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QIAstat-Dx[®] SARS-CoV-2/Flu A/B/RSV Panel Instructions for Use (Handbook)



Version 1

For in vitro diagnostic use



R1

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Intended Use

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel is a qualitative test intended for the detection and differentiation of SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV) A+B viral nucleic acid from nasopharyngeal swabs (NPS) collected from patients suspected of a respiratory viral infection consistent with COVID-19 or an Influenza-like-illness (ILI). The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel is able to accept both dry swabs and transport medium liquid samples. The assay is designed for use with the QIAstat-Dx Analyzer 1.0 for integrated nucleic acid extraction and multiplex real-time RT-PCR detection.

The results from the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel must be interpreted within the context of all relevant clinical and laboratory findings.

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel is intended for professional use only and is not intended for self-testing.

For in vitro diagnostic use.

Summary and Explanation

QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel cartridge description

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge is a disposable plastic device that allows performance of fully automated molecular assays for the detection of respiratory pathogens. The main features of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge include compatibility with respiratory dry swabs (Copan® FLOQSwabs®, cat. no. 503CS01) and transport medium liquid samples, 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 SARS-CoV-2/Flu A/B/RSV Panel Cartridge. The user does not need to come in contact with and/or manipulate any reagents. During the test, reagents are handled within the cartridge in the Analytical Module of the QIAstat-Dx Analyzer 1.0 by pneumatically-operated microfluidics and make no direct contact with the actuators. The QIAstat-Dx Analyzer 1.0 houses air filters for both incoming and outgoing air, further safeguarding the environment. After testing, the cartridge stays hermetically closed at all times, greatly enhancing its safe disposal.

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

After the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge containing the sample is introduced into the QIAstat-Dx Analyzer 1.0, the following assay steps occur automatically:

- Resuspension of Internal Control
- Cell lysis using mechanical and/or chemical means
- Membrane-based nucleic acid purification
- Mixing of the purified nucleic acid with lyophilized master mix reagents
- Transfer of defined aliquots of eluate/master mix to different reaction chambers
- Performance of multiplex real-time RT-PCR testing within each reaction chamber

Note: An increase in fluorescence, indicating detection of the target analyte, is detected directly within each reaction chamber.

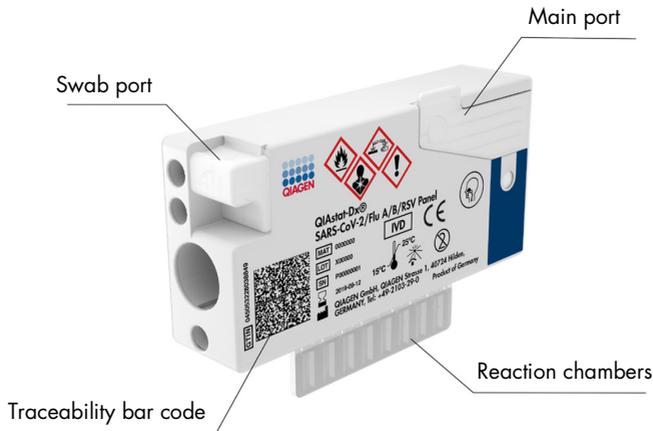


Figure 1. Layout of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge and its features.

Pathogen Information

Acute respiratory infections can be caused by a variety of pathogens, including 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 SARS-CoV-2/Flu A/B/RSV Panel Cartridge is a single-use cartridge that includes all reagents needed for nucleic acid extraction, nucleic acid amplification, and detection of 4 viruses (SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV) that cause respiratory symptoms. Testing requires a small sample volume and minimal hands-on time, and the results are available in approximately one hour.

The SARS-CoV-2 target in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel was designed in early 2020 upon alignment of more than 170 genomic sequences available in public databases from the SARS-CoV-2 identified as the causative agent of the viral pneumonia (COVID-19) outbreak originated in Wuhan, Hubei, China. To date, a coverage of more than 3.5 million of available genome sequences support the inclusivity of the SARS-CoV-2 detection. The SARS-CoV-2 in this panel targets 2 genes of the virus genome (Orf1b poly gene (Rdrp gene) and E genes) detected with the same fluorescence channel. The two gene targets are not differentiated and amplification of either or both gene targets lead to a fluorescence signal.

Pathogens that can be detected and identified with the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel are listed in Table 1.

Table 1. Pathogens detected by the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel

Pathogen	Classification (genome type)
Influenza A	Orthomyxovirus (RNA)
Influenza B	Orthomyxovirus (RNA)
SARS-CoV-2	Coronavirus (RNA)
Respiratory Syncytial Virus A/B	Paramyxovirus (RNA)

Principle of the Procedure

Description of the process

Diagnostic tests with the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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. Samples are collected and loaded manually into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge, depending on the sample type:

Option 1: Inserting the swab into the swab port when using a dry swab sample type (Figure 2).

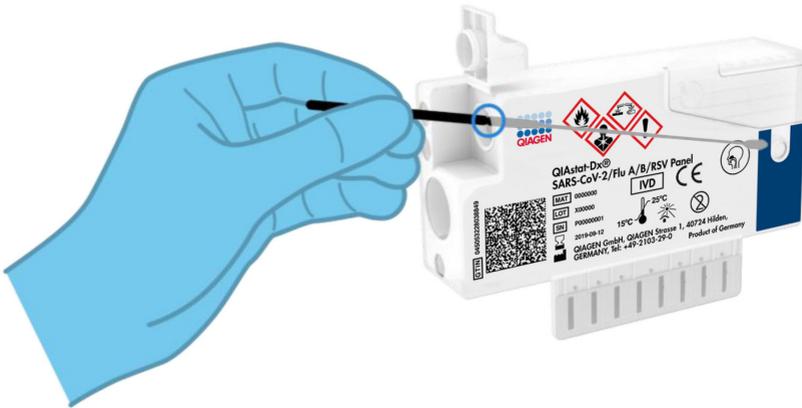


Figure 2. Loading the dry swab sample type into the swab port.

Option 2: A transfer pipette provided with the test kit is used for dispensing transport medium liquid sample into the main port (Figure 3).

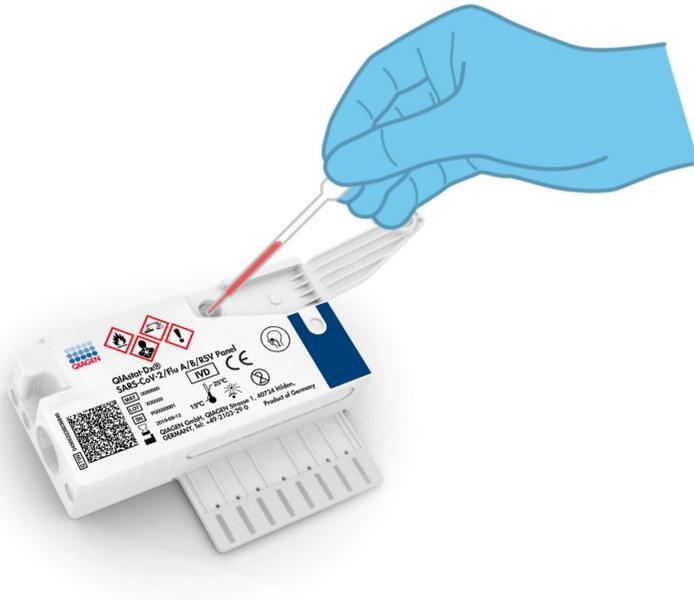


Figure 3. Dispensing transport medium liquid sample into the main port.

Sample collection and cartridge loading

The collection of samples and their subsequent loading into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge should be performed by personnel trained in safe handling of biological samples.

The following steps are involved and must be executed by the user:

1. A single-use nasopharyngeal swab sample is collected.
2. The nasopharyngeal swab is placed into a single use tube filled with transport medium only in the case of transport medium liquid sample type.

3. The sample information is manually written on or a sample label is affixed to the top of a QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge.
4. Sample is loaded manually into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge:
 - Dry swab sample type: The nasopharyngeal swab sample is inserted into the swab port of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge.
 - Transport medium liquid sample type: 300 µl of sample is transferred into the main port of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge using one of the included transfer pipettes.

IMPORTANT: When loading transport medium liquid sample, the user performs a visual check of the sample inspection window (see image below) to confirm that the liquid sample has been loaded (Figure 4).

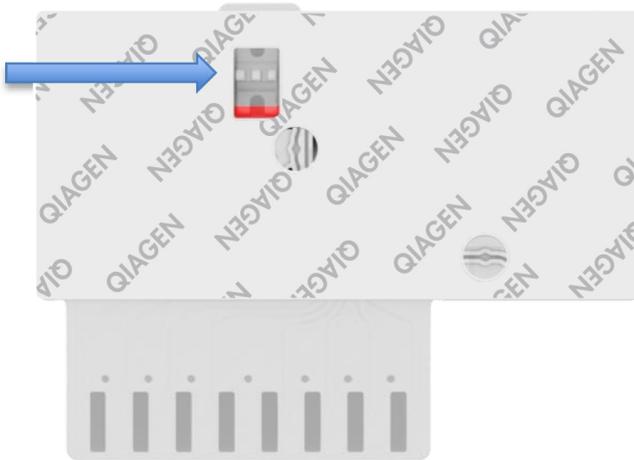


Figure 4. Sample inspection window (blue arrow).

5. The sample bar code and QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge bar code are scanned in the QIAstat-Dx Analyzer 1.0.
6. The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge is introduced into the QIAstat-Dx Analyzer 1.0.
7. The test is started on the QIAstat-Dx Analyzer 1.0.

Sample preparation, nucleic acid amplification, and detection

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

1. The liquid sample is homogenized and cells are lysed in the lysis chamber of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge, which includes a rotor that turns at high speed.
2. Nucleic acids are purified from the lysed sample via binding to a silica membrane in the purification chamber of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel Cartridge.
4. The mixture of sample and PCR reagents is dispensed into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge PCR chambers, which contains lyophilized, 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 SARS-CoV-2/Flu A/B/RSV Panel	
Catalog no.	691216
Number of tests	6
QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge*	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 SARS-CoV-2/Flu A/B/RSV Panel Cartridge.

