	19/20
Norovirus GII	GII.4 (recombinant)	ZeptoMetrix 0810087CF	8998	1.1 $TCID_{50}$/ml	20/20
Rotavirus A	69M	ZeptoMetrix 0810280CF	5787	436.1 $TCID_{50}$/ml	19/20
	Wa	ZeptoMetrix 0810041CF	5201	14.1 $TCID_{50}$/ml	19/20
Sapovirus	Genogroup I, genotype 1	QIAGEN Barcelona - Clinical sample GI-88	187506	N/A	20/20
	Genogroup V	Universitat de Barcelona 160523351	3007	N/A	20/20

Exclusivity (Analytical Specificity)

The analytical specificity study was carried out by *in vitro* testing and *in silico* analysis (9) to assess the potential cross-reactivity and exclusivity of the QIAstat-Dx Gastrointestinal Panel 2. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity and Off-panel organisms were tested to evaluate cross-reactivity with organisms not covered by the panel content. The On-panel and Off-panel organisms tested are shown in Table 7 and Table 8, respectively.

Samples were prepared by single spiking organisms into negative stool resuspended in Cary-Blair at the highest concentration possible based on the organism stock, preferably at 10^5 $TCID_{50}$/ml for viral, 10^5 cells/ml for parasite targets and 10^6 CFU/ml for bacterial targets. The pathogens were tested in 3 replicates. There was no intra-panel or Off-panel cross-reactivity for all pathogens tested *in vitro*, except for two non-targeted *Campylobacter* species (*C. helveticus* and *C. lari*) that cross-reacted with the Campylobacter assay oligonucleotides included in the QIAstat-Dx Gastrointestinal Panel 2.

Table 7. List of Analytical Specificity on-panel pathogens tested

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

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

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

In silico predictions of potential cross-reactions showed that the following cross-reactions may occur when testing stool samples with the QIAstat-Dx Gastrointestinal Panel 2 (Table 9) (5, 15–17).

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

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

* Note that these potential cross-reactions affect designs with target genes responsible of the pathogenicity of the corresponding QIAstat-Dx Gastrointestinal Panel 2 target pathogens which can be acquired within species in a known biological process in bacteria called horizontal gene transfer.

† Rare or less common *eae* intimin carrier organisms.

‡ On-panel target.

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

¶ Rare or less common Stx toxins producers.

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

Inclusivity (Analytical Reactivity)

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

In vitro (Wet) testing

QIAstat-Dx Gastrointestinal Panel 2 is inclusive for 100% (143 out of 143) of the pathogen strains tested *in vitro*. Most pathogen strains evaluated in wet testing (133/143) were detected at ≤ 3-fold of the corresponding LoD reference strain. (Table 10).

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

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

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Campylobacter	***Campylobacter coli***	**76-GA2 [LMG 21266]**	**ATCC**	**43478***	**1x LoD**
	Campylobacter coli	Z293	ZeptoMetrix	0804272	1x LoD
	Campylobacter coli	CIP 7080 [1407, CIP 70.80]	ATCC	33559*	3x LoD
	Campylobacter jejuni	**Z086**	**ZeptoMetrix**	**0801650***	**1x LoD**
	Campylobacter jejuni	subsp. *jejuni* RM3193	ATCC	BAA-1234*	0.1x LoD
	Campylobacter jejuni subsp. jejuni	O:19 HL7; D3180	ATCC	BAA-218	0.1x LoD
	Campylobacter jejuni subsp. jejuni	AS-83-79	ATCC	33291	0.1x LoD
	Campylobacter jejuni subsp. doylei	NCTC 11951	ATCC	49349	0.1x LoD
	Campylobacter upsaliensis	**NCTC 11541**	**ZeptoMetrix**	**0801999***	**1x LoD**
	Campylobacter upsaliensis	RM 3195 (1994)	ATCC	BAA-1059*	0.3x LoD
	Campylobacter upsaliensis	NCTC 11541 [C231]	ATCC	43954	1x LoD

* Strain tested during LoD verification study.

Table 10b. Inclusivity test results for *Clostridium difficile* strains.

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Clostridium difficile toxin A/B	***Clostridium difficile***	**(90556-M6S) Toxinotype 0 A+ B+**	**ATCC**	**9689***	**1x LoD**
	Clostridium difficile	NAP1, toxinotype IIIb A+B+	ATCC	BAA-1805	1x LoD
	Clostridium difficile	5325, toxinotype V A+B+	ATCC	BAA-1875	1x LoD
	Clostridium difficile	1470, toxinotype VIII A-B+	ATCC	43598	1x LoD
	Clostridium difficile	toxinotype XII A+B+	ATCC	BAA-1812	1x LoD
	Clostridium difficile	toxinotype XXII A+B (unknown)	ATCC	BAA-1814	1x LoD
	Clostridium difficile	NAP1A, toxinotype III A+B+	ATCC	0801619*	0.1x LoD
	Clostridium difficile	NAP1, toxinotype III A+B+	ZeptoMetrix	0801620	3x LoD

*Strain tested during LoD verification study.

Table10c. Inclusivity test results for *Plesiomonas shigelloides* strains.

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

*Strain tested during LoD verification study.

Table 10d. 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	Subps. Salame, II NCTC 10310 [JT945, SS140/61]	ATCC	700151	0.1x LoD
	Salmonella enterica	Subps. diarizonae IIIb, 62	ATCC	29934	0.1x LoD
	Salmonella enterica	Subps. houtenae IV, CIP 82.32 [264.66]	ATCC	43974	0.1x LoD
	Salmonella enterica	Subps. Indica VI, CIP 102501 [F. Kauffmann 1240]	ATCC	43976	0.1x LoD
	Salmonella enterica	Subsp. Enterica, serovar Agona, CDC 873 [CDC 1111-61]	ATCC	51957	0.1x LoD

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Table 10d. Inclusivity test results for Salmonella strains (continued from previous page)

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

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Vibrio cholerae	***Vibrio cholerae***	**Z133; non-toxigenic**	**ZeptoMetrix**	**801902***	**1x LoD**
	Vibrio cholerae	Pacini 1854; NCTC 8021, O:1 Ogawa	CECT	514	1x LoD
	Vibrio cholerae	Z132; toxigenic	ZeptoMetrix	0801901*	0.3x LoD

* Strain tested during LoD verification study.

Table 10f. Inclusivity test results for *Vibrio parahaemolyticus* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Vibrio parahaemolyticus	***Vibrio parahaemolyticus***	**EB101 [P. Baumann 113] (Japan)**	**ATCC**	**17802***	**1x LoD**
	Vibrio parahaemolyticus	VP250,O1:KUT	ATCC	BAA-242	1x LoD
	Vibrio parahaemolyticus	205 [9302]	ATCC	33846	3x LoD
	Vibrio parahaemolyticus	Z134	ZeptoMetrix	0801903*	0.3x LoD

* Strain tested during LoD verification study.

