GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® Xpress CoV-2/Flu/RSV *plus*

REF **XP3COV2FLURSV-GB10**

Instructions for Use

For Use with GeneXpert® System with Touchscreen Running Cepheid OS

UK CA IVD

Cepheid®

In Vitro Diagnostic Medical Device

303-0026, Rev. B
2025-08

Trademark, Patents and Copyright Statements

See Section 23, Revision History for a description of changes.

Xpert® Xpress CoV-2/Flu/RSV *plus*

1 Proprietary Name

Xpert® **Xpress** CoV-2/Flu/RSV ***plus***

2 Common or Usual Name

Xpert Xpress CoV-2/Flu/RSV *plus*

3 Intended Use

The Xpert Xpress CoV-2/Flu/RSV *plus* test, performed on the GeneXpert System with Touchscreen Running Cepheid OS (a touchscreen configuration within the GeneXpert Instrument Family), is a multiplexed real-time RT-PCR test intended for use in the simultaneous *in vitro* qualitative detection and differentiation of RNA from SARS-CoV-2, influenza A, influenza B and/or respiratory syncytial virus (RSV) in nasopharyngeal swab or anterior nasal swab specimens collected from individuals with signs and/or symptoms of respiratory viral infection.

SARS-CoV-2, influenza A, influenza B and RSV RNA identified by this test are generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test.

Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus and/or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

3.1 Intended User/Environment

The Xpert® **Xpress** CoV-2/Flu/RSV ***plus*** test is intended to be performed by trained users in both laboratory and near patient testing settings.

4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV), which has since spread globally, resulting in a pandemic of coronavirus disease 2019 (COVID-19). COVID-19 is associated with a variety of clinical outcomes, including asymptomatic infection, mild upper respiratory infection, severe lower respiratory disease including pneumonia and respiratory failure, and in some cases, death. The International Committee on Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2]

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily via aerosolized droplets (i.e., coughing or sneezing) and the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, malaise, cough and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting or diarrhea) may also occur, primarily in children, but are less common. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication due to influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.[3,4]

Influenza viruses are classified into types A, B, and C, the former two of which cause the most human infections. Influenza A (Flu A) is the most common type of influenza virus in humans and is generally responsible for seasonal flu epidemics and potentially pandemics. Flu A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B (Flu B) virus are generally restricted to humans and less frequently cause epidemics.[5] Flu A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by influenza A subtypes H1, H2, H3, N1 and N2.

Respiratory Syncytial Virus (RSV), a member of the *Pneumoviridae* family (formerly *Paramyxoviridae*), consisting of two strains (subgroups A and B), is also the cause of a contagious disease that affects primarily infants, the elderly, and those who are immunocompromised (e.g., patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system).[6] The virus can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.[6] By the age of two years, most children have already been infected by RSV and because only weak immunity develops, both children and adults can be re-infected.[6] RSV remains the leading cause for hospitalizations in infants worldwide.[7] Symptoms appear four to six days after infection and are usually self-limiting, lasting approximately one to two weeks in infants. In adults, infection lasts about 5 days and presents as symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season usually mirrors influenza as infections begin to rise during the fall and last through early spring.[5,6]

SARS-CoV-2, influenza, and RSV viruses can cause infections that present with very similar symptoms, making clinical differentiation between them very difficult.[8] Active surveillance programs in conjunction with infection prevention precautions are important components for preventing transmission of SARS-CoV-2, influenza and RSV. The use of assays providing rapid results to identify patients infected with these viruses can be an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

5 Principle of the Procedure

The Xpert Xpress CoV-2/Flu/RSV *plus* test is an automated *in vitro* diagnostic test for the simultaneous qualitative detection and differentiation of RNA from SARS-CoV-2, Flu A, Flu B, and RSV using reverse transcription PCR (RT-PCR). The Xpert Xpress CoV-2/Flu/RSV *plus* test is performed on GeneXpert Instrument Systems. The primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test are designed to amplify and detect unique sequences in the following: nucleocapsid (N) and envelope (E) and RNA-dependent RNA polymerase (RdRP) genes of the SARS-CoV-2 virus genome, influenza A matrix (M), influenza A basic polymerase (PB2), influenza A acidic protein (PA), influenza B matrix (M), influenza B non-structural protein (NS), and the RSV A and RSV B nucleocapsid genes.

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR and RT-PCR assays. The systems consist of an instrument, touchscreen, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the PCR/RT-PCR reagents and host the PCR/RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the *GeneXpert System with Touchscreen Operator Manual*.

The Xpert Xpress CoV-2/Flu/RSV *plus* test includes reagents for the detection of SARS-CoV-2, Flu A, Flu B and RSV viral RNA in either nasopharyngeal or anterior nasal swab specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The specimen is collected and placed into a transport tube containing 3 mL of viral transport medium, 3 mL of saline or 2 mL of eNAT™. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Xpress CoV-2/Flu/RSV *plus* kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Xpress CoV-2/Flu/RSV *plus* Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent	1.0 mL per cartridge
• Binding Reagent	1.0 mL per cartridge
• Elution Reagent	3.0 mL per cartridge
• Wash Reagent	0.4 mL per cartridge
Disposable Transfer Pipettes	**10-12 per kit**
Flyer	**1 per kit**

- Instructions to locate (and import) the ADF and documentation such as the Product Insert on www.cepheid.com.

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The protein stabilizer of bovine origin in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

7 Kit Storage and Handling

- Store the Xpert Xpress CoV-2/Flu/RSV *plus* cartridges at 2–28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.

8 Materials Required but Not Provided

- Nylon flocked swab (Copan P/N 502CS01, 503CS01) or equivalent
- Viral transport medium, 3 mL (Copan P/N 330C) or equivalent
- 0.85-0.9% (w/v) saline, 3 mL
- Nasopharyngeal Sample Collection Kit for Viruses (Cepheid P/N SWAB/B-100, Copan P/N 305C) or equivalent
- Nasal Sample Collection Kit for Viruses (Cepheid P/N SWAB/F-100, Copan P/N 346C) or equivalent
- GeneXpert instrument, touchscreen with built-in barcode scanner, operator manual.
- Cepheid OS

9 Materials Available but Not Provided

External controls in the form of inactivated virus(es) are available from ZeptoMetrix (Buffalo, NY).