Materials Required but Not Provided

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 1.3 or higher*
- *QIAstat-Dx Analyzer 1.0 User Manual* (for use with software version 1.3 or higher)
- QIAstat-Dx latest Assay Definition File software for SARS-CoV-2/Flu A/B/RSV Panel installed on the Operational Module

* DiagCORE® Analyzer instruments running QIAstat-Dx software version 1.3 or higher can be used as an alternative to QIAstat-Dx Analyzer 1.0 instruments.

Warnings and Precautions

For in vitro diagnostic use.

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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, consult the appropriate safety data sheets (SDSs). These are available online in 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 (United States of America)
- COSHH: Control of Substances Hazardous to Health (United Kingdom)

Follow your institution's safety procedures for handling biological samples. Dispose of samples, QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridges, and transfer pipettes according to the appropriate regulations.

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel 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 European Centre for Disease Prevention and Control (<https://www.ecdc.europa.eu/en/about-us/networks/disease-and-laboratory-networks/erlinet-biosafety>).

The following hazard and precautionary statements apply to components of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge



Contains: ethanol; guanidine hydrochloride; guanidine thiocyanate; isopropanol; proteinase K; t-Octylphenoxypolyethoxyethanol. Danger! Highly flammable liquid and vapour. 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/vapours/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.

Reagent Storage and Handling

Store the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridges in a dry, clean storage space at room temperature (15–25°C). Do not remove the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridges or the transfer pipettes from their individual packaging until actual use. Under these conditions, QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridges can be stored until the expiration date printed on the individual packaging. The expiration date is also included in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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.

Specimen Handling, Storage and Preparation

Transport medium liquid samples

Nasopharyngeal swab samples should be collected and handled according to the manufacturer's recommended procedures.

Recommended storage conditions for NPS (nasopharyngeal swab) resuspended in Universal Transport Medium (UTM) specimens are listed below:

- Room temperature up to 4 hours at 15–25°C
- Refrigerated up to 3 days at 2–8°C
- Frozen up to 30 days at –25 to –15°C

Dry swab samples

Use freshly collected dry swab specimens for best test performance. If immediate testing is not possible and to maintain best performance, recommended storage conditions for dry swabs are listed below:

- Room temperature up to 2 hours and 45 minutes at 15–25°C
- Refrigerated up to 7 hours at 2–8°C

Procedure

Internal Control

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge includes a full process Internal Control, which is titered MS2 bacteriophage. The MS2 bacteriophage is a single-stranded RNA virus 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 resuspension/homogenization, lysis, nucleic acid purification, reverse transcription, and PCR.

A positive signal for the Internal Control indicates that all processing steps performed by the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge were successful.

A negative signal 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.

Protocol: Dry swab samples

Sample collection, transport, and storage

Collect nasopharyngeal swab samples using Copan FLOQSwabs (cat. no. 503CS01) according to the manufacturer's recommended procedures.

Loading a sample into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge

1. Open the package of a QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge using the tear notches on the sides of the packaging (Figure 5).

IMPORTANT: After the package is opened, sample should be introduced inside the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge and loaded into the QIAstat-Dx Analyzer 1.0 within 120 minutes.



Figure 5. Opening the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge.

5. Insert the swab into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge until the breakpoint is aligned with the access opening (i.e., the swab will go no further) (Figure 8).

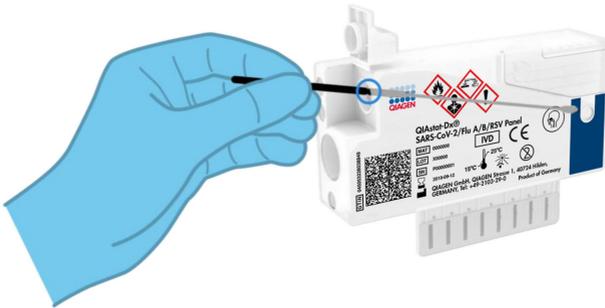


Figure 8. Inserting swab into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge.

6. Break the swab shaft at the breakpoint, leaving the rest of the swab in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge (Figure 9).

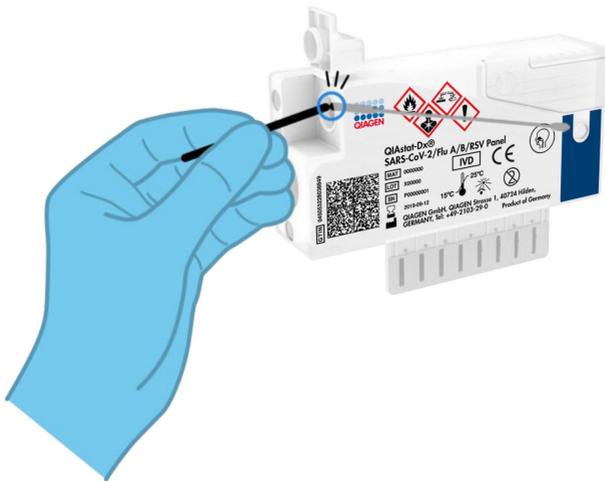


Figure 9. Breaking swab shaft.

7. Firmly close the sample lid of the swab port until it clicks (Figure 10).

IMPORTANT: After the sample is placed inside the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge, the cartridge must be loaded into the QIAstat-Dx Analyzer 1.0 within 90 minutes.

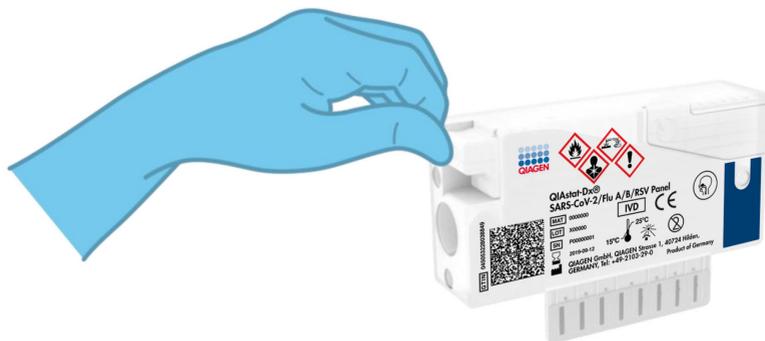


Figure 10. Closing the sample lid of the swab port.

Starting the QIAstat-Dx Analyzer 1.0

8. 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.

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

10. 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.

11. 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”, page 71, for additional information).

Running a test

12. Press the **Run Test** button in the top right corner of the touchscreen of the QIAstat-Dx Analyzer 1.0.
13. When prompted, scan the sample ID bar code on the nasopharyngeal swab sample (located on the swab blister packaging), or scan the specimen information bar code located on the top of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge (see step 3) using the integrated front bar code reader of the QIAstat-Dx Analyzer 1.0 (Figure 11).

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 11. Scanning sample ID bar code.

14. When prompted, scan the bar code of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge to be used (Figure 12). The QIAstat-Dx Analyzer 1.0 automatically recognizes the assay to be run based on the cartridge bar code and will automatically populate the Assay Type Field with 'R4PP' (Respiratory 4 plex Panel) .

Note: The QIAstat-Dx Analyzer 1.0 will not accept QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel Cartridge will be rejected. Refer to the *QIAstat-Dx Analyzer 1.0 User Manual* for further details on how to install assays.



Figure 12. Scanning QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge bar code.

15. Select the appropriate sample type from the list (Figure 13).

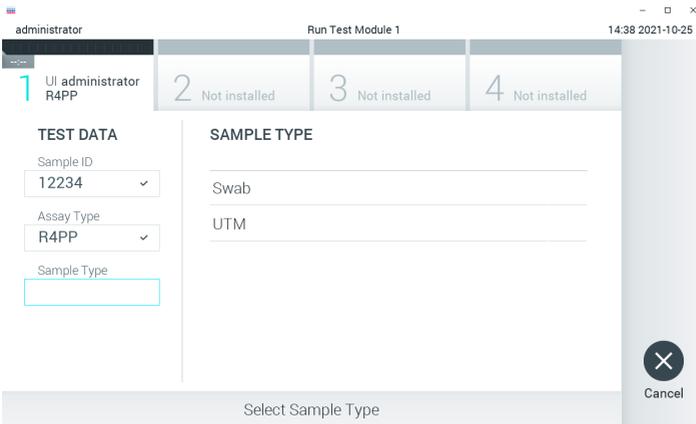


Figure 13. Selecting sample type.

16. 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.
17. 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 14).

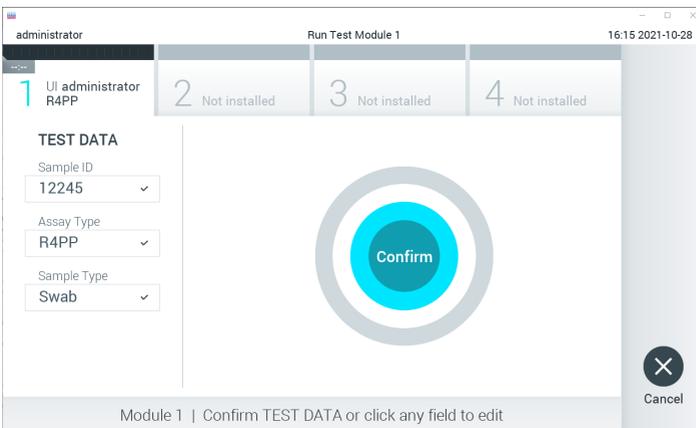


Figure 14. Confirming data entry.

18. Make sure that both sample lids of the swab port and main port of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge are firmly closed. When the cartridge entrance port on the top of the QIAstat-Dx Analyzer 1.0 automatically opens, insert the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge with the bar code facing to the left and the reaction chambers facing down (Figure 15).

Note: There is no need to push the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge into the QIAstat-Dx Analyzer 1.0. Position it correctly into the cartridge entrance port and the QIAstat-Dx Analyzer 1.0 will automatically move the cartridge into the Analytical Module.



Figure 15. Inserting QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge into QIAstat-Dx Analyzer 1.0.

19. Upon detecting the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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: The QIAstat-Dx Analyzer 1.0 will not accept a QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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: 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.

Note: Depending on the system configuration, the operator may be required to re-enter their user password to start the test run.

Note: The lid of the cartridge entrance port will close automatically after 30 seconds if a QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge is not positioned in the port. If this occurs, repeat the procedure starting with step 16.

20. While the test is running, the remaining run time is displayed on the touchscreen.

21. After the test run is completed, the **Eject** screen will appear (Figure 16, next page) 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.