Table 10g. Inclusivity test results for *Vibrio vulnificus* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Vibrio vulnificus	***Vibrio vulnificus***	**324 [CDC B9629]**	**ATCC**	**27562***	**1x LoD**
	Vibrio vulnificus	329 [CDC B3547],Biotype 2	ATCC	33817*	1x LoD
	Vibrio vulnificus	Z473	ZeptoMetrix	0804349	3x LoD

* Strain tested during LoD verification study.

Table 10h. Inclusivity test results for Yersinia enterocolitica strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Yersinia enterocolitica	***Yersinia enterocolitica***	**Z036**	**ZeptoMetrix**	**801734***	**1x LoD**
	Yersinia enterocolitica	NTCC 11175, Biotype 4, serotype 3 (O:3)	ATCC	700822*	1x LoD
	Yersinia enterocolitica	33114 [CCUG 11291, CCUG 12369, CIP 80.27, DSM 4780, LMG 7899, NCTC 12982], Biovar 1, O:8	ATCC	9610	1x LoD
	Yersinia enterocolitica	O:9	ATCC	55075	3x LoD

* Strain tested during LoD verification study.

Table 10i. Inclusivity test results for Enteroaggregative *E. coli* (EAEC) strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Enteroaggregative *E. coli (EAEC)*	***Enteroaggregative E. coli (EAEC)***	**92.0147**	**ZeptoMetrix**	**0801919***	**1x LoD**
	Enteroaggregative *E. coli* (EAEC)	CDC3250-76, O111a, 111b: K58:H21, CVD432+, agg R+, stx1-, stx2-, eae-	ATCC	29552*	1x LoD
	Enteroaggregative E. coli (EAEC)	–	Vall d'Hebrón	Clinical sample; VH 5291403690015	3x LoD

* Strain tested during LoD verification study.

Table 10j. Inclusivity test results for Enteropathogenic *E. coli* (EPEC) strains

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

* Strain tested during LoD verification study.

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

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

* Strain tested during LoD verification study.

Table 10l. Inclusivity test results for Enteroinvasive *E. coli* (EIEC)/*Shigella* strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Enteroinvasive *E. coli (EIEC)/ Shigella*	**Enteroinvasive *E. coli* (EIEC)**	**CDC EDL 1282, O29:NM**	**ATCC**	**43892***	**1x LoD**
	Enteroinvasive *E. coli* (EIEC)	O172:H-	SSI Diagnostica	82171	3x LoD
	Shigella boydii	**Z004**	**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	801627	3x LoD
	Shigella boydii (Serogroup C)	AMC 43-G-58 [M44 (Type 170)]	ATCC	9207	10x LoD

* Strain tested during LoD verification study

Table 10m. Inclusivity test results for Shiga-like toxin-producing *E. coli* (STEC)(stx1-carrier strains)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Shiga-like toxin producing *E. coli* (STEC) - stx1	**Shiga-like toxin producing *E. coli* (STEC) - stx1**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622***	**1x LoD**
	Shiga-like toxin producing *E. coli* (STEC) - stx1	O26:H4,stx1 (+)	ZeptoMetrix	0801748*	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	O22:H8,stx1c (+), stx2b (+)	SSI Diagnostica	91350	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	O8,stx1d (+)	SSI Diagnostica	91349	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	Reference ATCC 35150 (EDL 931), O157:H7,stx1 (+), stx2 (+)	Microbiologics	617	1x LoD
Shiga-like toxin producing *E. coli* (STEC) - stx1	Shiga-like toxin producing *E. coli* (STEC) - stx1	Reference CDC 00-3039, O45:H2,unknown	Microbiologics	1098	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	O103:H2,stx1 (+)	SSI Diagnostica	82170	3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx1	O128ac:H-,stx2f (+)	SSI Diagnostica	91355	10x LoD

* Strain tested during LoD verification study

Table 10n. Inclusivity test results for Shiga-like toxin-producing *E. coli* (STEC) (stx2-carrier strains)

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Shiga-like toxin producing *E. coli* (STEC) - stx2	**Shiga-like toxin producing *E. coli* (STEC) - stx2**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622***	**1x LoD**
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O22:H8,stx1c (+), stx2b (+)	SSI Diagnostica	91350	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O26:H11,stx2a (+)	SSI Diagnostica	95211	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O101:K32:H-,stx2e (+)	SSI Diagnostica	91354	0.3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx2	Reference ATCC 35150 (EDL 931),O157:H7,stx1 (+), stx2 (+)	Microbiologics	617	3x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O92,O107:K+:H48, stx2d (+)	SSI Diagnostica	91352	10x LoD
	Shiga-like toxin producing *E. coli* (STEC) - stx2	O128ac:H-,stx2f (+)	SSI Diagnostica	91355	10x LoD

* Strain tested during LoD verification study

Table 10o. Inclusivity test results for Shiga-like toxin producing *E. coli* (STEC) *stx1/stx2* O157 strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalogue ID	Times LoD
Shiga-like toxin producing *E. coli* (STEC) O157	**Shiga-like toxin producing *E. coli* (STEC) - O157**	**O157:H7; EDL933**	**ZeptoMetrix**	**0801622***	**1x LoD**
	Shiga-like toxin producing *E. coli* (STEC) O157	O128ac:H-,stx2f (+)	SSI Diagnostica	91355†	1x LoD
	Shiga-like toxin producing *E. coli* (STEC) O157	Reference ATCC 35150 (EDL 931), O157:H7, stx1 (+), stx2 (+)	Microbiologics	617	1x LoD

* Strain tested during LoD verification study.

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

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

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

* Strain tested during LoD verification study

Table 10q. Inclusivity test results for *Cyclospora cayetanensis* strains

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

* Strain tested during LoD verification study

Table 10r. Inclusivity test results for *Entamoeba histolytica* strains

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

* Strain tested during LoD verification study

Table 10s. Inclusivity test results for *Giardia lamblia* strains

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

* Strain tested during LoD verification study.

Table 10t. Inclusivity test results for Adenovirus F40/F41 targets

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

* Strain tested during LoD verification study

Table 10u. Inclusivity test results for Astrovirus strains

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

* Strain tested during LoD verification study.

Table 10v. Inclusivity test results for Norovirus GI/GII strains

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

* Strain tested during LoD verification study.

Table 10w. Inclusivity test results for Rotavirus A strains

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

* Strain tested during LoD verification study

Table 10x. Inclusivity test results for Sapovirus strains

QIAstat-Dx target	Pathogen	Strain	Supplier	Catalog ID	Times LoD
Sapovirus	**Human Sapovirus Genogroup I**	**–**	**QIAGEN Barcelona**	**Clinical sample; GI-88***	**1x LoD**
	Human Sapovirus Genogroup V	n/a	Universitat Barcelona	Clinical Sample; 160523351*	1x LoD
	Human Sapovirus Genogroup I	GI.1	Universitat de Barcelona	Clinical sample; 171016324	1x LoD
	Human Sapovirus Genogroup II	GII.3	Universitat de Barcelona	Clinical sample; 215512	1x LoD

* Strain tested during LoD verification study.