- External Positive Control: Catalog #NATFRC-6C (NATtrol Flu/RSV/SARS-CoV-2)
- External Negative Control: Catalog #NATCV9-6C (NATtrol Coxsackievirus A9)

eNAT Molecular Collection and Preservation Medium from Copan Italy S.p.A. (Brescia, IT):

- eNAT Molecular Collection and Preservation Medium, Copan Catalog #6U073S01
- eNAT Molecular Collection and Preservation Medium, Copan Catalog #6U074S01

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- Positive results are indicative of presence of Flu A, Flu B, RSV, or SARS-CoV-2 RNA.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[9] and the Clinical and Laboratory Standards Institute.[10]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Refer to Copan eNAT® Package Insert for safety and handling information.
- Avoid direct contact between guanidine thiocyanate and sodium hypochlorite (bleach) or other highly reactive reagents such as acids and bases. These mixtures could release noxious gas.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

10.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

10.3 Assay/Reagent

- Do not open the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use reagents beyond their expiry date.
- Each single-use Xpert Xpress CoV-2/Flu/RSV *plus* cartridge is used to process one test. Do not reuse processed cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 10% freshly prepared household chlorine bleach. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.

11 Chemical Hazards[11, 12]

- **Signal Word: Warning**
- **UN GHS Hazard Statements**

- Harmful if swallowed
- May be harmful in contact with skin
- Causes eye irritation

- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash hands thoroughly after handling.
  - **Response**
    - Call a POISON CENTER or doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for nasopharyngeal swab collection procedure and Section 12.2 for anterior nasal swab collection procedure. Nasopharyngeal and anterior nasal swab specimens can be stored at room temperature (15–30 °C) for up to 48 hours in viral transport medium, saline, or eNAT until testing is performed on the GeneXpert Instrument Systems. Alternatively, nasopharyngeal and anterior nasal swab specimens can be stored refrigerated (2–8 °C) up to seven days in viral transport medium or saline, and up to six days in eNAT until testing is performed on the GeneXpert Instrument Systems.

Samples collected into saline should not be frozen. Refer to the WHO Laboratory Biosafety Guidance Related to the Coronavirus Disease 2019 (COVID-19).

https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19)

12.1 Nasopharyngeal Swab Collection Procedure

1. Insert the swab into either nostril, passing it into the posterior nasopharynx (see Figure 1).

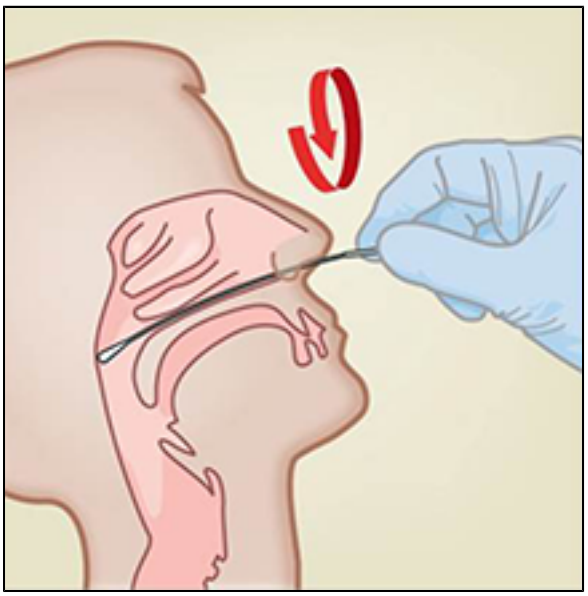


Figure 1. Nasopharyngeal Swab Collection

2. Rotate swab by firmly brushing against the nasopharynx several times.
3. Remove and place the swab into the tube containing 3 mL of viral transport medium, 3 mL of saline, or 2 mL of eNAT.
4. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.2 Nasal Swab Collection Procedure

1. Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril (see Figure 2).

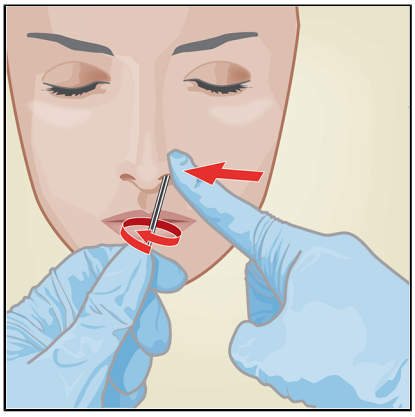


Figure 2. Nasal Swab Collection for First Nostril

2. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril (see Figure 3). To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.

Figure 3. Nasal Swab Collection for Second Nostril

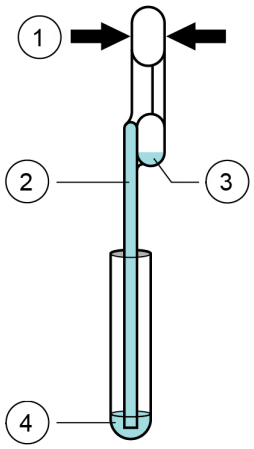
3. Remove and place the swab into the tube containing 3 mL of viral transport medium, 3mL of saline, or 2 mL of eNAT. Break swab at the indicated break line and cap the specimen collection tube tightly.

13 Procedure

13.1 Preparing the Cartridge

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

1. Remove a cartridge from the package.
2. Check the specimen transport tube is closed.
3. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open the cap on the specimen transport tube.
4. Open the cartridge lid.
5. Remove the transfer pipette from the wrapper.
6. Squeeze the top bulb of the transfer pipette **completely until the top bulb is fully flat**. While continuing to hold the bulb fully flat, place the pipette tip in the specimen transport tube (see Figure 4).



Number	Description
1	Squeeze here
2	Pipette
3	Overflow Reservoir Bulb
4	Sample

Figure 4. Transfer Pipette

7. Keeping the pipette below the surface of the liquid, release the top bulb of the pipette slowly to fill the pipette with sample before removing from the tube. It is okay if liquid goes into the overflow reservoir (see Figure 4). Check that the pipette does not contain bubbles.
8. To transfer the sample to the cartridge, squeeze the top bulb of the pipette completely again until it is fully flat to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) of the cartridge shown in Figure 5. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.

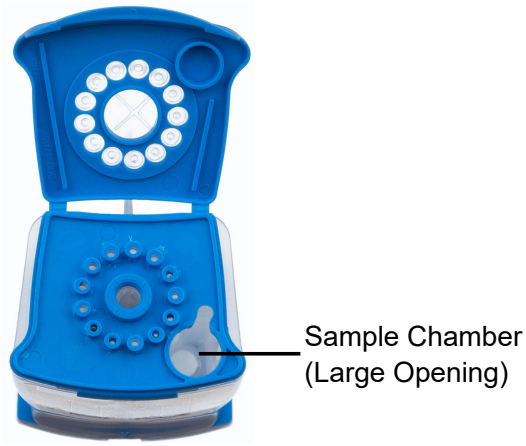


Figure 5. Xpert Xpress CoV-2/Flu/RSV *plus* Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample volume is added to the cartridge.