Figure 16. Eject screen display.

22. Press  **Eject** on the touchscreen to remove the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel Cartridges must be discarded. It is not possible to re-use cartridges for tests for which the execution was started but then subsequently cancelled by the operator, or for which an error was detected.

23. After the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge has been ejected, the results **Summary** screen will appear. Refer to “Interpretation of Results”, page 39, for further details. To begin the process for running another test, press **Run Test**.

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

Protocol: Transport medium liquid samples

Sample collection, transport and storage

Collect nasopharyngeal swab samples according to the swab manufacturer's recommended procedures and place the swab into UTM.

Loading a sample into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge

1. Open the package of a QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge using the tear notches on the sides of the packaging (Figure 17).

IMPORTANT: After the package is open, sample should be introduced inside the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge and loaded into the QIAstat-Dx Analyzer 1.0 within 120 minutes.



Figure 17. Opening the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge.

2. Remove the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel Cartridge. Make sure that the label is properly positioned and does not block the lid opening (Figure 18).



Figure 18. Sample information placement on top of QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge.

4. Open the sample lid of the main port on the front of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge (Figure 19).

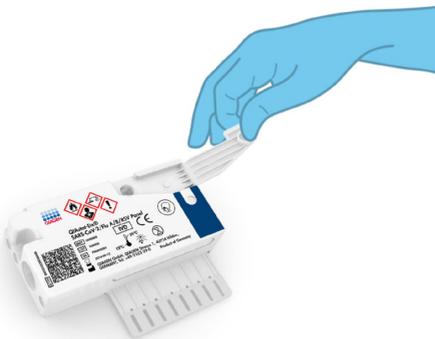


Figure 19. Opening the sample lid of main port.

5. Open the tube with the sample to be tested. Use the supplied transfer pipette to draw up fluid to the third fill line on the pipette (i.e., 300 μ l) (Figure 20).

IMPORTANT: Take care to avoid drawing air into the pipette. If Copan UTM® Universal Transport Medium is used as transport medium, take care not to aspirate any of the beads present in the tube. If air or beads are drawn into the pipette, carefully expel the sample fluid in the pipette back into the sample tube and draw up fluid again. Use alternative sterile and graduated pipettes in case all six pipettes provided with the kit have been used.

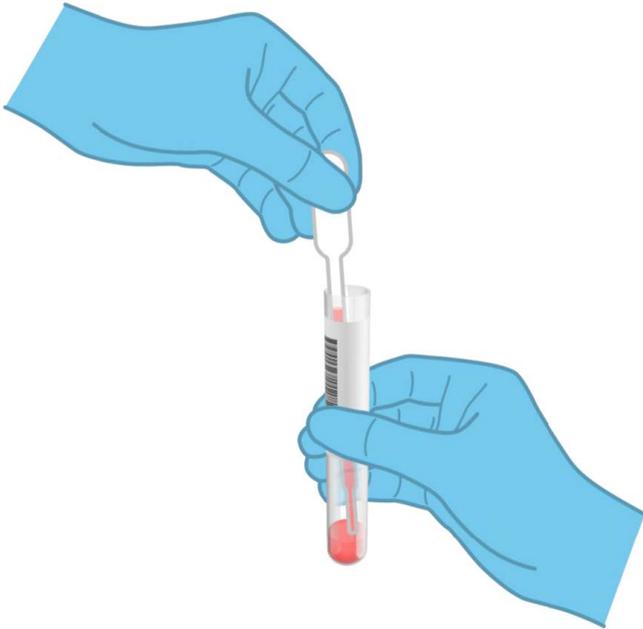


Figure 20. Drawing up sample into the supplied transfer pipette.

- Carefully transfer 300 μ l of sample volume into the main port of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge using the supplied single-use transfer pipette (Figure 21).

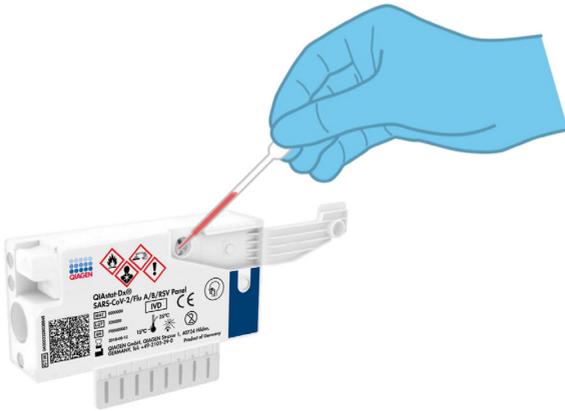


Figure 21. Transferring sample to main port of QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge.

- Firmly close the sample lid of the main port until it clicks (Figure 22).



Figure 22. Closing the sample lid of the main port.

8. Visually confirm that the sample has been loaded by checking the sample inspection window of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge (Figure 23).

IMPORTANT: After the sample is placed inside the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge, the cartridge must be loaded into the QIAstat-Dx Analyzer 1.0 within 90 minutes.

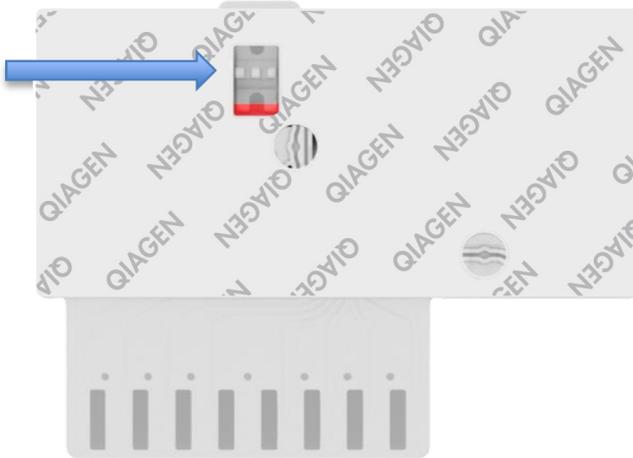


Figure 23. Sample inspection window (blue arrow).

Starting the QIAstat-Dx Analyzer 1.0

9. 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.

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

11. 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.

12. 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, page 71, for additional information).

Running a test

13. Press the **Run Test** button in the top right corner of the touchscreen of the QIAstat-Dx Analyzer 1.0.
14. When prompted, scan the sample ID bar code on the UTM tube containing the sample, or scan the specimen information bar code located on the top of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge (see step 3), using the integrated front bar code reader of the QIAstat-Dx Analyzer 1.0 (Figure 24).

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 24. Scanning sample ID bar code.

15. When prompted, scan the bar code of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge to be used (Figure 25, next page). The QIAstat-Dx Analyzer 1.0 automatically recognizes the assay to be run based on the cartridge bar code.

Note: The QIAstat-Dx Analyzer 1.0 will not accept QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel Cartridge will be rejected. Refer to the *QIAstat-Dx Analyzer 1.0 User Manual* for further details on how to install assays.



Figure 25. Scanning QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge bar code.

16. Select the appropriate sample type from the list (Figure 26).

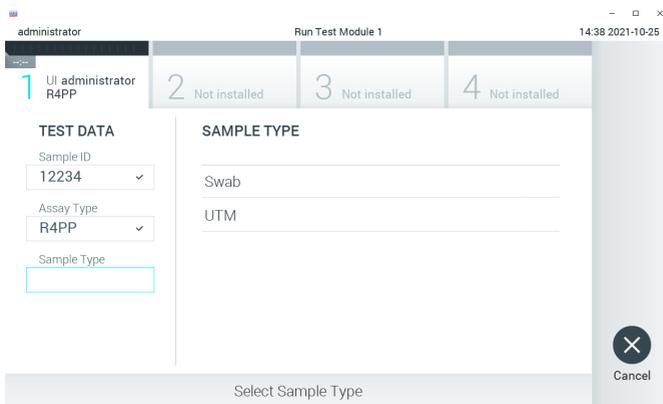


Figure 26. Selecting sample type.

17. 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.
18. 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 27).

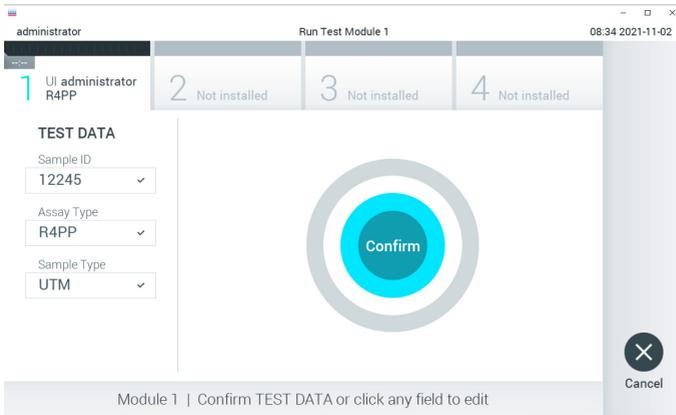


Figure 27. Confirming data entry.

19. Make sure that both sample lids of the swab port and main port of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge are firmly closed. When the cartridge entrance port on the top of the QIAstat-Dx Analyzer 1.0 automatically opens, insert the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge with the bar code facing to the left and the reaction chambers facing down (Figure 28, next page).

Note: There is no need to push the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge into the QIAstat-Dx Analyzer 1.0. Position it correctly into the cartridge entrance port and the QIAstat-Dx Analyzer 1.0 will automatically move the cartridge into the Analytical Module.



Figure 28. Inserting QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge into QIAstat-Dx Analyzer 1.0.

20. Upon detecting the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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: The QIAstat-Dx Analyzer 1.0 will not accept a QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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: 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.

Note: Depending on the system configuration, the operator may be required to re-enter their user password to start the test run.

Note: The lid of the cartridge entrance port will close automatically after 30 seconds if a QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge is not positioned in the port. If this occurs, repeat the procedure starting with step 17.

21. While the test is running, the remaining run time is displayed on the touchscreen.

22. After the test run is completed, the **Eject** screen will appear (Figure 29) 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.