In silico analysis

In silico analysis of potential reactivity showed that the following organisms (including species, subspecies, subtypes, serotypes or serovars) are predicted to be detected with the QIAstat-Dx Gastrointestinal Panel 2 (Table 11).

Table 11. Organisms with predicted reactivity based on *in silico* analysis

QIAstat-Dx GI Panel 2 Target	Organisms with predicted reactivity (species, subspecies, subtypes, serotypes or serovars)
Bacteria	
Campylobacter	*Campylobacter coli Campylobacter jejuni, Campylobacter jejuni* subsp. *jejuni, Campylobacter jejuni* subsp. doylei, *Campylobacter upsaliensis*
Clostridium difficile	*Clostridium difficile* (including ribotypes 01 and 17 and strains BI1, BI9, NAP1, SD1, SD2, M68, M120)
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)
Plesiomonas shigelloides	*Plesiomonas shigelloides* (e.g. strains NCTC10360, ATCC 14029T, R4605035)

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Table 11. Organisms with predicted reactivity based on in silico analysis (continued from previous page)

QIAstat-Dx GI Panel 2 Target	Organisms with predicted reactivity (species, subspecies, subtypes, serotypes or serovars)
Bacteria (continued)	
Vibrio cholerae	*Vibrio cholerae* (including serotypes O:1 and non-O:1 (O:37) and biovars El Tor, Bengal)
Vibrio parahaemolyticus	*Vibrio parahaemolyticus*
Vibrio vulnificus	*Vibrio vulnificus*
Yersinia enterocolitica	*Yersinia enterocolitica, Yersinia enterocolitica* subsp. palearctica, *Yersinia enterocolitica* subsp. enterocolitica
Enteroaggregative *E. coli* (EAEC)	Enteroaggregative *E. coli* (EAEC) (including serotypes O104:H4, O111:HND, O126:HND, O25:H4, O86:H2, O86:HND, OUT:H4, OUT:HND)
Enteroinvasive *E. coli* (EIEC)/*Shigella*	Enteroinvasive *E. coli* (EIEC), *Escherichia coli* sp., *Shigella flexneri, Shigella dysenteriae, Shigella boydii, Shigella sonnei*
Enteropathogenic *E. coli* (EPEC)	Enteropathogenic *E. coli* (EPEC) (e.g. including serotypes OUT: HND, OUT:H6, OUT:H34, OUT:H21, O55:H7, O119:HNM, O117) Other eae-carriers bacteria: some Shiga-like toxin-producing *E. coli* (STEC), STEC O157:H7 and few *Shigella boydii* strains
Enterotoxigenic *E. coli* (ETEC)	Enterotoxigenic *E. coli* (ETEC) (including H10407 and E24377A strains and serotypes O169:H41, O25:H42, O148:H28, O6:H16)
Shiga-like toxin-producing *E. coli* (STEC) - *stx1*	Shiga-like toxin-producing *E. coli* (STEC) (including non-O157 serotypes O111:NM, O111:H-, O26:H11, O145:NM, O145:H28, O45:H2, O26:H11, ONT:NM, and including STEC O157 serotypes O157:H7) Stx1 toxin subtypes predicted to be detected include stx1a, stx1c and stx1d Other stx-carriers bacteria: *Shigella sonnei, Shigella dysenteriae*
Shiga-like toxin-producing *E. coli* (STEC) - *stx2*	Shiga-like toxin-producing *E. coli* (STEC) (including non-O157 serotypes O111:NM, O104:H4, O111:H-, O26:H11, O121:H19, O145:H34, O113:H21, ONT:H-, O128:H2, OUT:HNM, O124:HNM and including STEC O157 serotypes O157:H7, O157:NM) Stx2 toxin subtypes predicted to be detected include stx2a, stx2b, stx2c, stx2d, stx2e, stx2f and stx2g
Shiga-like toxin-producing *E. coli* (STEC) O157	*Escherichia coli* O157 including: STEC O157:H7 strains (e.g. EDL933) and *E. coli* O157: non-H7 groups including Non-Shiga-toxigenic *E. coli* O157 bacteria (e.g. serotype O157:H45) Other bacteria with O157 O-antigen: *Escherichia fergusonii* O157

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Table 11. Organisms with predicted reactivity based on in silico analysis (continued from previous page)

QIAstat-Dx GI Panel 2 Target	Organisms with predicted reactivity (species, subspecies, subtypes, serotypes or serovars)
Parasites	
Cryptosporidium	*Cryptosporidium parvum, Cryptosporidium meleagridis, Cryptosporidium canis, Cryptosporidium felis, Cryptosporidium* sp. Rare or non-human species: *Cryptosporidium wrairi*
Cyclospora cayetanensis	*Cyclospora cayetanensis* (including strains LG, CY9, NP20 and NP21)
Entamoeba histolytica	*Entamoeba histolytica* (e.g. strains HM-1: IMSS, EHMfas1, HK-9)
Giardia lamblia	*Giardia lamblia* (aka *Giardia duodenalis, Giardia intestinalis*)[i]
Viruses	
Adenovirus	Human Adenovirus F 40/41
Astrovirus	Human Astrovirus (including types 1, 2, 3, 4, 5, 6, 7, 8)
Norovirus GI/GII	Norovirus genogroup II genotypes: GII.1, GII.2, GII.3, GII.4, GII.4_Sydney 2012, GII.P4_New Orleans 2009, GII.4_DenHaag, GII.4_Hong Kong, GII.5, GII.6, GII.7, GII.8, GII.10, GII.12, GII.13, GII.17, GII.21. Norovirus genogroup I genotypes: GI.1, GI.3, GI.4, GI.5, GI.6, GI.7, GI.8, GI.9.
Rotavirus	Rotavirus A (including strains Wa, ST3, 69M, DS-1, RVA and serotypes G1P[8], G12P[6], G2P[4], G3P[6], G4P[6], G6P[6], G8P[8], G9P[19])
Sapovirus	Genogroups GI (including genotypes GI.1, GI.2, GI.3, GI.4, GI.6), GII (including genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.6), GIV (including genotype GIV.1) and GV (including genotypes GV.1).

Interfering Substances

The effect of potentially interfering substances on the detectability of the QIAstat-Dx Gastrointestinal Panel 2 organisms was evaluated. Forty-three (43) potentially interfering substances were spiked into the sample mixes at a level predicted to be above the concentration of the substance likely to be found in stool specimens. Each organism was tested at 3x LoD and testing was performed in triplicates. Endogenous substances such as human whole blood, human genomic DNA and several pathogens were tested alongside exogenous substances like antibiotics, other gastrointestinal-related medications and different technique-specific substances.

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

Mucin from bovine submaxillary was found to interfere with the detection of *Vibrio cholerae*, EAEC and *Entamoeba* at concentrations above 2.5% w/v.

Human genomic DNA was found to interfere with the detection of *E. coli* O157 and *Entamoeba* at concentrations above 5 µg/ml.

Bisacodyl was found to interfere with the detection of EAEC at concentrations above 0.15% w/v.