9. Close the cartridge lid.

13.2 External Controls

External controls described in Section 9 are available but not provided and should be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert Xpress CoV-2/Flu/RSV *plus* test, perform the following steps:

1. Mix control by rapidly inverting the external control tube 5 times. Open the cap on external control tube.
2. Open the cartridge lid.
3. Using a clean transfer pipette, transfer one draw of the external control sample (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 5.
4. Close cartridge lid.

13.3 Starting the Test in Cepheid OS 1.0

Important **Before you start the test, make sure that the system contains modules with Cepheid OS 1.0 and that the appropriate Assay Definition File is imported into the software.**

Important **This section lists the default steps to operate the GeneXpert system with touchscreen. For detailed instructions, see the *GeneXpert System with Touchscreen Operator Manual*.**

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert system with touchscreen:
   a) Turn on the GeneXpert II or GeneXpert IV Instrument. The power switch is located on the back of the instrument. Press the switch to the **ON (|)** position.
   b) Turn on the touchscreen. The power switch is located on the back of the touchscreen. Press the switch to the **ON (|)** position.
2. Log on to the Cepheid OS Software using your username and password.
3. On the HOME screen, touch **NEW TEST**.
4. Enter a patient ID.
5. Touch **CONTINUE** and **CONFIRM**.
6. Enter a sample ID.
7. Touch **CONTINUE** and **CONFIRM**.
8. Scan cartridge barcode. Hold the cartridge about 10 cm (4 inches) away from the scanner.

Note If the barcode on the cartridge does not scan, then repeat the test with a new cartridge.

9. After scanning, touch **CONFIRM**.
10. If you are not logged in, the Enter Credentials to Continue screen appears. Enter your user name and password and touch **Login**.
11. The Cartridge Preparation screen appears. Watch video (if necessary) and prepare cartridge if you haven't already done so. Touch **CONTINUE**.
12. Load the prepared cartridge.
13. Open the instrument module door below the flashing green light.
14. Place the cartridge on the module bay floor with the cartridge label facing out.

Note Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or touchscreen will stop the test.

15. Press the module door closed. The door latches and the flashing green light turns solid green and the Test Loading screen appears, followed by the Test Running screen.
    When the test completes, the Test Completed screen appears.
16. Remove the cartridge and dispose of according to your institution's hazardous waste disposal guidelines.
17. Touch **REPORT** to view a test report.

13.4 Viewing Results in Cepheid OS 1.0

This section lists the basic steps for viewing and printing results in Cepheid OS 1.0 software. For more detailed instructions on how to view and print the results, see the *GeneXpert System with Touchscreen Operator Manual.*

Note If reporting results using a LIS, confirm that LIS results match system results for the patient ID field; if results conflict, report the system results only.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the **View Results** window to view and/or generate a PDF report file.

13.5 Starting the Test in Cepheid OS 2.1 or Higher

Before you start the test, make sure that:

Important

- The system is running the correct Cepheid OS software version shown in section - Materials Required but Not Provided.
- The correct assay definition file is imported into the software.

Note The default workflow is shown. Your system administrator may alter the workflow.

1. Turn on GeneXpert system with touchscreen.
2. Log on to system software using your username and password.
3. On the Modules tab, touch **Start Test**.
4. Follow onscreen prompts to create new test and enter patient and sample information.
5. Scan or manually input the cartridge serial number. If scanning, hold the cartridge about 1-3 inches (3-7 cm) away from the scanner. The scanner projects a green crosshair, which you center on the barcode. Scanning is complete when you hear an audible beep. Touch **Continue**.
6. Select the desired test and touch **Continue**.
7. Watch the cartridge preparation video, if needed.
8. On the Confirm screen, review all data and touch **Confirm**.
9. Open the module door under flashing green light and insert the cartridge.
10. Close cartridge module door completely by pressing until it latches. The test starts.
11. When the test completes, the **Results Summary** screen appears. Open the module door and remove cartridge.
12. Dispose of used cartridge in appropriate waste container according to your institution’s standard practices.

13.6 Viewing Results in Cepheid OS 2.1 or Higher

The Cepheid OS 2.1 results screen will automatically interpret test results for you and clearly show them in the **View Results** window.

1. Tap **Results**.
2. Tap the test to be viewed in the Results screen.
3. Click **OK**.
4. To generate a PDF report file, touch **View Report**.
   More detailed instructions for viewing and uploading results are available in your system operator manual.

14 Quality Control

14.1 Internal Controls

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) – Before the start of the PCR reaction, the GeneXpert system measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

14.2 External Controls

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

15 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress CoV-2/Flu/RSV *plus* test provides test results based on the detection of respective gene targets according to the algorithms.

The format of the test results presented will vary depending on the user's choice to run either an Xpress SARS-CoV-2-Flu-RSV-plus, Xpress SARS-CoV-2-Flu-plus or Xpress SARS-CoV-2-plus test.

Table 1 shows the possible result outcomes when the Xpress SARS-CoV-2-Flu-RSV-plus test mode is selected.

Table 1. Xpress SARS-CoV-2-Flu-RSV-plus Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred ● Probe Check: PASS; all probe check results pass
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting ● SPC: NA; SPC is ignored because the Flu A target amplification occurred ● Probe Check: PASS; all probe check results pass
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA; SPC is ignored because the Flu B target amplification occurred ● Probe Check: PASS; all probe check results pass
RSV POSITIVE	The RSV target RNA is detected. ● The RSV signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA; SPC is ignored because the RSV target amplification occurred ● Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE;** **RSV NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. ● SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected ● SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
INVALID	SPC or other analysis settings do not meet acceptance criteria and all targets are not detected. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. ● SPC: FAIL; SPC and SARS-CoV-2, Flu A, Flu B and RSV signals do not have a Ct within valid range and endpoint is below minimum setting ● SARS-CoV-2 amplification fails specification ● Probe Check: PASS; all probe check results pass

Result	Interpretation
ERROR	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● RSV: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL[1]; all or one of the probe check results fail [1]If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● RSV: NO RESULT ● SPC: NO RESULT ● Probe Check: NA

If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another approved test in the UK, if coinfection would change clinical management.

Table 2 shows the possible result outcomes when the Xpress SARS-CoV-2-Flu-plus test mode is selected.

Table 2. Xpress SARS-CoV-2-Flu-plus Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred ● Probe Check: PASS; all probe check results pass
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting ● SPC: NA; SPC is ignored because Flu A target amplification occurred ● Probe Check: PASS; all probe check results pass
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA; SPC is ignored because the Flu B target amplification occurred ● Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected. ● SARS-CoV-2, Flu A, and Flu B target RNAs are not detected ● SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
INVALID	SPC or other analysis settings do not meet acceptance criteria and all targets are not detected. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. ● SPC: FAIL; SPC and SARS-CoV-2, Flu A and Flu B signals do not have a Ct within valid range and endpoint is below minimum setting. ● SARS-CoV-2 amplification fails specification ● Probe Check: PASS; all probe check results pass
ERROR	Presence or absence of SARS-CoV-2, Flu A and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.