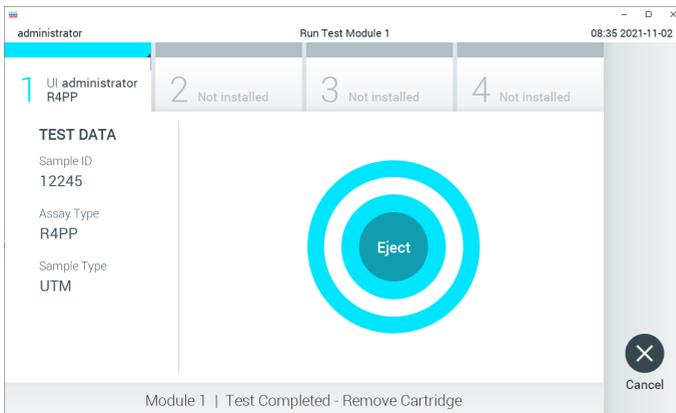


Figure 29. Eject screen display.

23. Press  **Eject** on the touchscreen to remove the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel 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 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 SARS-CoV-2/Flu A/B/RSV Panel 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.

24. After the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge has been ejected, the results **Summary** screen will appear. Refer to “Interpretation of Results”, page 39, for further details. To begin the process for running another test, press **Run Test**.

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

Interpretation of Results

Viewing results

The QIAstat-Dx Analyzer 1.0 automatically interprets and saves test results. After ejecting the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge, the results **Summary** screen is automatically displayed (Figure 30).

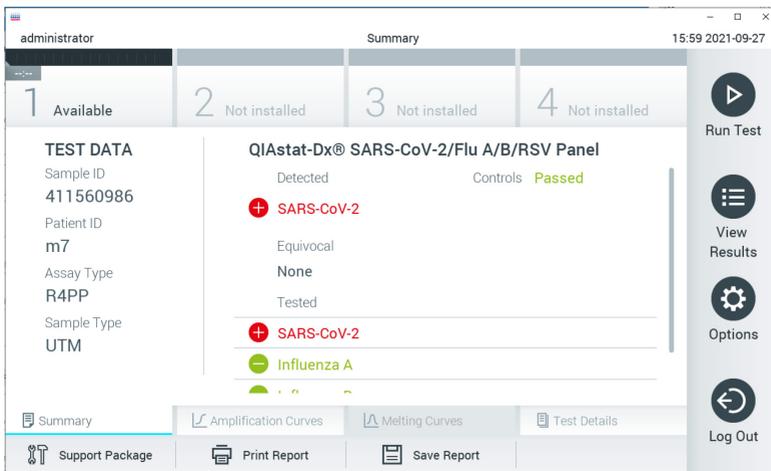


Figure 30. Results Summary screen example showing Test Data on the left panel and Test Summary in the main panel.

The main part of the screen provides the following three 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 SARS-CoV-2/Flu A/B/RSV Panel. 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.

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
- 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 31).



Figure 31. Amplification Curves screen (PATHOGENS tab).

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

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

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

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

Press the **CONTROLS** tab on the left side to view the controls in the amplification plot. Press the circle next to the control name to select or deselect it (Figure 32).



Figure 32. 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 33, next page):

- 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 respiratory pathogen is detected/identified)
 - Negative (no respiratory pathogen is detected)
 - Invalid
- 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

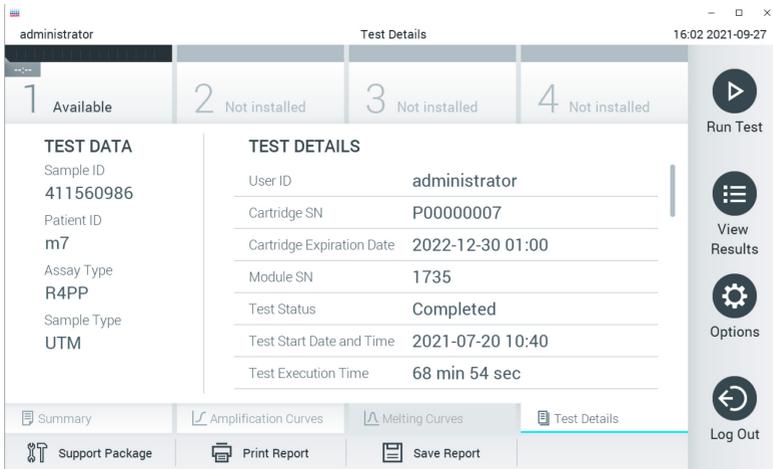


Figure 33. Example screen showing Test Data on the left panel and Test Details in the main panel.

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 34).

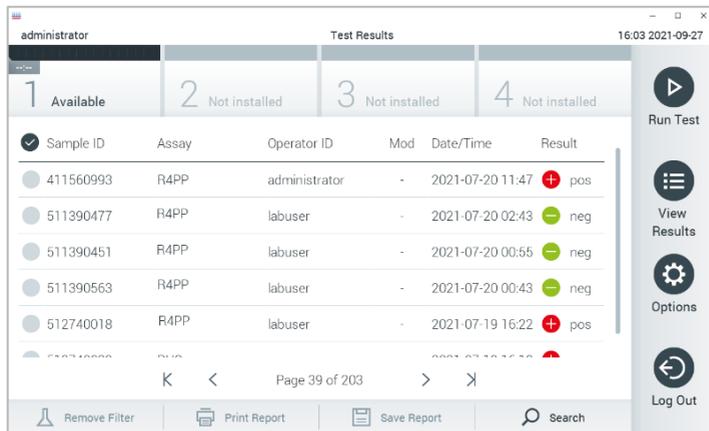


Figure 34. Example View Results screen.

The following information is available for every executed test (Figure 35):

- Sample ID
- Assay (name of test assay, which is “R4PP” for QIAstat-Dx® SARS-CoV-2/Flu A/B/RSV Panel)
- 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], negative [neg], failed [fail] or successful [suc])

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

Select one or more test results by pressing the **gray circle** to left of the sample ID. A **checkmark** will appear next to selected results. Unselect test results by pressing this **checkmark**. The entire list of results can be selected by pressing the **☑ checkmark circle** in the top row (Figure 35).

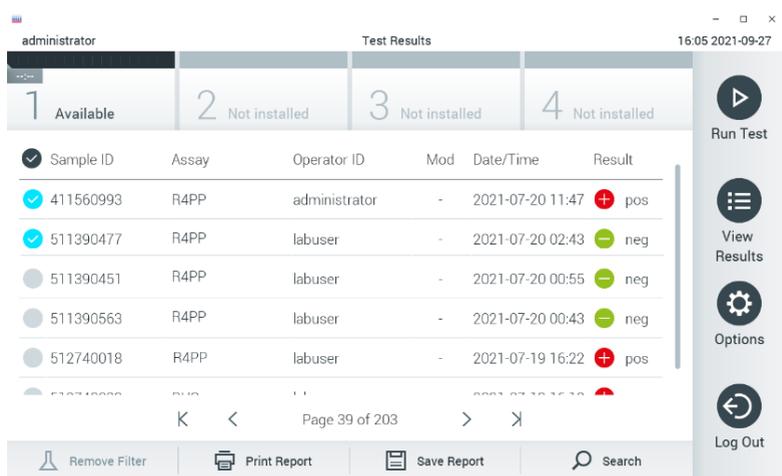


Figure 35. Example of selecting Test Results in the View Results screen.

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

Outcome	Result	Description
Positive	 pos	At least one pathogen is positive
Negative	 neg	No pathogens were detected
Failed	 fail	The test failed because either an error occurred or the test was canceled by the user
Successful	 suc	The test is either positive or negative, but the user does not have the access rights to view the test results

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

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

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

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

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

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

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

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

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

Exporting results to a USB drive

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

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.

Result interpretation

For every pathogen that can be detected with the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel, only one signal will be generated if the pathogen is present in the sample.

Internal Control interpretation

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

Table 3. Interpretation of Internal Control results

Control result	Explanation	Action
Passed	The Internal Control amplified successfully	The run was completed with success. All results are valid 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 QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel is tested against predetermined specifications to ensure consistent product quality.

Limitations

- Results from the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions.
- Positive results do not rule out co-infection with organisms not included in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel. The agent detected may not be the definitive cause of the disease.
- Negative results do not preclude infection of the upper respiratory tract. Not all agents of acute respiratory infection are detected by this assay and sensitivity in some clinical settings may differ from that described in the package insert.
- A negative result with the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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, therapies, or agents.
- The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel is not intended for testing of samples other than those described in these Instructions for Use. Test performance characteristics have been established only with nasopharyngeal swab samples collected in transport medium, from individuals with acute respiratory symptoms.
- The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel must be interpreted by a trained healthcare professional within the context of all relevant clinical, laboratory, and epidemiological findings.
 - The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel can be used only with the QIAstat-Dx Analyzer 1.0.*
 - The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel is a qualitative assay and does not provide a quantitative value for detected organisms.
 - Viral nucleic acids may persist in vivo, even if the organism is not viable or infectious. Detection of a target marker does not imply that the corresponding organism is the causative agent of the infection or the clinical symptoms.
 - Detection of viral nucleic acids depends on proper sample collection, handling, transportation, storage, and loading into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge. Improper operations for any of the aforementioned processes can cause incorrect results, including false-positive or false-negative results.
 - The assay sensitivity and specificity for the specific organisms and for all organisms combined are intrinsic performance parameters of a given assay and do not vary depending on prevalence. In contrast, both the negative and positive predictive values of a test result are dependent on the disease/organism prevalence. Please note that a higher prevalence favors the positive predictive value of a test result, while a lower prevalence favors the negative predictive value of a test result.

* DiagCORE Analyzer instruments running QIAstat-Dx software version 1.3 or higher can be used as an alternative to QIAstat-Dx Analyzer 1.0 instruments.

Performance Characteristics

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel (cat. no. 691216) assay is a new configuration of the QIAstat-Dx Respiratory SARS-CoV-2 Panel assay (cat. No. 691214) with only 4 targets (SARS-CoV-2/Flu A/Flu B/RSV) called and the other targets masked by the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Assay Definition File. The studies shown below were not repeated, but instead, existing data were re-analyzed using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Assay Definition File.

Clinical performance

Transport medium liquid specimens

The performance characteristics of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel assay were assessed in a multicenter clinical trial conducted at eight (8) geographically diverse study sites: five (5) U.S. sites and three (3) EU sites. The performance of nasopharyngeal swab specimen was assessed in universal transport medium (UTM) (Copan Diagnostics); MicroTest™ M4®, M4RT®, M5®, and M6™ (Thermo Fisher Scientific); BD™ Universal Viral Transport (UVT) System (Becton Dickinson and Company); HealthLink Universal Transport Medium (UTM) System (HealthLink Inc.); Universal Transport Medium (Diagnostic Hybrids Inc.); V-C-M Medium (Quest Diagnostics); UniTranz-RT Universal Transport Media (Puritan Medical Products Company); and dry nasopharyngeal swab specimens (FLOQSwabs, Copan, cat. no. 503CS01).