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

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

Rotavirus reassortants WC3:2-5, R574(9) and WI79-4,9 used in Rotavirus A vaccines were predicted to be reactive with Rotavirus A in the QIAstat-Dx Gastrointestinal Panel 2. Final concentrations without observable interfering effects on the detection of targets at 3x LoD concentration for WC3:2-5, R574(9) and WI79-4,9 were 8.89×10^{-5} $TCID_{50}$/ml and 1.10 PFU/ml, respectively (see Table 12) for other concentrations tested.

Competitive interference was tested in a subset of pathogens. No interference was observed when evaluating competitive interference by target pathogens when two QIAstat-Dx Gastrointestinal Panel target pathogens were tested by spiking samples with one pathogen target at 3x LoD and one at 50x LoD. Results from the pathogen targets tested are provided in Table 14.

Results from the 43 interfering substances that could be present or introduced in a stool specimen are provided in Table 12.

Table 12. Final highest concentration without observable inhibitory effect

Substance tested	Concentration tested	Result
Endogenous substances		
Bovine and ovine bile	12% w/v	No Interference
Cholesterol	1.5% w/v	No Interference
Fatty acids (palmitic acid)	0.2% w/v	No Interference
Fatty acids (stearic acid)	0.4% w/v	No Interference
Human genomic DNA	20 µg/ml 10 µg/ml 5 µg/ml	Interference Interference No Interference
Human stool (overfill of Cary Blair vial)	300 mg/ml	No Interference
Human urine	50% v/v	No Interference
Human whole blood with Na Citrate	40% v/v	No Interference
Mucin from bovine submaxillary	5% w/v 2.5% w/v	Interference No Interference
Triglycerides	5% w/v	No Interference
Non-target microorganisms		
Aeromonas hydrophila	1 x 10^6 units/ml	No Interference
Bacteroides vulgatus	1 x 10^6 units/ml	No Interference
Bifidobacterium bifidum	1 x 10^6 units/ml	No Interference
Enterovirus Species D, Serotype EV-D68	1 x 10^5 units/ml	No Interference
Non-pathogenic *E. coli*	1 x 10^6 units/ml	No Interference
Helicobacter pylori	1 x 10^6 units/ml	No Interference
Saccharomyces cerevisiae (deposited as *S. boulardii*)	1 x 10^5 units/ml	No Interference
Exogenous substances		
Bacitracin	250U/ml	No Interference
Bisacodyl	0.3% w/v 0.15% w/v	Interference No Interference
Bismuth subsalicylate	0.35% w/v	No Interference
Calcium carbonate (TUMS® Extra Strength 750)	5%w/v 0.5% w/v	Interference No Interference

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

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

Table 13. QIAstat-Dx Gastrointestinal Panel 2 results for competitive interference

Sample Mix	Target	Final concentration tested x LoD	Co-infection detected
Norovirus 50x - Rotavirus 3x	Norovirus GI/GII	50x	Yes
	Rotavirus A	3x	
Norovirus 3x - Rotavirus 50x	Norovirus GI/GII	3x	Yes
	Rotavirus A	50x	
Giardia 50x - Adenovirus 3x	*Giardia lamblia*	50x	Yes
	Adenovirus F40/F41	3x	
Adenovirus 50x - *Giardia* 3x	*Giardia lamblia*	3x	Yes
	Adenovirus F40/F41	50x	
Norovirus 50x -*C.diff* 3x	Norovirus GII	50x	Yes
	Clostridium difficile toxin A/B	3x	
Norovirus 3x - *C.diff* 50x	Norovirus GII	3x	Yes
	Clostridium difficile toxin A/B	50x	
EPEC 50x - EAEC 3x	EPEC	50x	Yes
	EAEC	3x	
EPEC 3x - EAEC 50x	EPEC	3x	Yes
	EAEC	50x	
EPEC 50x - *C.diff* 3x	EPEC	50x	Yes
	Clostridium difficile toxin A/B	3x	
EPEC 3x - *C.diff* 50x	EPEC	3x	Yes
	Clostridium difficile toxin A/B	50x	
EPEC 50x - ETEC 3x	EPEC	50x	Yes
	ETEC	3x	
EPEC 3x - ETEC 50x	EPEC	3x	Yes
	ETEC	50x	
ETEC 50x - EIEC 3x	ETEC	50x	Yes
	EIEC/ *Shigella*	3x	
ETEC 3x - EIEC 50x	ETEC	3x	Yes
	EIEC/ *Shigella*	50x	

Carryover

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

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

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

Reproducibility

Reproducibility testing of contrived samples was performed at three test sites including one internal site (Site A) and two external sites (Site B and Site C). The study incorporated a range of potential variation introduced by sites, days, replicates, cartridge lots, operators, and QIAstat-Dx analyzers. For each site, testing was performed across 5 non-consecutive days with 6 replicates per day (leading to a total of 30 replicates per target, concentration and site), 4 QIAstat-Dx Analyzers (2 analyzers per operator and per site), and at least 2 operators on each testing day. A total of 5 sample mixes (two combined samples at 1x LoD and 3x LoD plus one negative sample) were prepared. For each mix, 6 replicates were tested and evaluated.

Table 14 shows the detection rate per target and concentration for each site of the Reproducibility study. In addition, data obtained at all three sites have been compiled to calculate the exact 2-sided 95% Confidence Interval by target and concentration.

Table 14. Detection rate per target and concentration for each site of the Reproducibility study and exact 2-sided 95% Confidence Interval by target and concentration

			% Agreement with Expected Result			
Pathogen Tested	**Concentration Tested**	**Expected Result**	**Site A**	**Site B**	**Site C**	**All Sites (95% Confidence Interval)**
Adenovirus F41 ZeptoMetrix 0810085CF	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Clostridium difficile ZeptoMetrix 0801619	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Campylobacter ZeptoMetrix 0801650	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
***Escherichia coli* EPEC** ZeptoMetrix 0801747	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	29/30 96.67 %	30/30 100%	89/90 100% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)

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Table 14. 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 from previous page)

			% Agreement with Expected Result			
Pathogen Tested	**Concentration Tested**	**Expected Result**	**Site A**	**Site B**	**Site C**	**All Sites (95% Confidence Interval)**
Entamoeba histolytica ATCC 30459	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	29/30 96.67%	89/90 100% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Giardia lamblia ATCC 30888	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Norovirus GII ZeptoMetrix 0810087CF	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	29/30 96.67%	30/30 100%	30/30 100%	89/90 100% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Rotavirus A ZeptoMetrix 0810280CF	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	29/30 96.67%	30/30 100%	89/90 100% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)

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Table 14. 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 from previous page)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
***Escherichia coli* (STEC) O157:H7** ZeptoMetrix 0801622	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	29/30 96.67%	89/90 100% (93.96 – 99.97%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Escherichia coli (STEC) *stx1* ZeptoMetrix 0801622	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Escherichia coli (STEC) *stx2* ZeptoMetrix 801622	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
Salmonella enterica ZeptoMetrix 801437	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	29/30 96.67%	29/30 96.67%	88/90 100% (92.20 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 – 99.73%)

(continued on the next page)

Table 14. 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 from previous page)

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Vibrio parahaemolyticus *ATCC 17802*	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 – 99.73%)
Yersinia enterocolitica Zeptometrix 801734	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	1x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 - 100.00%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (95.98 – 99.73%)

Clinical Performance

The clinical performance shown below was demonstrated using QIAstat-Dx Analyzer 1.0. A multi-center international observational clinical study was conducted using prospectively and retrospectively collected samples to evaluate the performance of QIAstat-Dx Gastrointestinal Panel 2 during normal conditions of use. The study was conducted in 13 clinical sites across 5 countries (4 sites in Europe and 9 sites in USA) from May 2021 to July 2021.