Result	Interpretation
NO RESULT	Presence or absence of SARS-CoV-2, Flu A and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● SPC: NO RESULT ● Probe Check: NA

If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another approved test in the UK, if coinfection would change clinical management.

Table 3 shows the possible result outcomes when the Xpress SARS-CoV-2-plus test mode is selected.

Table 3. Xpress SARS-CoV-2-plus Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred ● Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE	SARS-CoV-2 target RNA is not detected. ● SARS-CoV-2 target RNA is not detected ● SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
INVALID	SPC or other analysis settings do not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. ● SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting ● SARS-CoV-2 amplification fails specification ● Probe Check: PASS; all probe check results pass
ERROR	Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. ● SPC: NO RESULT ● Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.

Result	Interpretation
NO RESULT	Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 16.2 of the IFU. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● SPC: NO RESULT ● Probe Check: NA

The Xpert Xpress CoV-2/Flu/RSV *plus* test can be run to detect SARS-CoV-2, Flu and RSV by selecting **Xpress SARS-CoV-2-Flu-RSV-plus** from the **Select Test** menu; SARS-CoV-2 and Flu only by selecting **Xpress SARS-CoV-2-Flu-plus**; or SARS-CoV-2 only by selecting **Xpress SARS-CoV-2-plus**. The Xpress SARS-CoV-2-plus test mode includes an Early Assay Termination (EAT) function that will provide earlier time to result in high titer specimens if the signal from the SARS-CoV-2 target reaches a predetermined threshold before the full 45 PCR cycles have been completed. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen, and its results may not be reported.

16 Retests

16.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 16.2.

- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected. Alternatively, other assay analysis settings intended to produce a valid test result were not met.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, no sample added, or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.
- Because the incidence of co-infection with three or more viruses (Influenza A, Influenza B, RSV, and SARS-CoV-2) is low, it is recommended that specimens undergo repeat testing if nucleic acids from three or more viruses are detected in a single specimen.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid Technical Support for assistance.

16.2 Retest Procedure

To retest a non-determinate result (**INVALID**, **NO RESULT**, or **ERROR**), use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or new external control tube.

1. Put on a clean pair of gloves. Obtain a new Xpert Xpress CoV-2/Flu/RSV *plus* cartridge and a new transfer pipette.
2. Check the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly inverting the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.

17 Limitations

- Performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test has only been established in nasopharyngeal and anterior nasal swab specimens. Use of the Xpert Xpress CoV-2/Flu/RSV *plus* test with other specimen types has not been assessed and performance characteristics are unknown.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.
- As with any molecular test, mutations within the target regions of the Xpert Xpress CoV-2/Flu/RSV *plus* test could affect primer and/or probe binding resulting in failure to detect the presence of virus or the virus being detected less predictably.
- In some samples with very high SARS-CoV-2 viral concentrations, analysis settings intended to reduce the risks of false positive results caused by non-specific or irregular fluorescence detection may trigger an **INVALID** test result.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; or sample mix-up. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- False negative results may occur if virus is present at levels below the analytical limit of detection.
- Negative results do not preclude SARS-CoV-2, influenza or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Xpress CoV-2/Flu/RSV *plus* test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral nucleic acid may persist *in vivo*, independent of virus infectivity. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- This test has been evaluated for use with human specimen material only.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for patients without signs and symptoms of respiratory tract infection.
- This test has not been evaluated for monitoring treatment of infection.
- This test has not been evaluated for screening of blood or blood products for the presence of SARS-CoV-2, influenza, or RSV.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- Results from analytical studies with contrived co-infected samples showed potential for competitive interference of influenza B or RSV A at low concentrations (~3X LoD) when influenza A concentration is >1.7e5 RNA copies/mL or 1.7e6 RNA copies/mL, respectively. In addition, there is potential for competitive interference of influenza B at low concentration (~3X LoD) when SARS-CoV-2 concentration is >1e5 RNA copies/mL.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
- Zicam at 15% (w/v) may interfere with the detection of low levels of influenza B and RSV A.
- As the Xpert Xpress CoV-2/Flu/RSV *plus* test does not differentiate between the N2, RdRP and E gene targets, the presence of other coronaviruses in the B lineage, *Betacoronavirus* genus, including SARS-CoV may cause a false positive result. None of these other coronaviruses is known to currently circulate in the human population.
- This test is not intended to differentiate RSV subgroups, influenza A subtypes or influenza B lineages. If differentiation of specific RSV or influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Performance has not been established with media containing guanidine thiocyanate (GTC) other than eNAT.

18 Performance Characteristics

18.1 Clinical Evaluation

The performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated using archived clinical nasopharyngeal (NP) swab and nasal swab (NS) specimens in viral transport medium or universal transport medium. Archived specimens were selected consecutively by date and previously known analyte result. A total of 279 NP swab and 239 NS specimens were tested with Xpert Xpress CoV-2/Flu/RSV *plus* side by side with a CE-marked SARS-CoV-2 RT-PCR test and a CE-marked influenza/RSV RT-PCR test in a randomized and blinded fashion.

Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and non-determinate rate were determined by comparing the results of the Xpert Xpress CoV-2/Flu/RSV *plus* test relative to the results of a SARS-CoV-2 CE-marked RT-PCR test for the SARS-CoV-2 target, and a CE-marked RT-PCR test for the Flu A, Flu B, and RSV targets, respectively.

For the NP swab specimens, Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 100.0% and 100.0% for SARS-CoV-2, respectively; 100.0% and 100.0% for Flu A, respectively; 100.0% and 100.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 4). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.7% (2/279). On repeat testing, both (2) specimens yielded valid results. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.0% (0/279).

Table 4. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results Using NP Swab Specimens

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	279	66	0	213	0	100.0% (94.5% - 100.0%)	100.0% (98.2% - 100.0%)
Flu A	264	51	0	213	0	100.0% (93.0% - 100.0%)	100.0% (98.2% - 100.0%)
Flu B	264	46	0	218	0	100.0% (92.3% - 100.0%)	100.0% (98.3% - 100.0%)
RSV	264	47	0	217	0	100.0% (92.4% - 100.0%)	100.0% (98.3% - 100.0%)
TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval							

For the NS specimens, Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 100.0% and 100.0% for SARS-CoV-2, respectively; 100.0% and 99.5% for Flu A, respectively; 100.0% and 100.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 5). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 1.3% (3/240). Two (2) of the three (3) specimens gave valid results upon retest. One specimen was not re-tested due to insufficient volume. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.4% (1/240).