This study was designed as an observational, prospective-retrospective study using leftover samples obtained from subjects with signs and symptoms of an acute respiratory infection. Participating sites were asked to test fresh and/or frozen clinical samples according to a protocol and site/site-specific instructions.

Samples tested using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel were compared with the results of the standard of care (SOC) method(s) at the sites, as well as with a range of validated and commercially available molecular methods. This approach provided results for

pathogens not detected by SOC and/or allowed for final discrepancy resolution of discordant results. The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel assay results were compared against FilmArray® Respiratory Panel 1.7 & 2 and the SARS-CoV-2 RT-PCR assay developed by the Charité – Universitätsmedizin Berlin Institute of Virology, Berlin, Germany.

A total of 3,065 clinical UTM patient samples were enrolled into the study and 1 sample was excluded from the final analysis with QIAstat-Dx SARS-CoV-2/Flu A/B/RSV ADF.

Clinical Sensitivity or Positive Percent Agreement (PPA) was calculated as $100\% \times (TP/[TP + FN])$. True positive (TP) indicates that both the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel and comparator(s) methods had a positive result for the organism, and false negative (FN) indicates that the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel result was negative while the comparator methods results were positive. Specificity or Negative Percent Agreement (NPA) was calculated as $100\% \times (TN/[TN + FP])$. True negative (TN) indicates that both the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel and the comparator method had negative results, and a false positive (FP) indicates that the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel result was positive but the comparator methods results were negative. For the calculation of the clinical specificity of the individual pathogens, the total available results were used with the concerning true- and false-positive organism results subtracted. The exact binomial two-sided 95% confidence interval was calculated for each point estimate.

Overall Clinical Sensitivity (PPA) and overall Clinical Specificity (NPA) were calculated from 3064 samples.

In total, 906 true positive and 7608 true negative QIAstat-Dx Respiratory Panel and QIAstat-Dx Respiratory SARS CoV-2 Panel results were found, as well as 20 false-negative and 42 false-positive results.

Table 4 (next page) displays QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Clinical Sensitivity (or Positive Percent Agreement) and Clinical Specificity (or Negative Percent Agreement) with 95% Confidence Intervals.

Table 4. QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel performance data

Pathogen	Positive Percent Agreement			Negative Percent Agreement		
	TP/TP+FN	%	95% CI	TN/TN+FP	%	95% CI
All	906/926	97.84	96.68-98.68	7608/7650	99.45	99.26-99.60
Influenza A	327/329	99.39	97.81-99.83	2406/2429	99.05	98.58-99.37
Influenza B	177/184	96.20	92.36-98.15	2590/2590	100.00	99.85-100.00
Respiratory Syncytial Virus A+B	319/325	98.15	96.03-99.15	2441/2442	99.96	99.77-99.99
SARS-CoV-2	83/88	94.32	87.38-97.55	171/189	90.48	85.45-93.89

The QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel assay detected multiple organisms in 15 samples, all were double infections.

Dry swab specimen

A total of 333 paired clinical specimens (NPS in UTM and NPS dry swab) were tested to assess the clinical performance characteristics of the dry swab specimens in comparison to the UTM specimen. This testing was conducted at 4 clinical sites in the EU. The objective was to demonstrate equivalency between performance characteristics of the dry swab and the UTM specimens using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

Patients enrolled in the studies provided 2 nasopharyngeal swabs (one from each nostril). One swab was directly inserted into the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge, and the other swab was transferred into UTM for comparator testing with a separate QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge (paired samples).

The Clinical Sensitivity (or PPA) was calculated as $100\% \times (TP/[TP + FN])$. True positive (TP) indicates that both the dry swab and the UTM specimen had a positive result for a specific organism and false negative (FN) indicates that the dry swab result was negative while the UTM specimen result was positive for a specific organism. Specificity (or NPA) was calculated as $100\% \times (TN/[TN + FP])$. True negative (TN) indicates that both the dry swab and UTM

specimen had negative result for a specific organism and a false positive (FP) indicates that the dry swab result was positive but the UTM specimen result was negative for a specific organism. The two-sided Wilson-Score 95% confidence interval was calculated for each point estimate.

A total of 319 evaluable paired sample results were available for analysis from the original 333 paired samples enrolled. The remaining 14 paired samples did not fulfill the inclusion criteria.

Overall Clinical Sensitivity (or PPA) could be calculated from a total 67 positive target results obtained with the UTM specimen. The overall Clinical Specificity (or NPA) was calculated from 1,112 individual negative target results were obtained with the UTM specimen. The positive results included different targets in the panel and were representative of the epidemiology of the population tested during the clinical performance study (including SARS-CoV-2 for 2 of the sites).

In total, 65 true-positive and 1,105 true-negative dry swab results were found, as well as 2 false-negative (positive for UTM specimen/negative for dry swab specimen) and 7 false-positive (positive for dry swab/negative for UTM specimen) results. Overall, the PPA was 97.01% (95% CI, 89.75%-99.18%) and NPA was 99.37% (95% CI, 98.71%-99.69%), showing a high overall correlation between dry swab and UTM specimen types (Table 5).

Table 5. Agreement Between overall QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel dry swab result and overall QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel UTM result overall sensitivity and specificity assessment.

Grouping Variables	Proportion		Two-sided 95% Confidence Limits	
	Fraction	%	Lower	Upper
PPA	65/67	97.01	89.75	99.18
NPA	1105/1112	99.37	98.71	99.69

Specifically, for the SARS-CoV-2 target, 40 true-positive results were found in the comparison between UTM specimens and dry-swab specimens tested using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel. No false-negatives were found during this comparison between specimens.

Additionally, 181 true-negative and 3 false-positive (dry-swab positive and UTM negative) results were found for SARS-CoV-2.

Differences in results between UTM specimen and dry-swab specimens could be attributable to sampling differences between specimens and the dilution effect of dry swabs in transport media. Dry-swab specimens can only be tested once using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel, therefore discordance testing was not possible for this sample type.

Conclusion

Extensive multicenter studies sought to assess the performance of the UTM specimen, as well as the equivalency of the dry swab, with the UTM specimen performance in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel assay.

The overall Clinical Sensitivity of the UTM specimen was found to be 97.84% (95% CI, 96.68%–98.68%). The overall Clinical Specificity 99.45% (95% CI, 99.26%-99.60%).

The overall Clinical Sensitivity of the dry swab specimen was found to be 97.01% (95% CI, 89.75%–99.18%). The overall Clinical Specificity for the dry swab specimen was 99.37% (95% CI, 98.71%-99.69%).

Analytical performance

Sensitivity (Limit of Detection)

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

The LoD for each QIAstat-Dx SARS-CoV-2/ Flu A/B/RSV Panel pathogen was assessed by analyzing serial dilutions of analytical samples prepared from high-titer stocks obtained from commercial suppliers (ZeptoMetrix® and ATCC®) or quantified clinical samples.

Simulated NPS sample matrix (cultured human cells in Copan UTM) for transport medium liquid samples and simulated dry swab sample matrix (cultured human cells in artificial NPS) for dry swabs were spiked with 1 or more pathogens and tested in 20 replicates. Liquid sample workflow uses NPS eluted in UTM and a transfer of 300 μl to the cartridge, whereas dry swab workflow allows transfer of the NPS directly to the cartridge. Dry swab mock swabs were prepared by pipetting 50 μl of each diluted virus stock onto a swab and were left to dry for a minimum of 20 minutes. Swab was tested following the Dry Swab Sample protocol (page 18).

The LoD concentration was determined for a total of 17 pathogen strains. The LoD per analyte was determined using selected strains representing individual pathogens that are possible to detect with the QIAstat-Dx SARS-CoV-2/ Flu A/B/RSV Panel. To confirm the established LoD concentration, the detection rate of all replicates must be $\geq 95\%$ (at least 19/20 replicates must generate a positive signal).

At least three different cartridge lots and at least three different QIAstat-Dx Analyzers were used for LoD determination for every pathogen. Individual LoD values for each target is shown in Table 6.

Table 6. LoD values obtained for the different respiratory target strains in NPS sample matrix (cultured human cells in Copan UTM) and/or dry swab (cultured human cells in artificial NPS) tested with the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel

Pathogen	Strain	Source	Concentration	Detection rate
Influenza A	A/New Jersey/8/76 (H1N1)	ATCC VR-897	341 CEID ₅₀ /ml	20/20
	A/Brisbane/59/07 (H1N1)	ZeptoMetrix 0810244CFH	4 TCID ₅₀ /ml	20/20
	A/New Caledonia/20/99 (H1N1)*	ZeptoMetrix 0810036CFHI	28.7 TCID ₅₀ /ml	20/20
	A/Virginia/ATCC6/2012 (H3N2)†	ATCC VR-1811	0.1 PFU/ml	20/20
	A/Wisconsin/67/2005 (H3N2)†	ZeptoMetrix 0810252CFHI	3.8 TCID ₅₀ /ml	20/20
	A/Port Chalmers/1/73 (H3N2)*	ATCC VR-810	3000 CEID ₅₀ /ml	20/20
	A/Virginia/ATCC1/2009 (H1N1 pdm09)*	ATCC VR-1736	6.7 PFU/ml	20/20
	A/SwineNY/03/2009 (H1N1 pdm09)	ZeptoMetrix 0810249CFHI	56 TCID ₅₀ /ml	20/20
Influenza B	B/Virginia/ATCC5/2012†	ATCC VR-1807	0.03 PFU/ml	20/20
	B/FL/04/06*	ATCC VR-1804	2050 CEID ₅₀ /ml	20/20
	B/Taiwan/2/62	ATCC VR-295	5000 CEID ₅₀ /ml	19/20
RSV A	A2†/*	ATCC VR-1540	720 PFU/ml	20/20
	Long†	ATCC VR-26	33.0 PFU/ml	20/20
RSV B	18537†	ATCC VR-1580	0.03 PFU/ml	20/20
	CH93(18)-18	ZeptoMetrix 0810040CFHI	0.4 TCID ₅₀ /ml	19/20
SARS-CoV-2	Clinical Sample†	Hospital from Barcelona (Spain)	500 copies/mL	20/20
	England/02/2020†	NIBSC, 20/146	19000 copies/mL	20/20

* Tested with liquid sample and mock dry swab sample type.