The final data set consisted of a total of 2,085 leftover de-identified specimens, which were prospectively collected from patients who underwent stool specimen collection for clinical indications of diarrhea caused by gastrointestinal infection at the 13 investigational sites. In addition, testing was performed on archived known positives and contrived specimens to further augment the positive specimen numbers (Table 15). Samples used in the study were all

stool samples in Cary-Blair transport media collected using either Para-Pak C&S (Meridian Bioscience), FecalSwab® (COPAN), Fecal Transwab® (Medical Wire & Equipment Co. (Bath) Ltd), or C & S Medium (Medical Chemical).

Table 15. Prospective and Retrospective Specimen summary across each clinical site of all specimens used in the clinical study

Site/Country	Specimen Type		Total
	Prospective (Fresh)	Retrospective (Archived Frozen)	
Germany	339	21	360
Denmark	293	37	330
Spain	246	60	306
France	63	7	70
USA site 1	186	6	192
USA site 2	43	9	52
USA site 3	281	84	365
USA site 4	177	0	177
USA site 5	44	0	44
USA site 6	39	0	39
USA site 7	148	0	148
USA site 8	131	0	131
USA site 9	95	0	95
Total	2085	224	2309

All prospectively collected specimens that had age, sex, and patient population status were collected by the site. Subject demographics (evaluable specimens) are summarized in Table 16 below.

Table 16. Demographic data for enrolled prospective specimens

Demographic data	N	%
Gender		
Female	1158	55.5
Male	927	44.5
Age Group		
0-6 years	221	10.6
6-21 years	167	8.0
22-49 years	540	25.9
50+ years	1150	55.2
Not Reported	7	0.3

Patient population		
Emergency room	114	5.5
Hospitalized	500	24.0
Immunocompromised	3	0.1
No information available	560	26.9
Outpatient	908	43.5
No. of days between symptom onset and QIAstat-Dx testing		
> 7 days	152	7.3
≤ 7 days	222	10.6
Not Reported	1711	82.1

Performance of the QIAstat-Dx Gastrointestinal Panel 2 was compared to the reference method: BioFire® FilmArray® GI Panel for all targets. For the majority of targets, direct comparison of the two results could be made as a binary result (positive or negative). However, for certain targets the QIAstat-Dx GI Assay provides additional differentiation, so further comparators were required to determine agreement., The appropriate comparator/reference method used for each member of the panel are detailed in the Table 17 below.

Table 17. QIAstat-Dx Gastrointestinal Panel 2 Clinical studies reference method

QIAstat-Dx Gastrointestinal Panel 2 target	Reference method
Adenovirus F40/F41 Astrovirus Norovirus GI/GII Rotavirus A Sapovirus (GI, GII, GIV, GV) *Campylobacter (C. jejuni, C. coli* and *C. upsaliensis)* *Clostridium difficile* (toxin A/B) Enteroaggregative *Escherichia coli* (EAEC) *Shigella*/Enteroinvasive *Escherichia coli* (EIEC) Enteropathogenic *Escherichia coli* (EPEC) Enterotoxigenic *Escherichia coli* (ETEC) *lt/st* Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* *E. coli* O157 serogroup *Salmonella*	BioFire FilmArray Gastrointestinal (GI) Panel

Plesiomonas shigelloides *Vibrio cholerae* *Yersinia enterocolitica* Cryptosporidium *Cyclospora cayetanensis* *Entamoeba histolytica*	
Vibrio parahaemolyticus	BioFire FilmArray GI Panel *Vibrio* + PCR-BDS assay to identify *V. parahaemolyticus*
Vibrio vulnificus	BioFire FilmArray GI Panel *Vibrio* + PCR-BDS assay to identify *V. vulnificus*

When PCR-BDS is referred to: This is a targeted Polymerase Chain Reaction (PCR) assay which was developed and validated for the performance evaluation, when amplification is observed in the PCR, the amplicon was verified by Bi-Directional Sequencing (BDS).

Discordant Results Resolution

Upon discordance with the reference method, resolution testing was performed to determine the presence/absence of specific targets. Table 18 below details the methods used for discordance resolution.

Table 18. Discordant Specimen Testing

QIAstat-Dx Gastrointestinal Panel 2	Discordant testing method
Adenovirus F40/F41 Astrovirus Norovirus GI/GII Rotavirus A Sapovirus (GI, GII, GIV, GV)	BD-MAX Enteric Viral Panel
Campylobacter (C. jejuni, C. coli and *C. upsaliensis)* *Shigella*/Enteroinvasive *E. coli* (EIEC) *Salmonella*	BD-MAX Enteric Bacterial Panel
Enterotoxigenic *E. coli* (ETEC) *lt/st* *Plesiomonas shigelloides* *Yersinia enterocolitica*	BD-MAX Extended Enteric Bacterial Panel
Clostridium difficile (toxin A/B) Enteroaggregative *E. coli* (EAEC) Enteropathogenic *E. coli* (EPEC) Shiga-like toxin- *E. coli* (STEC) *stx1* Shiga-like toxin- *E. coli* (STEC) *stx2* *Vibrio cholerae* *Vibrio parahaemolyticus* *Vibrio vulnificus* *Cryptosporidium* *Giardia lamblia*	PCR with Bi-directional sequencing (PCR-BDS)*

* All Polymerase Chain Reaction (PCR)- Bidirectional Sequencing (BDS) assays represent a validated nucleic acid amplification test (NAAT) followed by bi-directional sequencing. For *Vibrio parahaemolyticus* and *Vibrio vulnificus*, the same PCR-BDS method was used for both discordant testing and differentiation testing..

Clinical Performance – PPA and NPA

A total of 2,309 prospective and archived clinical samples were evaluated to determine the Clinical Performance characteristics of the QIAstat-Dx Gastrointestinal Panel 2. The Positive Percentage Agreement (PPA) and Negative Percent Agreement (NPA) was calculated for each target after discordance resolution for all clinical samples (prospective and retrospective).