Table 5. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results Using NS Specimens

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	239	47	0	192	0	100.0% (92.4% - 100.0%)	100.0% (98.0% - 100.0%)
Flu A	239	48	1	191	0	100.0% (92.6% - 100.0%)	99.5% (97.1% - 99.9%)
Flu B	239	48	0	191	0	100.0% (92.6% - 100.0%)	100.0% (98.0% - 100.0%)
RSV	239	47	0	192	0	100.0% (92.4% - 100.0%)	100.0% (98.0% - 100.0%)

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval							

18.2 Analytical Sensitivity (Limit of Detection)

The analytical sensitivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was first estimated using two reagent lots by testing limiting dilutions of seven respiratory viruses (NATtrol SARS-CoV-2, Flu A H1, Flu A H3, Flu B Victoria lineage, Flu B Yamagata lineage, RSV A and RSV B) into pooled negative clinical NP swab matrix, following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The estimated LoD values as determined by Probit regression analysis were verified using two lots of Xpert Xpress CoV-2/Flu/RSV *plus* reagents. The verified LoD values for the viruses tested are summarized in Table 6.

Table 6. Xpert Xpress CoV-2/Flu/RSV *plus* Limit of Detection

Virus/Strain	LoD Concentration
SARS-CoV-2 (USA-WA1/2020)	138 copies/mL
Influenza A/Idaho/07/2018	0.007 $TCID_{50}$/mL
Influenza A/Hong Kong/45/2019	0.44 FFU/mL
Influenza B/Washington/2/2019	12.9 $CEID_{50}$/mL
Influenza B/Wisconsin/10/2016	2.4 $TCID_{50}$/mL
RSV A/2/Australia/61	0.33 $TCID_{50}$/mL
RSV B/9320/MA/77	0.37 $TCID_{50}$/mL

18.3 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress CoV-2/Flu/RSV *plus* was evaluated on September 27, 2021 using *in silico* analysis of the assay amplicons in relation to 2,685,478 SARS-CoV-2 sequences available in the GISAID gene database for three targets, E, N2 and RdRP.

For analysis of the E target, 3,818 sequences were excluded due to ambiguous nucleotides, which reduced the total to 2,681,660 sequences. Of the 2,681,660 GISAID sequences, 2,667,594 (99.48%) were an exact match to the SARS-CoV-2 E target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 13,990 sequences and two mismatches or more were observed for 76 sequences. Of the 76 sequences with two or more mismatches, 43 sequences contained 2 or 3 mismatches in the forward primer region; one sequence contained 3 mismatches in the reverse primer region; and one sequence contained 2 mismatches in the forward primer and 2 mismatches in the reverse primer. These double and triple mismatches could have a negative impact on the performance of the assay.

For analysis of the N2 target, 4,110 sequences were excluded due to ambiguous nucleotides, which reduced the total used in the evaluation to 2,681,368 sequences. Of the 2,681,368 GISAID sequences, 2,608,487 (97.3%) were an exact match to the SARS-CoV-2 N2 target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 70,212 sequences. Two or three mismatches were observed for 2,669 sequences. Of the 31 sequences with three variant positions, 5 sequences have two of the mismatched nucleotides in the probe region and 5 of the sequences have two of the mismatched nucleotides in the reverse primer region. These double mismatches could have an impact on probe or reverse primer binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

The RdRP is amplified using a semi-nested primer/probe set; only the inner amplicon is used for the *in silico* analysis. For analysis of the RdRP target, 1,374 sequences were excluded due to ambiguous nucleotides, which reduced the total to 2,684,104 sequences. Of the 2,684,104 GISAID sequences, 2,657,136 (99.0%) were an exact match to the SARS-CoV-2 RdRP target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 26,864 sequences and two or more mismatches were observed for 77 sequences. Two sequences have 5 mismatches,

three located in the probe region and two in the reverse primer region; 20 sequences have two nucleotide mismatches in the forward primer or probe region. These mismatches could have an impact on probe or reverse primer binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for inclusivity, the inclusivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench testing against multiple strains of SARS-CoV-2, influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and respiratory syncytial virus subgroups A and B (RSV A and RSV B) at levels near the analytical LoD. A total of 84 strains comprised of 5 SARS-CoV-2 virus strains, 4 SARS-CoV-2 in vitro RNA transcripts representing variant strains, 69 influenza viruses (48 influenza A and 21 influenza B) and 6 RSV strains (4 RSVA and 2 RSV B) were tested in this study with the Xpert Xpress CoV-2/Flu/RSV *plus* test. Three replicates were tested for each strain. All SARS-CoV-2, Flu and RSV strains tested positive in all three replicates. Results are shown in Table 7.

Table 7. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2/Flu/RSV *plus* Test

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
SARS-CoV-2	NATtrol SARS-CoV-2 USA-WA1/2020	412 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Hong Kong/VM20001061/2020	0.5 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Italy-INMI1	4 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/South_Africa/ KRISP-K005325/2020	0.2 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/England/ 204820464/2020	0.5 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA USA/WA2/2020(C09)[a]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2RNA/ England/205041766/ 2020(C14)[a]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /England/ MILK-9E05B3/2020 (C15)[a]	200 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /Japan (Brazil)/IC-0564/2021 (C17)[a]	100 copies/mL	POS	NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	30 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/WS/33	5.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/PR/8/34	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Mal/302/54	0.156 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Denver/1/57	10 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/New Jersey/8/76	5.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/New Caledonia/20/1999	0.10 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/New York/55/2004	30 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Solomon Island/3/2006	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Taiwan/42/06	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/59/2007	0.060 $TCID_{50}$/mL	NEG	POS	NEG	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	A/Swine/NY/02/2009	20 $TCID_{50}$/mL	NEG	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/Colorado/14/2012	0.13 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/45/2015	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Iowa/53/2015	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/272/2017	1.0 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Idaho/07/2018	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/505/2018	0.25 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/66/2019	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/02/2020	NA[b]	NEG	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	2.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hong Kong/8/68	2.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Port Chalmers/1/73	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/15/2001	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/67/05[c]	0.22 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/10/2007	0.025 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Minnesota/11/2010	30 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/08/2011	0.25 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/50/2012	0.050 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Alaska/232/2015	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Singapore/ INFIMH-16-0019/2016	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/71/2017	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Kansas/14/2017	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Wisconsin/04/2018	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Arizona/45/2018	2.0 FFU/mL	NEG	POS	NEG	NEG
	A/Hong Kong/45/2019	2.0 FFU/mL	NEG	POS	NEG	NEG
Avian influenza A[d]	A/Mallard/NY/6750/78 (H2N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/duck/Hunan/ 795/2002 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Vietnam/1194/ 2004 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Anhui/01/ 2005 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Japanese white eye/Hong Kong/1038/2006 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	<1 pg/µL	NEG	POS	NEG	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	A/duck/LTC-10-82743 (H7N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/chicken/New Jersey/15086/3 (H7N3)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	0.612 ng/µL	NEG	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	NA[e]	NEG	POS	NEG	NEG
	A/chicken/Korea/38349-p96323/1996 (H9N2)	<1 pg/µL	NEG	POS	NEG	NEG
Influenza B	B/Lee/40	1.0 PFU/mL	NEG	NEG	POS	NEG
	B/Allen/45	0.25 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/GL/1739/54	0.50 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/1/59	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Taiwan/2/62	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hong Kong/5/72	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
Influenza B Victoria Lineage	B/Panama/45/90	1.0 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Malaysia/2506/04	0.025 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/02/06	0.025 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Brisbane/60/2008	0.05 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/15/2016	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Colorado/6/2017	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hawaii/01/2018	8.0 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Missouri/12/2018(NA D197E)	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Washington/02/2019	60 $TCID_{50}$/mL	NEG	NEG	POS	NEG
Influenza B Yamagata Lineage	B/Florida/07/2004	0.50 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/04/06	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/01/2010	0.50 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/10/2016	20 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Indiana/17/2017	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Oklahoma/10/2018	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG
RSV A	RSV-A/NY	0.386 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A/WI-629.8.2/2007	0.50 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A/WI/629-11-1_2008	0.50 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A, Strain: 4/2015 Isolate #1	0.25 $TCID_{50}$/mL	NEG	NEG	NEG	POS
RSV B	RSV-B/WV14617/85	0.10 $TCID_{50}$/mL	NEG	NEG	NEG	POS