† The LoD has been obtained in simulated matrix.

‡ Tested with mock dry swab sample type.

Assay robustness

The verification of robust assay performance was assessed by analyzing the Internal Control performance in clinical nasopharyngeal swab samples. Thirty (30) individual nasopharyngeal swab samples, negative for all pathogens possible to detect, were analyzed with the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

All samples tested showed a positive result and valid performance for the Internal Control of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

Exclusivity (Analytical Specificity)

The analytical specificity study was carried out by *in silico* analysis and *in vitro* testing to assess the cross-reactivity and exclusivity of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity and off-panel organisms were tested to evaluate panel exclusivity. The off-panel organisms selected were clinically relevant organisms (colonizing the upper respiratory tract or causing respiratory symptoms), common skin flora or laboratory contaminants, or microorganisms for which much of the population may have been infected. The on-panel and off-panel organisms tested are shown in Table 7.

Samples were prepared by spiking potential cross-reactive organisms into simulated nasopharyngeal swab sample matrix at the highest concentration possible based on the organism stock – at least 10^5 TCID₅₀/ml for viral targets and 10^6 CFU/ml for bacterial and fungal targets. These concentrations represent levels approximately 800–1,000,000-fold higher than the LoD of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

Table 7. List of Analytical Specificity pathogens tested

Type	Pathogen	
On-panel viruses	Influenza A (H3N2)	
	Influenza A (H1N1)	
	Influenza A (H1N1 pdm09)	
	Influenza B	
	RSV A	
Off-panel viruses	Coronavirus 229E	Enterovirus D68
	Coronavirus OC43	Rhinovirus 1A
	Coronavirus NL63	Bocavirus†
	Coronavirus HKU1*	Cytomegalovirus
	Parainfluenza virus 1	Epstein-Barr Virus
	Parainfluenza virus 2	Herpes Simplex Virus 1
	Parainfluenza virus 3	Herpes Simplex Virus 2
	Parainfluenza virus 4A	Measles Virus
	hMPV A	Middle East Respiratory Syndrome Coronavirus‡
	Adenovirus C	Mumps
	Adenovirus B	SARS Coronavirus§

Continued on the next page

Table 7 (continued from the previous page)

Type	Pathogen	
Off-panel bacteria	<i>Acinetobacter calcoaceticus</i>	<i>Legionella micdadei</i>
	<i>Bordetella avium</i>	<i>Legionella pneumophila</i>
	<i>Bordetella bronchiseptica</i>	<i>Moraxella catarrhalis</i>
	<i>Bordetella hinzii</i>	<i>Mycobacterium tuberculosis</i> **
	<i>Bordetella holmesii</i>	<i>Mycoplasma genitalium</i>
	<i>Bordetella parapertussis</i>	<i>Mycoplasma hominis</i>
	<i>Bordetella pertussis</i>	<i>Mycoplasma orale</i>
	<i>Chlamydia trachomatis</i>	<i>Mycoplasma pneumoniae</i>
	<i>Chlamydia pneumoniae</i>	<i>Neisseria elongata</i>
	<i>Corynebacterium diptheriae</i>	<i>Neisseria gonorrhoeae</i>
	<i>Enterobacter aerogenes</i>	<i>Neisseria meningitidis</i>
	<i>Escherichia coli</i> (O157)	<i>Proteus mirabilis</i>
	<i>Haemophilus influenzae</i>	<i>Pseudomonas aeruginosa</i>
	<i>Haemophilus aegyptus</i>	<i>Serratia marcescens</i>
	<i>Klebsiella oxytoca</i>	<i>Staphylococcus aureus</i>
	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus epidermidis</i>
	<i>Lactobacillus acidophilus</i>	<i>Stenotrophomonas maltophilia</i>
	<i>Lactobacillus plantarum</i>	<i>Streptococcus agalactiae</i>
	<i>Legionella bozemanii</i>	<i>Streptococcus pneumoniae</i>
<i>Legionella dumofii</i>	<i>Streptococcus pyogenes</i>	
<i>Legionella feeleeii</i>	<i>Streptococcus salivarius</i>	
<i>Legionella longbeachae</i>	<i>Ureaplasma urealyticum</i>	
Off-panel fungi	<i>Aspergillus flavus</i>	<i>Candida albicans</i>
	<i>Aspergillus fumigatus</i>	<i>Cryptococcus neoformans</i>

* Coronavirus HKU1 clinical specimen tested.

† Bocavirus Type 1 clinical specimens tested.

‡ Middle East Respiratory Syndrome Coronavirus synthetic RNA tested.

§ SARS Coronavirus was tested using custom gBlocks from the two regions targeted by the SARS-CoV-2 designs.

** *Mycobacterium tuberculosis* genomic DNA tested.

In silico, sequence hits were analyzed together in order to detect unique specific sequences matching with all primers and probes to be considered as positive amplifications. Primers and probes were considered as reactive if the following parameters were fulfilled:

- At least one forward, one probe, and one reverse primer of the SARS-CoV-2 assay match with the target BLAST hit sequence.
- At least 70% of query cover/identity between the BLAST hit sequence and every single primer/probe sequence.
- A maximum of 500 bp of amplicon size.

This analysis showed that no cross-reaction can be found in cases of Flu A, Flu B, and RSV. In case of SARS-CoV-2, designs show that a potential unspecific signal can be produced by a cross-reaction with a group of SARS coronaviruses found in bats or pangolins not infective for humans. Those results can be expected based on the zoonotic origin of this virus. These coronaviruses have only been detected in bats and have not been reported to infect or colonize humans. No unspecific signals were generated with critical off-panel human targets.

No cross-reactivity with any on-panel/off-panel pathogen tested from the table above has been observed for the Influenza A, Influenza B, and RSV targets included in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel. In addition, based on the *in silico* analysis, no cross-reaction has been predicted for the on-panel targets included in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel and any off-panel pathogen.

Inclusivity (Analytical Reactivity) *

An inclusivity study was performed to analyze the detection of a variety of strains that represent the genetic diversity of each respiratory panel target organism (“inclusivity strains”). Inclusivity strains for all analytes were included in the study, representative of the species/types for the different organisms (e.g., a range of Influenza A strains isolated from different geographical areas and in different calendar years were included). Table 8 (next page) shows the list of respiratory pathogens tested in this study.

* Laboratory testing not applicable to the SARS-CoV-2 target due to the presence of a single strain at time of study. SARS-CoV-2 Inclusivity has been performed bioinformatically among lineages and variants.

Table 8. List of Analytical Reactivity pathogens tested

Pathogen	Subtype/ serotype	Strain	Source	x LoD Detected	Result	
Influenza A	H1N1	A/PR/8/34	ATCC VR-1469	0.1x	Influenza A	
		New Jersey/8/76*	ATCC VR-897	1x	Influenza A	
		A/Brisbane/59/07*	ZeptoMetrix 0810244CFHI	1x	Influenza A	
		A/New Caledonia/20/99*	ZeptoMetrix 0810036CFHI	0.3x	Influenza A	
		A/Denver/1/57	ATCC VR-546	0.1x	Influenza A	
		A/Weiss/43	ATCC VR-96	0.1x	Influenza A	
		A/Fort Monmouth/1/1947	ATCC VR-1754	0.1x	Influenza A	
		A/WS/33	ATCC VR-1520		Influenza A	
		A/Swine/Iowa/15/1930	ATCC VR-333		Influenza A	
		A/Mal/302/54	ATCC VR-98	1x	Influenza A	
		H3N2	A/Virginia/ATCC6/2012*	ATCC VR-1811	1x	Influenza A
			A/Wisconsin/67/2005*	ZeptoMetrix 0810252CFHI	1x	Influenza A
			A/Port Chalmers/1/73*	ATCC VR-810	1x	Influenza A
			A/Victoria/3/75	ATCC VR-822		Influenza A
	A/Aichi/2/68		ATCC VR-1680	3x	Influenza A	
	A/Hong Kong/8/68		ATCC VR-1679	0.3x	Influenza A	
	A/Alice (recombinant, carries A/England/42/72)		ATCC VR-776	1x	Influenza A	
	MRC-2 (recombinant A/England/42/72 and A/PR/8/34 strains)		ATCC VR-777		Influenza A	
	A/Switzerland/ 9715293/2013		ATCC VR-1837	1x	Influenza A	
	A/Wisconsin/15/2009		ATCC VR-1882		Influenza A	
	H1N1 (pandemic 2009)	A/Virginia/ATCC1/2009*	ATCC VR-1736	1x	Influenza A	
		A/SwineNY/03/2009	ZeptoMetrix 0810249CFHI	1x	Influenza A	
		Canada/6294/09	ZeptoMetrix 0810109CFJHI	0.01x	Influenza A	
		Mexico/4108/09	ZeptoMetrix 0810166CFHI	0.1x	Influenza A	
		Netherlands/2629/2009	BEI Resources NR-19823	0.3x	Influenza A	
		A/Virginia/ATCC2/2009	ATCC VR-1737	0.1x	Influenza A	
		A/Virginia/ATCC3/2009	ATCC VR-1738	0.1x	Influenza A	
		Swine NY/01/2009	ZeptoMetrix 0810248CFHI	0.3x	Influenza A	
		Swine NY/02/2009	ZeptoMetrix 0810109CFNHI0	1x	Influenza A	
		A/California/07/2009	ATCC VR-1884	0.1x	Influenza A	
		NYMC X-179A				
		H2N2	Japan/305/1957 (nucleic acid)	BEI Resources NR-2775	1x	Influenza A
			Korea/426/1968xPuerto Rico/8/1934 [isolate ID/source=recombinant] (nucleic acid)	BEI Resources NR-9679	0.3x	Influenza A

Continued on next page

Table 8 (continued from previous page)