Additionally, to supplement the prospective and archived clinical samples data, an evaluation of contrived specimens was performed for several pathogens (Adenovirus F40/F41, Astrovirus, Rotavirus, Sapovirus, *Campylobacter*, ETEC, EIEC/Shigella, STEC *stx1*/*stx2*, *E. coli*

O157, *Plesiomonas shigelloides*, *Salmonella*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Yersinia enterocolitica*, *Cryptosporidium*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, and *Giardia lamblia*), due to low number of prospective and archived clinical samples found during the study. Surrogate specimens were prepared using residual clinical specimens that had previously tested negative for all GI panel analytes targeted by QIAstat-Dx Gastrointestinal Panel 2 and comparator methods. Specimens were spiked around the assay LoD and at clinically relevant levels using different quantified strains for each organism. The analyte status of each contrived specimen was blinded to the users analyzing the specimens. A total of 1,254 cartridge test runs were performed for the contrived samples providing additional data on the rarer pathogens measured by QIAstat-Dx Gastrointestinal Panel 2. PPA was established for the mentioned targets on contrived samples.

The total combined PPA and NPA per pathogen and overall was calculated alongside with the corresponding exact binomial two-sided 95% confidence interval. The results are summarized in Table 19 below.

Table 19. Summary of Clinical Study Results for all clinical specimens (prospective and retrospective), contrived samples and total combined, including the exact binomial two-sided 95% CI

			Sensitivity (PPA)				Specificity (NPA)			
			Fraction	%	95% CI		Fraction	%	95% CI	
Pathogen Type	Target	Sample Type	TP/(TP+FN)		Lower	Upper	TN/(TN+FP)		Lower	Upper
Viruses	Adenovirus F40/F41	Clinical specimens	9/9	100.00	66.37	100.00	2285/2286	99.96	99.76	100.00
		Contrived specimens	68/70	97.14	90.06	99.65	N/A	N/A	N/A	N/A
		Total specimens	**77/79**	**97.47**	**91.15**	**99.69**	**2285/2286**	**99.96**	**99.76**	**100.00**
	Astrovirus	Clinical specimens	13/14	92.86	66.13	99.82	2282/2282	100.00	99.84	100.00
		Contrived specimens	67/68	98.53	92.08	99.96	N/A	N/A	N/A	N/A
		Total specimens	**80/82**	**97.56**	**91.47**	**99.70**	**2282/2282**	**100.00**	**99.84**	**100.00**

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Table 19. Summary of Clinical Study Results for all clinical specimens (prospective and retrospective), contrived samples and total combined, including the exact binomial two-sided 95% CI (continued from previous page)

Pathogen Type	Target	Sample Type	Sensitivity (PPA)				Specificity (NPA)			
			Fraction	%	95% CI		Fraction	%	95% CI	
			TP/(TP+FN)		Lower	Upper	TN/(TN+FP)		Lower	Upper
Viruses	Norovirus GI/GII	Clinical specimens	69/73	94.52	86.56	98.49	2221/2222	99.95	99.75	100.00
		Contrived specimens	**0/0**	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Total specimens	**69/73**	**94.52**	**86.56**	**98.49**	**2221/2222**	**99.95**	**99.75**	**100.00**
	Rotavirus A	Clinical specimens	34/36	94.44	81.34	99.32	2256/2259	99.87	99.61	99.97
		Contrived specimens	69/70	98.57	92.30	99.96	N/A	N/A	N/A	N/A
		Total specimens	**103/106**	**97.17**	**91.95**	**99.41**	**2256/2259**	**99.87**	**99.61**	**99.97**
	Sapovirus	Clinical specimens	16/16	100.00	79.41	100.00	2280/2281	99.96	99.76	100.00
		Contrived specimens	69/69	100.00	94.79	100.00	N/A	N/A	N/A	N/A
		Total specimens	**85/85**	**100.00**	**95.75**	**100.00**	**2280/2281**	**99.96**	**99.76**	**100.00**
Bacteria	*Campylobacter*	Clinical specimens	146/146	100.00	97.51	100.00	2148/2152	99.81	99.52	99.95
		Contrived specimens	45/46	97.83	88.47	99.94	N/A	N/A	N/A	N/A
		Total specimens	**191/192**	**99.48**	**97.13**	**99.99**	**2148/2152**	**99.81**	**99.52**	**99.95**
	Clostridium difficile toxin A/B	Clinical specimens	234/245	95.51	92.11	97.74	2053/2056	99.85	99.57	99.97
		Contrived specimens	**0/0**	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Total specimens	**234/245**	**95.51**	**92.11**	**97.74**	**2053/2056**	**99.85**	**99.57**	**99.97**
	Enteroaggregative *E. coli* (EAEC)	Clinical specimens	83/96	86.46	77.96	92.59	2196/2201	99.77	99.47	99.93
		Contrived specimens	**0/0**	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Total specimens	**83/96**	**86.46**	**77.96**	**92.59**	**2196/2201**	**99.77**	**99.47**	**99.93**

(continued on the next page)

Table 19. Summary of Clinical Study Results for all clinical specimens (prospective and retrospective), contrived samples and total combined, including the exact binomial two-sided 95% CI (continued from previous page)

Pathogen Type	Target	Sample Type	Sensitivity (PPA) Fraction	Sensitivity (PPA) %	Sensitivity (PPA) 95% CI Lower	Sensitivity (PPA) 95% CI Upper	Specificity (NPA) Fraction	Specificity (NPA) %	Specificity (NPA) 95% CI Lower	Specificity (NPA) 95% CI Upper
Bacteria	Enteropathogenic *E. coli* (EPEC)	Clinical specimens	236/256	92.1	88.1	95.16	1980/1984	99.8	99.4	99.95
		Contrived specimens	0/0	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Total specimens	**236/256**	**92.1**	**88.1**	**95.16**	**1980/1984**	**99.8**	**99.4**	**99.95**
	Enterotoxigenic *E. coli* (ETEC) *lt/st*	Clinical specimens	59/62	95.16	86.50	98.99	2235/2236	99.96	99.75	100.00
		Contrived specimens	43/43	100.00	91.78	100.00	N/A	N/A	N/A	N/A
		Total specimens	**102/105**	**97.14**	**91.88**	**99.41**	**2235/2236**	**99.96**	**99.75**	**100.00**
	Shigella/ Enteroinvasive *E. coli* (EIEC)	Clinical specimens	37/38	97.37	86.19	99.93	2259/2259	100.00	99.84	100.00
		Contrived specimens	69/69	100.00	94.78	100.00	N/A	N/A	N/A	N/A
		Total specimens	**106/107**	**99.07**	**94.90**	**99.98**	**2259/2259**	**100.00**	**99.84**	**100.00**
	Shiga-like toxin *E.coli* (STEC) *stx1/stx2**	Clinical specimens	43/50	86.00	73.26	94.18	2244/2246	99.91	99.68	99.99
		Contrived specimens	200/200	100.00	98.17	100.00	N/A	N/A	N/A	N/A
		Total specimens	**243/250**	**97.20**	**94.32**	**98.87**	**2244/2246**	**99.91**	**99.68**	**99.99**
	E. coli O157	Clinical specimens	2/2	100.00	15.81	100.00	38/38	100.00	90.75	100.00
		Contrived specimens	67/69	97.1	89.9	99.65	N/A	N/A	N/A	N/A
		Total specimens	**69/71**	**97.1**	**90.1**	**99.66**	**38/38**	**100.00**	**90.7**	**100.00**