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	RSV-B-CH93(18)-18-01	0.10 $TCID_{50}$/mL	NEG	NEG	NEG	POS

[a] *in vitro* RNA transcripts

[b] Titer A/Indiana/02/2020 virus was without titer and was diluted 100,000-fold in simulated background matrix for testing.

[c] One of three replicates reported ERROR. The run was successfully repeated to obtain three valid replicates.

[d] Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.

[e] Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000-fold in simulated background matrix and tested due to biosafety regulations.

18.4 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 8 was conducted by mapping the SARS-CoV-2 primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 8 is expected based on the *in silico* analysis.

Table 8. Microorganisms Analyzed in the *in silico* Analysis for the SARS-CoV-2 Target

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza viruses 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-*pneumophila*
	Bacillus anthracis (Anthrax)

Microorganisms from the Same Genetic Family	High Priority Organisms
	Moraxella catarrhalis
	Neisseria elongata and *N. meningitidis*
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for cross-reactivity, the analytical specificity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench-testing a panel of 48 microorganisms comprising 4 human coronaviruses, 1 MERS coronavirus and 43 common respiratory pathogens or those potentially encountered in the nasopharynx. The panel was tested in different pools of microorganisms; if a pool produced a positive result, then each member of the pool would have been tested individually. Three replicates of each pool were tested. A sample was considered negative if all three replicates were negative. The bacterial and yeast strains were tested at concentrations of ≥ 1 x 10^6 CFU/mL with the exception of *Chlamydia pneumoniae* which was tested at 1.2 x 10^6 IFU/mL and *Lactobacillus reuteri* which was tested at 5 x 10^7 copies/mL of genomic DNA. Viruses were tested at concentrations of ≥ 1 x 10^5 $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 9.

Table 9. Respiratory Microorganisms and Human Coronavirus Tested, Concentrations and Xpert Xpress CoV-2/Flu/RSV *plus* Test Results

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Negative Control	NA	NEG	NEG	NEG	NEG
Positive Control	NA	POS	POS	POS	POS
Human coronavirus NL63	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
MERS-coronavirus	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus 229E	1.21e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus OC43	1.02e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus HKU1	1.23e6 copies/mL	NEG	NEG	NEG	NEG
Adenovirus Type 1	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Adenovirus Type 7	1.14e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Cytomegalovirus	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Echovirus	1.14e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Enterovirus	2.80e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Epstein Barr Virus	5.60e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
HSV	1.97e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human metapneumovirus	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 1	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 2	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Human parainfluenza Type 3	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 4	1.19e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Measles	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Mumps virus	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Rhinovirus Type 1A	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Acinetobacter baumannii	1.30e7 CFU/mL	NEG	NEG	NEG	NEG
Bordetella pertussis	6.40e7 CFU/mL	NEG	NEG	NEG	NEG
Burkholderia cepacia	1.90e8 CFU/mL	NEG	NEG	NEG	NEG
Candida albicans	6.30e6 CFU/mL	NEG	NEG	NEG	NEG
Candida parapsilosis	1.45e6 CFU/mL	NEG	NEG	NEG	NEG
Citrobacter freundii	1.73e8 CFU/mL	NEG	NEG	NEG	NEG
Corynebacterium sp.	1.27e7 CFU/mL	NEG	NEG	NEG	NEG
Enterococcus faecalis	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
Escherichia coli	1.55e8 CFU/mL	NEG	NEG	NEG	NEG
Hemophilus influenzae	6.62e6 CFU/mL	NEG	NEG	NEG	NEG
Lactobacillus reuteri	5.0e7 copies/mL	NEG	NEG	NEG	NEG
Legionella spp.	1.42e8 CFU/mL	NEG	NEG	NEG	NEG
Moraxella catarrhalis	2.46e6 CFU/mL	NEG	NEG	NEG	NEG
Mycoplasma pneumoniae	2.7e6 CFU/mL	NEG	NEG	NEG	NEG
Neisseria meningitides	4.2e6 CFU/mL	NEG	NEG	NEG	NEG
Neisseria mucosa	1.0e8 CFU/mL	NEG	NEG	NEG	NEG
Propionibacterium acnes	8.25e7 CFU/mL	NEG	NEG	NEG	NEG
Pseudomonas aeruginosa	1.05e7 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus haemolyticus	2.66e6 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus aureus	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus epidermidis	2.47e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus agalactiae	1.75e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus pneumoniae	2.26e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus pyogenes	9.0e6 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus salivarius	4.19e6 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus sanguinis	8.67e6 CFU/mL	NEG	NEG	NEG	NEG
Chlamydia pneumoniae	1.20e6 CFU/mL	NEG	NEG	NEG	NEG
Mycobacterium tuberculosis (avirulent)	1.20e6 CFU/mL	NEG	NEG	NEG	NEG

18.5 Microbial Interference

Microbial interference of the Xpert Xpress CoV-2/Flu/RSV *plus* test caused by the presence of bacterial or viral strains that might be encountered in human upper respiratory tract specimens, was evaluated by testing a panel of 10 commensal microorganisms, consisting of 7 viral strains and 3 bacterial strains. Contrived samples consisted of SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B viruses seeded at 3x the Limit of Detection (LoD) into simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix in the presence of Adenovirus Type 1C, Human Coronavirus OC43, Rhinovirus Type 1A, Human metapneumovirus, Human parainfluenza Types 1, 2, and 3 (each seeded at $1x10^5$ units/mL), *Hemophilus influenzae* (seeded at $1x10^6$ CFU/mL), *Staphylococcus aureus* or *Staphylococcus epidermidis* (each seeded at $1x10^7$ CFU/mL).