Pathogen	Subtype/ serotype	Strain	Source	x LoD Detected	Result
Influenza A	H5N3	A/Duck/Singapore/645/1997 [isolate ID/source=avian]	BEI Resources NR-9682	1x	Influenza A
	H10N7	Chicken/Germany/N/49 [isolate ID/source=avian] (nucleic acid)	BEI Resources NR-2765	10x	Influenza A
	H1N2	Recombinant Kilbourne F63, A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA) [isolate ID/source=recombinant] (nucleic acids)	BEI Resources NR-9677	100x	Influenza A
Influenza B	Not available	B/Virginia/ATCC5/2012*	ATCC VR-1807	1x	Influenza B
		B/FL/04/06	ATCC VR-1804	1x	Influenza B
		B/Taiwan/2/62	ATCC VR-295	0.3x	Influenza B
		B/Allen/45	ATCC VR-102	Not detected [†]	Negative
		B/Hong Kong/5/72	ATCC VR-823	Not detected [†]	Negative
		B/Maryland/1/59	ATCC VR-296	0.1x	Influenza B
		B/GL/1739/54	ATCC VR-103	1x	Influenza B
		B/Wisconsin/1/2010	ATCC VR-1883	0.1x	Influenza B
		B/Massachusetts/2/2012	ATCC VR-1813	3x	Influenza B
		B/Florida/02/06	ZeptoMetrix 0810037CFHI	Impaired detectability [†]	Influenza B or Negative
	B/Brisbane/60/2008	BEI Resources NR-42005	0.1x	Influenza B	
	B/Malaysia/2506/2004	BEI Resources NR-9723	0.3x	Influenza B	
Respiratory Syncytial Virus A+B	Not available	18537*	ATCC VR-1580	1x1x	RSV A+B
		A2*	ATCC VR-1540	0.3x	RSV A+B
		Long*	ATCC VR-26	1x	RSV A+B
		CH93(18)-18*	ZeptoMetrix 0810040CFHI	1x	RSV A+B
		Not available	ZeptoMetrix 0810040ACFHI	0.1x	RSV A+B
		B/WV/14617/85	ATCC VR-1400	1x	RSV A+B

* Strain tested during LoD verification study.

[†] Both strains are derivative from B/Lee/40 ancestral lineage and, according to *in silico* analysis, they were predicted to be detected by the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

[†] *In silico* analysis showed that this strain should be detected by QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

Note: Influenza A/Brisbane/59/07 (H1N1), Influenza a/Port Chalmers/1/73 (H3N2), and Influenza A H1N1 pdm09 A/Virginia/ATCC1/2009 (H1N1 pdm09) were taken as reference strain to calculate the x-fold LoD detected for the corresponding serotype.

All pathogens tested showed positive results at the concentration tested.

In addition, *in silico* analysis for SARS-CoV-2 assay shows in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel a 100% sequence identity to 2,277,575 out of the 2,357,125 (96.63%) SARS-CoV-2 available complete genomes since 01.01.2020 until 13.07.2021.

For those genomes with any mismatch in any oligonucleotide, 73,135 genomes (3.10%) showed any mismatch in non-critical positions with no expected impact in the PCR, whereas only 6,508 genomes (0.28%) presented mismatches with potentially critical impact.

However, the influence of those most abundant critical mismatches has been flagged for experimental check with no impact on assay performance detected. These single mismatches are tolerated by the PCR workflow in the QIAstat-Dx system.

As a conclusion, no safety and performance issues with the QIAstat-Dx Respiratory SARS-CoV-2 assay were identified among all SARS-CoV-2 genomic sequences available as of July 2021, including all Variants of Concern (VOCs), Variants of Interest (VOIs), and Variants Under Investigation (VUIs) described up to this date.

Interfering substances

The effect of potentially interfering substances on the detectability of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel organisms was evaluated. Thirty (30) potentially interfering substances were added to contrived samples at a level predicted to be above the concentration of the substance likely to be found in an authentic NPS specimen. The contrived samples (also referred to as combined samples) were each comprised of a mix of organisms tested at a concentration of 5x LoD.

Endogenous substances such as whole blood, human genomic DNA, and several pathogens were tested alongside exogenous substances like antibiotics, nasal sprays, and different workflow contaminants.

The combined samples were tested with and without addition of an inhibitory substance allowing direct sample-to-sample comparison. Combined samples not spiked with any test substance served as a positive control. Additionally, for substances that may contain genetic material (such as blood, mucin, DNA, and microorganisms), negative specimens (blank sNPS sample matrix with no organism mix) were spiked with only the test substance to evaluate the potential for false positive results due to the test substance itself.

Combined samples not spiked with any test substance served as a positive control and blank sNPS sample matrix with no organism mix as negative controls.

All pathogen-containing samples without spiked interferent generated positive signals for all pathogens present in the respective combined sample. Negative signals were obtained for all pathogens not present in the same sample but detected by the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

None of the substances tested showed inhibition, except for the nasal influenza vaccines. This was due to the fact that the selection of substances concentration was higher than the concentrations expected to be present in a sample. In addition, nasal influenza vaccines (Fluenz[®] Tetra and FluMist[®]) were predicted to be reactive with the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Influenza A and Influenza B assays. Final dilution without observable interfering effect was 0.000001% v/v for both vaccines.

No impact on performance is expected when clinical liquid samples are examined in the presence of the substances tested.

Clinically relevant co-infections testing demonstrated that when at least two QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel pathogens of different concentrations are simultaneously present in one sample, all targets can be detected by the assay.

Although SARS-CoV-2 was not assessed in the interference study, inhibitory effects of several substances were assessed for four viruses of the Coronaviridae family (229E, HKU1, OC43, and NL63) in previous studies. Results demonstrated that detection of coronavirus was not inhibited when using the tested substances, and then it is expected that SARS-CoV-2 is equivalently detected when these substances are present in NPS samples.

The results of interfering substance testing are provided in Table 9.

Table 9. Final highest concentration without observable inhibitory effect

Substance tested	Concentration tested	Results
Endogenous substances		
Human genomic DNA 200 ng/μL	20 ng/μl	No Interference
Human blood (+NaCitrate)	1% v/v	No Interference
Mucin from bovine submaxillary	1% v/v	No Interference
Competitive microorganisms		
<i>Staphylococcus aureus</i>	1.00E+06 CFU/ml	No Interference
<i>Neisseria meningitidis</i>	5.00E+04 CFU/ml	No Interference
<i>Corynebacterium diphtheriae</i>	5.00E+03 CFU/ml	No Interference
Human Cytomegalovirus	1.00E+05 TCID ₅₀ /ml	No Interference
Exogenous substances		
Tobramycin	0.6 mg/ml	No Interference
Mupirocin	2% w/v	No Interference
Saline nasal spray with preservatives	1% v/v	No Interference
Afrin® severe congestion nasal spray (Oxymetazoline HCl)	1% v/v	No Interference
Analgesic ointment (Vicks® VapoRub®)	1% w/v	No Interference
Petroleum Jelly (Vaseline®)	1% w/v	No Interference
FluMist nasal influenza vaccine	0.00001% v/v	Interference
FluMist nasal influenza vaccine	0.000001% v/v	No Interference
Fluenz Tetra nasal influenza vaccine	0.00001% v/v	Interference
Fluenz Tetra nasal influenza vaccine	0.000001% v/v	No Interference
Disinfecting/cleaning substances		
Disinfecting wipes	½ inches ² /1 ml UTM	No Interference
DNAzap™	1% v/v	No Interference
RNaseOUT™	1% v/v	No Interference
Bleach	5% v/v	No Interference
Ethanol	5% v/v	No Interference
Specimen collection materials		
Swab Copan 168C	1 swab/1 ml UTM	No Interference
Swab Copan FloQ®	1 swab/1 ml UTM	No Interference
Swab Copan 175KS01	1 swab/1 ml UTM	No Interference
Swab Puritan 25-801 A 50	1 swab/1 ml UTM	No Interference
VTM Sigma Virocult	100%	No Interference
VTM Remel® M4RT	100%	No Interference
VTM Remel M4	100%	No Interference
VTM Remel M5	100%	No Interference
VTM Remel M6	100%	No Interference
BD Universal Viral Transport	100%	No Interference

Carryover

A carryover study was performed to evaluate the potential occurrence of cross-contamination between consecutive runs when using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel on the QIAstat-Dx Analyzer 1.0.

Samples of simulated NPS matrix with alternating high-positive and negative samples were conducted on one QIAstat-Dx Analyzer 1.0.

No carryover between samples containing SARS-CoV-2 was observed in the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

Reproducibility

Reproducibility testing of transport medium contrived samples was performed at 3 test sites including two external sites (LACNY [Laboratory Alliance of Central New York] and INDIANA [Indiana University]) and one internal site (STAT). 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 days with 4 replicates per day (leading to a total of 20 replicates per target, concentration, and site), a minimum of 2 different QIAstat-Dx Analyzers per site, and at least 2 operators on each testing day.

A total of 12 transport medium liquid sample mixes were prepared with at least 3 replicates tested per sample mix. Each pathogen was spiked into HeLa in UTM combined samples in a final concentration of 0.1x LoD, 1x LoD, or 3x LoD, respectively. A summary of results for each analyte is provided in Table 10, Table 11, and Table 12, respectively.

Table 10 (next page) summarizes the results for 0.1x LoD concentration. Reproducibility and repeatability will impact the SARS-CoV-2 target in the same manner as other target organisms previously verified.

Table 10. Detection rate per target at 0.1x LoD concentration for each site of reproducibility study and 2-sided 95% Confidence Interval by target

Target (0.1x LoD)	Site	Detection rate (# positive)	% detection rate (# positive)	95% Confidence Interval
Influenza A (0810249CFHI, H1N1 pdm09)	STAT	19/20	95.0%	76.4–99.1%
	LACNY	18/20	90.0%	69.9–97.2%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	57/60	95.0%	86.3–98.3%
Influenza A (ATCC VR-810, H3N2)	STAT	10/20	50.0%	29.9–70.1%
	LACNY	9/19	47.4%	27.3–68.3%
	INDIANA	16/19	84.2%	62.4–94.5%
	All sites (overall)	35/58	60.3%	47.5–71.9%
Influenza A (ATCC VR-897, H1N1)	STAT	14/20	70.0%	48.1–85.5%
	LACNY	9/19	47.4%	27.3–68.3%
	INDIANA	12/20	60.0%	38.7–78.1%
	All sites (overall)	35/59	59.3%	46.6–70.9%
Influenza B (ATCC VR-295)	STAT	7/20	35.0%	18.1–56.7%
	LACNY	9/19	47.4%	27.3–68.3%
	INDIANA	8/20	40.0%	21.9–61.3%
	All sites (overall)	24/59	40.7%	29.1–53.4%
Respiratory Syncytial Virus A (ATCC VR-1540)	STAT	6/20	30.0%	14.5–51.9%
	LACNY	7/20	35.0%	18.1–56.7%
	INDIANA	9/20	45.0%	25.8–65.8%
	All sites (overall)	22/60	36.7%	25.6–49.3%
Respiratory Syncytial Virus B (0810040CF)	STAT	14/20	70.0%	48.1–85.5%
	LACNY	15/19	79.0%	56.7–91.5%
	INDIANA	10/20	50.0%	29.9–70.1%
	All sites (overall)	39/59	66.1%	53.4–76.9%

Table 11 summarizes the results for 1x LoD concentration.