Plesiomonas shigelloides	Clinical specimens	8/8	100.0	63.0	100.00	2283/2288	99.7	99.4	99.93
	Contrived specimens	67/68	98.5	92.0	99.96	N/A	N/A	N/A	N/A
	Total specimens	**75/76**	**98.6**	**92.8**	**99.97**	**2283/2288**	**99.7**	**99.4**	**99.93**
Salmonella	Clinical specimens	71/71	100.0	94.9	100.00	2225/2227	99.9	99.6	99.99
	Contrived specimens	33/33	100.0	89.4	100.00	N/A	N/A	N/A	N/A
	Total specimens	**104/104**	**100.0**	**96.5**	**100.00**	**2225/2227**	**99.9**	**99.6**	**99.99**

(continued on the next page)

Table 19. Summary of Clinical Study Results for all clinical specimens (prospective and retrospective), contrived samples and total combined, including the exact binomial two-sided 95% CI (continued from previous page)

			Sensitivity (PPA)				Specificity (NPA)			
			Fraction	%	95% CI		Fraction	%	95% CI	
Pathogen Type	Target	Sample Type	TP/(TP+FN)		Lower	Upper	TN/(TN+FP)		Lower	Upper
Bacteria	*Vibrio cholerae*	Clinical specimens	2/2	100.00	15.81	100.00	2294/2294	100.00	99.84	100.00
		Contrived specimens	67/70	95.71	87.98	99.11	N/A	N/A	N/A	N/A
		Total specimens	**69/72**	**95.83**	**88.30**	**99.13**	**2294/2294**	**100.00**	**99.84**	**100.00**
	Vibrio parahaemoly-ticus	Clinical specimens	3/4	75.00	19.41	99.37	2291/2292	99.96	99.76	100.00
		Contrived specimens	70/70	100.00	94.87	100.00	N/A	N/A	N/A	N/A
		Total specimens	**73/74**	**98.65**	**92.70**	**99.97**	**2291/2292**	**99.96**	**99.76**	**100.00**
	Vibrio vulnificus	Clinical specimens	0/0	N/A	N/A	N/A	2296/2296	100.00	99.84	100.00
		Contrived specimens	69/69	100.00	94.79	100.00	N/A	N/A	N/A	N/A
		Total specimens	**69/69**	**100.00**	**94.79**	**100.00**	**2296/2296**	**100.00**	**99.84**	**100.00**
	Yersinia enterocolitica	Clinical specimens	51/51	100.00	93.02	100.00	2232/2246	99.38	98.96	99.66
		Contrived specimens	68/69	98.55	92.19	99.96	N/A	N/A	N/A	N/A
		Total specimens	**119/120**	**99.17**	**95.44**	**99.98**	**2232/2246**	**99.38**	**98.96**	**99.66**
Parasites	*Cryptosporidium spp.*	Clinical specimens	19/21	90.48	69.62	98.83	2272/2275	99.87	99.62	99.97
		Contrived specimens	58/58	100.00	93.84	100.00	N/A	N/A	N/A	N/A
		Total specimens	**77/79**	**97.47**	**91.15**	**99.69**	**2272/2275**	**99.87**	**99.62**	**99.97**
	Cyclospora cayetanensis	Clinical specimens	25/26	96.15	80.36	99.90	2269/2269	100.00	99.84	100.00
		Contrived specimens	56/56	100.00	93.62	100.00	N/A	N/A	N/A	N/A
		Total specimens	**81/82**	**98.78**	**93.39**	**99.97**	**2269/2269**	**100.00**	**99.84**	**100.00**

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Table 19. Summary of Clinical Study Results for all clinical specimens (prospective and retrospective), contrived samples and total combined, including the exact binomial two-sided 95% CI (continued from previous page)

Pathogen Type	Target	Sample Type	Sensitivity (PPA)				Specificity (NPA)			
			Fraction	%	95% CI		Fraction	%	95% CI	
			TP/(TP+FN)		Lower	Upper	TN/(TN+FP)		Lower	Upper
Parasites	*Entamoeba histolytica*	Clinical specimens	0/0	N/A	N/A	N/A	2295/2295	**100.00**	**99.84**	**100.00**
		Contrived specimens	69/70	98.57	92.30	99.96	N/A	N/A	N/A	N/A
		Total specimens	**69/70**	**98.57**	**92.30**	**99.96**	**2295/2295**	**100.00**	**99.84**	**100.00**
	Giardia lamblia	Clinical specimens	36/36	100.00	90.26	100.00	**2254/2259**	99.78	99.48	99.93
		Contrived specimens	56/56	100.00	93.62	100.00	N/A	N/A	N/A	N/A
		Total specimens	**92 /92**	**100.00**	**96.07**	**100.00**	**2254/2259**	**99.78**	**99.48**	**99.93**
Overall Clinical Samples			**1196/1262**	**94.77**	**93.39**	**95.93**	**49188/49243**	**99.89**	**99.85**	**99.92**
Overall Contrived Specimens			**1310/1323**	**99.02**	**98.33**	**99.48**	**N/A**	**N/A**	**N/A**	**N/A**
Overall Total combined			**2506/2585**	**96.94**	**96.21**	**97.57**	**49188/49243**	**99.89**	**99.85**	**99.92**

*__Note__: The differentiation of *stx1* and *stx2* toxin genes from Shiga-like toxin-producing *E. coli* (STEC) was substantiated during the clinical evaluation of contrived specimens. Contrived specimens for STEC (*stx1/stx2*) evaluation were spiked with the following strains and toxinotypes: ZeptoMetrix #0801748 (stx1+), SSI #95211 (*stx2a*+) and ZeptoMetrix #0801622 (*stx1*+, *stx2*+). In total 134 and 135 contrived specimens were evaluated for STEC *stx1* and STEC *stx2* analytes, respectively, showing both a 100% detection rate. Analytical Reactivity studies evaluated additional STEC *stx1*-carrier and *stx2*-carrier strains (see Tables 10m-o).

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

Additional information about specific QIAstat-Dx Gastrointestinal Panel 2 error codes and messages can be found in Table 20.

Table 20. Information about specific QIAstat-Dx Gastrointestinal Panel 2 error codes and messages

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

Symbols

The following table describes the symbols that may appear on the labeling or in this document.

Symbols	Description
	Contains reagents sufficient for <N> reactions
	Use by
	For in vitro diagnostic use
	Manufacturer
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Gastrointestinal application
Rn	R is for revision of the Handbook and n is the revision number
	Temperature limitation
	Consult instructions for use
	Caution
	Serial number

	Do not reuse
	Keep away from sunlight
	Do not use if package is damaged
	Global Trade Item Number
	Flammable, risk of fire
	Corrosive, risk of chemical burn
	Health hazard, risk of sensitization, carcinogenicity
	Risk of harm

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Appendices

Appendix A: Installing the Assay Definition File

The Assay Definition File (ADF 1.1) of the QIAstat-Dx Gastrointestinal Panel 2 must be installed on the QIAstat-Dx Analyzer 1.0 prior to testing with QIAstat-Dx Gastrointestinal Panel 2 Cartridges.