Replicates of 8 positive samples were tested for each target virus (SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B) and each potential microbial interference strain combination. For each target, all 8 of 8 replicate samples were correctly identified using the Xpert Xpress CoV-2/Flu/RSV *plus* test. No interference by the commensal viral or bacterial strains was reported.

18.6 Competitive Interference

Competitive interference of the Xpert Xpress CoV-2/Flu/RSV *plus* caused by co-infections were evaluated by testing contrived samples of individual SARS-CoV-2, Flu A, Flu B or RSV strains at 3X LoD in the presence of different target strains at a higher concentration in a simulated background matrix. The concentration at 3X LoD was 414 copies/mL for SARS-CoV-2 (inactivated USA-WA1/2020); 0.021 $TCID_{50}$/mL for Flu A/Idaho/072018, 38.7 $CEID_{50}$/mL for Flu B/Washington/2/2019; 0.99 $TCID_{50}$/mL for RSV A/2/Australia/61), and 1.11 $TCID_{50}$/mL for RSV B/9320/MA/77. The competitive strains were evaluated at 10^4 or higher titer units (copies/mL, $TCID_{50}$/mL, $CEID_{50}$/mL or PFU/mL). The corresponding concentration of RNA (copies/mL) for the Flu and RSV strains was determined by droplet digital PCR (ddPCR). Replicates of 3 were tested for each target strain and each competitive strain combination. The virus at high concentration shows no competitive inhibitory effects if 3 of 3 replicates for the target strain report positive results. If the results reported less than 3 of 3 positive replicates, the concentration of the competing virus was reduced by 10-fold increments until no interference was observed. Below is a summary of the results:

Table 10. Summary of Competitive Interference Study with Flu A at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)			
		at 1.7e8 RNA copies/mL	at 1.7e7 RNA copies/mL	at 1.7e6 RNA copies/mL	at 1.7e5 RNA copies/mL
Flu B	Flu A	0/3	0/3	2/3	3/3
RSV A		0/3	0/3	3/3	Not tested
RSV B		3/3	Not tested	Not tested	Not tested
SARS-CoV-2		3/3	Not tested	Not tested	Not tested

Table 11. Summary of Competitive Interference Study with Flu B at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 1.4e5 RNA copies/mL
Flu A	Flu B	3/3
RSV A		3/3
RSV B		3/3
SARS-CoV-2		3/3

Table 12. Summary of Competitive Interference Study with RSV A at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 4.6e6 RNA copies/mL
Flu A	RSV A	3/3
Flu B		3/3

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 4.6e6 RNA copies/mL
SARS-CoV-2		3/3

Table 13. Summary of Competitive Interference Study with RSV B at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 1.9e5 RNA copies/mL
Flu A	RSV B	3/3
Flu B		3/3
SARS-CoV-2		3/3

Table 14. Summary of Competitive Interference Study with SARS-CoV-2 at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)	
		at 1e6 RNA copies/mL	at 1e5 RNA copies/mL
Flu A	SARS-CoV-2	3/3	Not tested
Flu B		1/3	3/3
RSV A		3/3	Not tested
RSV B		3/3	Not tested

The study showed that Flu A/Idaho/07/2018 at concentrations above 1.7e5 RNA copies/mL inhibited detection of Flu B at 3X LoD, and at concentrations above 1.7e6 RNA copies/mL inhibited detection of RSV A at 3X LoD (Table 10). In addition, SARS-CoV-2 at concentrations above 1e5 RNA copies/mL inhibited detection of Flu B at 3X LoD (Table 14). No other competitive interference was observed for the potential co-infections tested in the study at the concentrations tested.

18.7 Potentially Interfering Substances

Substances that could be present in the nasopharynx (or introduced during specimen collection and handling) and potentially interfere with accurate detection of SARS-CoV-2, Flu A, Flu B and RSV were evaluated with direct testing on the Xpert Xpress CoV-2/Flu/RSV *plus*.

Potentially interfering substances in the nasal passage and nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antivirals. Positive and negative samples were prepared in simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix. Negative samples (N = 8) were tested in the presence of each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (N = 8) were tested per substance with viruses spiked at 3X the LoD determined for each strain. Positive samples tested with the Xpert Xpress CoV-2/Flu/RSV *plus* included one SARS-CoV-2, one influenza A H1N1, one influenza A H3N2, one influenza B and two RSV (RSV A and RSV B) strains. The controls were samples with viruses spiked at 3X LoD into simulated NPS/ NS matrix containing no potentially interfering substance. The substances, with active ingredients, that were evaluated are listed in Table 15.

Table 15. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Substance/Active Ingredient
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/mL)
Afrin	Nasal Spray	Oxymetazoline, 0.05%
BD Universal Transport Medium	Transport Media	BD Universal Transport Medium
Copan 3U045N.PH (Cepheid Swab/M)	Transport Media	Copan 3U045N.PH (Cepheid Swab/M)

Substance ID	Substance/Class	Substance/Active Ingredient
Blood	Blood	Blood (Human)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate
Menthol	Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol
Mucin	Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)
Mupirocin	Antibiotic, nasal ointment	Mupirocin (20 mg/g=2%)
PHNY	Nasal Drops	Phenylephrine, 1%
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)
Remel M4RT	Transport Media	Remel M4RT
Remel M5	Transport Media	Remel M5
Tamiflu	Anti-viral drugs	Zanamivir
Tobramycin	Antibacterial, systemic	Tobramycin
Zicam	Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur (0.05%)
Zinc	Zinc supplement	Zinc Gluconate

The results from the study (Table 16) show that for most cases, 8 out of 8 replicates reported positive results for each combination of virus and substance tested and no interference was observed. When Zicam was initially tested at 15% w/v, interference was observed in the detection of Flu B and RSV A. However, when Zicam was tested at 7.5% w/v, no interference was observed.