Table 11. Detection rate per target at 1x LoD concentration for each site of reproducibility study and 2-sided 95% Confidence Interval by target

Target (1x LoD)	Site	Detection rate (# positive)	% detection rate (# positive)	95% Confidence Interval
Influenza A (0810249CFHI)	STAT	20/20	100%	83.9–100%
	LACNY	19/19	100%	83.2–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	59/59	100%	93.9–100%
Influenza A (ATCC VR-810)	STAT	19/20	95.0%	76.4–99.1%
	LACNY	18/18	100%	82.4–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	57/58	98.3%	90.9–99.7%
Influenza A (ATCC VR-897)	STAT	19/20	95.0%	76.4–99.1%
	LACNY	20/20	100%	83.9–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	59/60	98.3%	91.1–99.7%
Influenza B (ATCC VR-295)	STAT	19/20	95.0%	76.4–99.1%
	LACNY	20/20	100%	83.9–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	59/60	98.3%	91.1–99.7%
Respiratory Syncytial Virus A (ATCC VR-1540)	STAT	20/20	100%	83.9–100%
	LACNY	19/19	100%	83.2–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	59/59	100%	93.9–100%
Respiratory Syncytial Virus B (0810040CF)	STAT	20/20	100%	83.9–100%
	LACNY	20/20	100%	83.9–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	60/60	100%	94.0–100%

Table 12 summarizes the results for 3x LoD concentration.

Table 12. Detection rate per target at 3x LoD concentration for each site of reproducibility study and 2-sided 95% Confidence Interval by target

Target (3x LoD)	Site	Detection rate (# positive)	% detection rate (# positive)	95% Confidence Interval
Influenza A (0810249CFHI)	STAT	20/20	100%	83.9–100%
	LACNY	19/19	100%	83.2–100%
	INDIANA	19/19	100%	83.2–100%
	All sites (overall)	58/58	100%	93.8–100%
Influenza A (ATCC VR-810)	STAT	20/20	100%	83.9–100%
	LACNY	19/19	100%	83.2–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	59/59	100%	93.9–100%
Influenza A (ATCC VR-897)	STAT	20/20	100%	83.9–100%
	LACNY	20/20	100%	83.9–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	60/60	100%	94.0–100%
Influenza B (ATCC VR-295)	STAT	19/20	95.0%	76.4–99.1%
	LACNY	19/19	100%	83.2–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	58/59	98.3%	91.0–99.7%
Respiratory Syncytial Virus A (ATCC VR-1540)	STAT	20/20	100%	83.9–100%
	LACNY	19/19	100%	83.2–100%
	INDIANA	19/19	100%	83.2–100%
	All sites (overall)	58/58	100%	93.8–100%
Respiratory Syncytial Virus B (0810040CF)	STAT	20/20	100%	83.9–100%
	LACNY	20/20	100%	83.9–100%
	INDIANA	20/20	100%	83.9–100%
	All sites (overall)	60/60	100%	94.0–100%

Dry swab samples were tested in replicates using different lots of QIAstat-Dx Respiratory Panel Cartridges and tests were executed on different QIAstat-Dx Analyzers 1.0 by different operators, on different sites, and on different days.

A representative sample mix containing Influenza B and SARS-CoV-2 was prepared to test performance reproducibility in dry swab samples.

Table 13. List of respiratory pathogens tested for performance reproducibility in dry swab samples

Pathogen	Strain
Influenza B	B/FL/04/06
SARS-CoV-2	England/02/2020

Table 14. Summary of Positive Agreement/Negative Agreement for reproducibility testing in dry swab samples

Concentration	Pathogen	Site	Expected result	Detection rate	% Agreement with Expected Result
3x LoD	Influenza B	Site 1	Positive	30/30	100
		Site 2	Positive	30/30	100
		Site 3	Positive	30/30	100
		All	Positive	90/90	100
	SARS-CoV-2	Site 1	Positive	30/30	100
		Site 2	Positive	30/30	100
Site 3		Positive	30/30	100	
1X LoD	Influenza B	All	Positive	90/90	100
		Site 1	Positive	30/30	100
		Site 2	Positive	30/30	100
		Site 3	Positive	30/30	100
	SARS-CoV-2	Site 1	Positive	30/30	100
		Site 2	Positive	30/30	100
Site 3		Positive	30/30	100	
Negative	All	All	Negative	120/120	100
		Site 1	Negative	120/120	100
		Site 2	Negative	120/120	100
		Site 3	Negative	120/120	100
		All	Negative	360/360	100

All samples tested generated the expected result (95–100% agreement) showing reproducible performance of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

Reproducibility testing demonstrated that the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel running in the QIAstat-Dx Analyzer 1.0 provides highly reproducible test results when the same samples are tested in multiple runs, on multiple days, with multiple sites, with various operators using different QIAstat-Dx Analyzers 1.0, and multiple lots of QIAstat-Dx Cartridges.

Appendices

Appendix A: Installing the Assay Definition File

The Assay Definition File of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel must be installed on the QIAstat-Dx Analyzer 1.0 prior to testing with QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridges.

Note: Whenever a new version of the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel assay is released, the new QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Assay Definition File must be installed prior to testing.

Note: Assay Definition Files are 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 new assays 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 36).

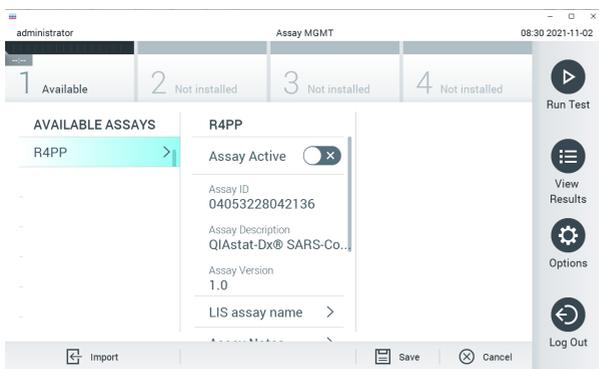


Figure 36. Assay Management screen.

3. Press the **Import** icon in the bottom left of the screen.
4. Select the file corresponding to the assay to be imported from the USB drive.
5. A dialog will appear to confirm upload of the file.
6. A dialog may appear to override the current version by a new one. Press **yes** to override.
7. The assay becomes active by selecting **Assay Active** (Figure 37).

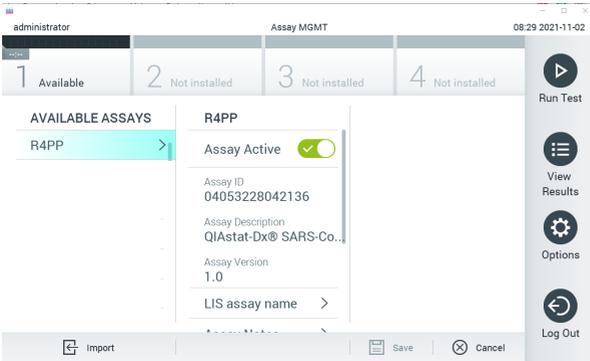


Figure 37. Activating the assay.

8. 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. Next, select **Assign Assays** from the "User Options". Enable the assay and press the **Save** button (Figure 38).

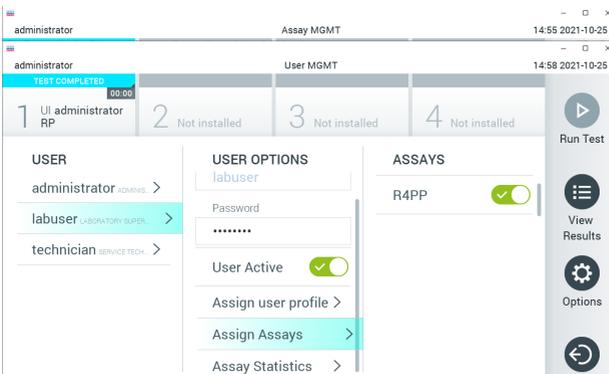


Figure 38. Assigning the active assay.

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 SARS-CoV-2/Flu A/B/RSV Panel 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 SARS-CoV-2/Flu A/B/RSV Panel 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 respiratory pathogens.

IFU: Instructions For Use.

Main port: In the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel 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

RT: Reverse Transcription

Swab port: In the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge, inlet for dry swabs.

User: A person who operates the QIAstat-Dx Analyzer 1.0/QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge in the intended way.

Appendix C: Disclaimer of warranties

EXCEPT AS PROVIDED IN QIAGEN TERMS AND CONDITIONS OF SALE FOR THE QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge, QIAGEN ASSUMES NO LIABILITY WHATSOEVER AND DISCLAIMS ANY EXPRESS OR IMPLIED WARRANTY RELATING TO THE USE OF THE QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridge INCLUDING LIABILITY OR WARRANTIES RELATING TO MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR INFRINGEMENT OF ANY PATENT, COPYRIGHT, OR OTHER INTELLECTUAL PROPERTY RIGHT ANYWHERE IN THE WORLD.

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Symbols

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



Contains reagents sufficient for <N> reactions



Use by



In vitro diagnostic medical device



Catalog number



Lot number



Material number (i.e., component labeling)



Upper respiratory application

Rn

R is for revision of the Handbook and n is the revision number



Temperature limitation



Manufacturer



Consult instructions for use



Caution



CE marking for European Conformity



Serial number



Do not reuse



Keep away from sunlight



Do not use if package is damaged



Global Trade Item Number

Ordering Information

Product	Contents	Cat. no.
QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel	For 6 tests: 6 individually packaged QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel Cartridges and 6 individually packaged transfer pipettes	691216
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
Revision 1 11/2021	Initial release.

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