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

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

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

1. Insert the USB stick containing the Assay Definition File into one of the USB ports on the QIAstat-Dx Analyzer 1.0.
2. Press the Options button and then select Assay Management. The Assay Management screen appears in the Content area of the display (Figure 25).

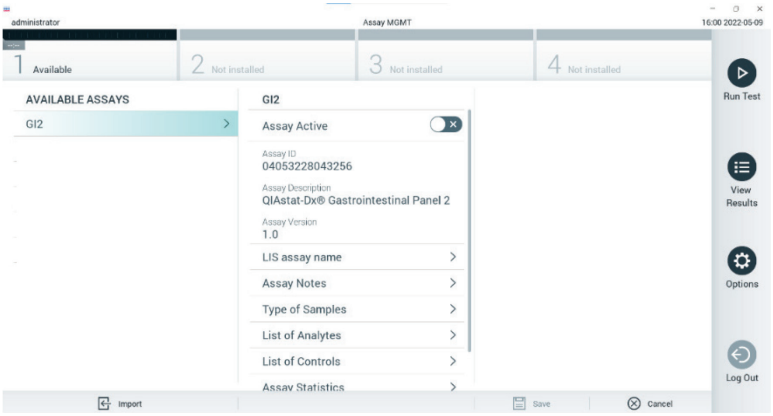

Figure 21. Assay Management screen.

3. Press the Import icon in the bottom left of the screen (Figure 54).
4. Select the file corresponding to the assay to be imported from the USB drive.
5. A dialog box will appear to confirm upload of the file.
6. A dialog box may appear to override the current version by a new one. Press **Yes** to override (Figure 26).

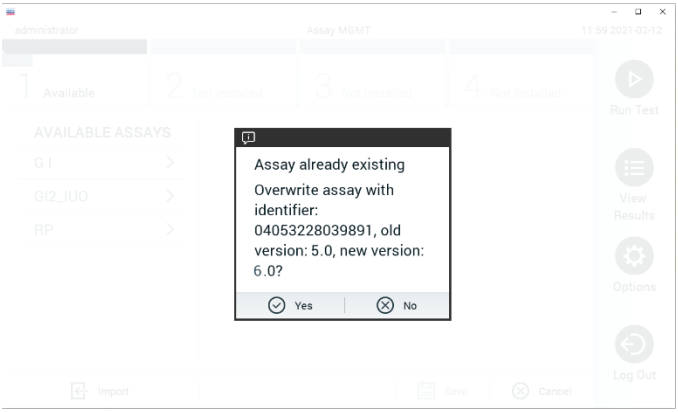

Figure 22. Dialog that appears when upgrading the ADF version.

7. The assay becomes active by selecting Assay Active (Figure 27).

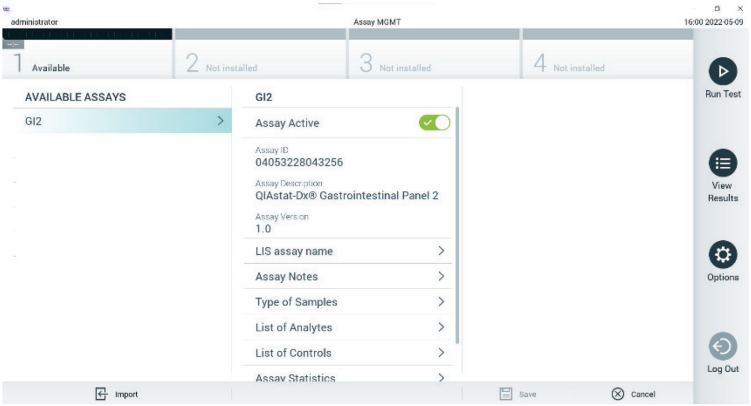

Figure 23. Activating the assay.

17. Assign the active assay to the user by pressing the Options button and then the User Management button. Select the user who should be allowed to run the assay. If it is needed, this action can be repeated for every user created in the system. Next, select Assign Assays from the "User Options". Enable the assay and press the Save button (Figure 28).

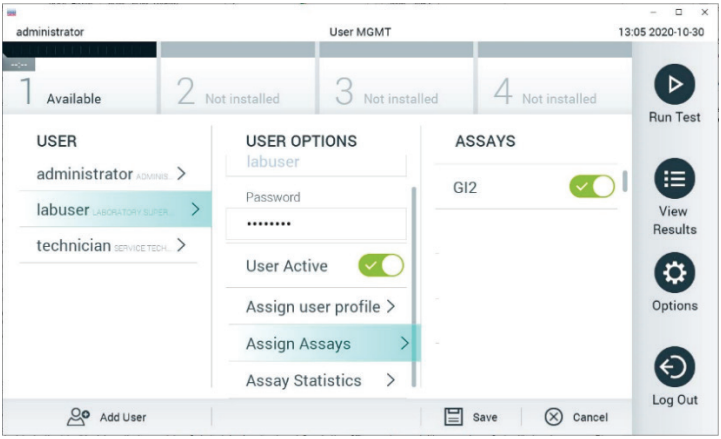

Figure 24. Assigning the active assay.

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

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

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

IFU: Instructions For Use.

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

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

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

PCR: Polymerase Chain Reaction.

IUO: For investigational use only

RT: Reverse Transcription.

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

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

Appendix C: Additional Instructions for use

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

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

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

1. Open the package of a new QIAstat-Dx Gastrointestinal Panel 2 Cartridge using the tear notches on the sides of the packaging.
2. Remove the QIAstat-Dx Gastrointestinal Panel 2 Cartridge from the packaging.
3. Manually write the sample information, or place a sample information label on the top of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge. Ensure that the label is properly positioned and does not block the lid opening.
4. Place the QIAstat-Dx Gastrointestinal Panel 2 Cartridge flat on the clean work surface so that the bar code on the label faces upwards. Open the sample lid of the main port on the front of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge.
5. Thoroughly mix the stool in the Cary-Blair 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)
7. 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.
8. Carefully transfer the sample into the main port of the QIAstat-Dx Gastrointestinal Panel 2 Cartridge using the supplied single-use transfer pipette (Figures 6 and 7).
9. Firmly close the lid of the main port until it clicks (Figure 8).

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

Ordering Information

Product	Contents	Cat. no.
QIAstat-Dx Gastrointestinal Panel 2	For 6 tests: 6 individually packaged QIAstat-Dx Gastrointestinal Panel 2 Cartridges and 6 individually packaged transfer pipettes	691412
Related Products		
QIAstat-Dx Analyzer 1.0	1 QIAstat-Dx Analytical Module, 1 QIAstat-Dx Operational Module and related hardware and software to run molecular diagnostic QIAstat-Dx assay cartridges	9002824

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

Document Revision History

Date	Changes
R1, 04/2023	Initial Release

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