Table 16. Mean Ct values for Xpert Xpress CoV-2/Flu/RSV *plus* Targets Tested in the Presence of Potentially Interfering Substances

Substance	Concentration Tested	Number of Correct Results/Number Tested					
		SARS-CoV-2/ USA-WA-1	Influenza A/Idaho/07/ 2018	H3N2 Flu A/ Hong Kong/ 45/2019	Flu B/ Washington /02/2019	RSV A/2/ Australia/61	RSV B/9320/ MA/77
Control Simulated NPS/NS Matrix (No substance)	100% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Afrin	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Albuterol Sulfate	0.83 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
BD Universal Transport Medium	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Blood	2% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Copan Swab M	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Fluticasone Propionate Nasal Spray	5 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8

Substance	Concentration Tested	Number of Correct Results/Number Tested					
		SARS-CoV-2/ USA-WA-1	Influenza A/Idaho/07/ 2018	H3N2 Flu A/ Hong Kong/ 45/2019	Flu B/ Washington /02/2019	RSV A/2/ Australia/61	RSV B/9320/ MA/77
Menthol	1.7 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Mucin	0.1% (w/v)	8/8	8/8	8/8	8/8	8/8	8/8
Mupirocin	10 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
PHNY	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Remel M4RT	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Remel M5	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Saline	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Tamiflu	7.5 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Tobramycin	4 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Zicam	15% (w/v)	8/8	8/8	8/8	5/8[a]	7/8[b]	8/8
Zinc	0.1 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8

[a] With 15% (w/v) Zicam, a statistically significant difference was observed between the control mean Ct and the test mean Ct. Testing was repeated with 7.5% (w/v) Zicam and no clinically significant difference was observed between the control mean Flu B Ct and the test mean Flu B Ct.

[b] With 15% (w/v) Zicam, a statistically significant difference was observed between the control mean Ct and the test mean Ct. Testing was repeated with 7.5% (w/v) Zicam and no statistically significant difference was observed between the control mean RSV A Ct and the test mean RSV A Ct.

18.8 Carry-over Contamination

A study was conducted to assess whether the single-use, self-contained Xpert Xpress CoV-2/Flu/RSV *plus* cartridge prevents specimen and amplicon carryover by testing a negative sample immediately after testing of a very high positive sample in the same GeneXpert module. The negative sample used in this study consisted of simulated NPS/NS matrix and the positive sample consisted of high Flu B and high SARS-CoV-2 virus concentrations (Flu B/Wisconsin/10/2016 at 1.0e6 $TCID_{50}$/mL and inactivated SARS-CoV-2 USA-WA1/2020 at 1e4 copies/mL) seeded into negative NPS/NS matrix. The negative sample was tested in a GeneXpert module at the start of the study. Following the initial testing of the negative sample, the high positive sample was processed in the same GeneXpert module immediately followed by another negative sample. This was repeated 20 times in the same module, resulting in 20 positives and 21 negatives for the module. The study was repeated using a second GeneXpert module for a total of 40 positive and 42 negative samples. All 40 positive samples were correctly reported as **SARS-CoV-2 POSITIVE**; **Flu A NEGATIVE**; **Flu B POSITIVE**; **RSV NEGATIVE**. All 42 negative samples were correctly reported as **SARS-CoV-2 NEGATIVE**; **Flu A NEGATIVE**; **Flu B NEGATIVE**; **RSV NEGATIVE** with the Xpert Xpress CoV-2/Flu/RSV *plus* test. No specimen or amplicon carry-over contamination was observed in this study.

18.9 Reproducibility

The reproducibility of the Xpert Xpress CoV-2/Flu/RSV *plus* test was established at three sites using a 9-member panel including one negative sample, four low positive (~1.5X LoD) and four moderate positive (~3X LoD) samples. The negative sample consisted of simulated matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated matrix using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix), cultured viruses Influenza A/ Idaho/07/2018, Influenza B/Wisconsin/10/2016, and RSV B/Wash/18537/62.

Testing was conducted over six (6) days, using three (3) lots of Xpert Xpress CoV-2/Flu/RSV *plus* cartridges at three (3) participating sites each with two (2) operators to yield a total of 144 observations per panel member (3 Sites x 2 Operators x 3 Lots x 2 Days/Lot x 2 Runs x 2 Replicates = 144 observations/panel member). The results from the study are summarized in Table 17.

Table 17. Summary of the Reproducibility Results - % Agreement

Sample	Site 1			Site 2			Site 3			% Total Agreement [95% CI]
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Negative	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
SARS-CoV-2 Low Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
SARS-CoV-2 Mod Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
Flu A Low Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
Flu A Mod Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
Flu B Low Pos	100% 24/24	100% 24/24	100% 48/48	95.8% 23/24	95.8% 23/24	95.8% 46/48	100% 24/24	100% 24/24	100% 48/48	98.6% (142/144) [95.1-99.6]
Flu B Mod Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 23/23	95.8% 23/24	97.9% 46/47	99.3% (142/143) [96.1-99.9]
RSV Low Pos	100% 24/24	100% 24/24	100% 48/48	95.8% 23/24	100% 24/24	97.9% 47/48	100% 24/24	100% 24/24	100% 48/48	99.3% (143/144) [96.2-99.9]
RSV Mod Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]

19 References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed February 9, 2020.
2. bioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 3, 2020.
3. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. J Infect Dis. 2006;194:S98-110.
4. Schweiger B, Zadow I, Heckler R, et al. Application of a fluorogenic PCR assay for typing and subtyping of influenza viruses in respiratory samples. J Clin Micro. 2000;38:1552-1558.
5. https://www.cdc.gov/flu/about/viruses-types.html. Accessed on May 19, 2016.
6. http://www.cdc.gov/RSV/index.html. Accessed on March 14, 2013.
7. Acero-Bedoya, S., Wozniak, P. S., Sánchez, P. J., Ramilo, O., & Mejias, A. (2019). Recent trends in RSV immunoprophylaxis: clinical implications for the infant. American journal of perinatology, 36(S 02), S63-S67.
8. Solomon, D. A., Sherman, A. C., & Kanjilal, S. (2020). Influenza in the COVID-19 Era. Jama, 324(13), 1342-1343.
9. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). https://www.cdc.gov/labs/bmbl/index.html
10. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).
11. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
12. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

20 Cepheid Headquarters Location

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

21 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag Number

United States Technical Support

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France Technical Support

Telephone: + 33 563 825 319
Email: support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

22 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	For prescription use only
	United Kingdom Conformity Assessed
	United Kingdom Responsible Person
	Country of Origin: Sweden
	Country of Origin: United States of America

UK REP

Cepheid UK Limited
Unit 4, Blythe Valley Innovation Centre
Central Boulevard, Blythe Valley Business Park
Solihull, B90 8AJ, United Kingdom

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

UK CA IVD

23 Revision History

Description of Changes: 303-0026, Rev. A to Rev. B

Section	Description of Change
6	Clarified the animal origin of the protein stabilizer used in the product.
15	Clarified INVALID results. Specified approved test in the UK. Clarified assay names.
16.1	Clarified INVALID results. Added recommendation.
17	Added potential INVALID results trigger.
19	Updated references.
20	Removed EU Headquarter.
22	Added country of origin symbols. Updated the symbols (according to EN ISO 15223-1:2021) and the Cepheid UK Limited address.