

Xpert[®] Xpress CoV-2/Flu/RSV *plus*

REF XP3COV2/FLU/RSV-10

Instructions for Use

For Use with GeneXpert[®] Dx or GeneXpert[®] Infinity Systems

IVD CE

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See Section 24, 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 Instrument Systems, 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.

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 (Dx and Infinity 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, computer, 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 Dx System Operator Manual* or the *GeneXpert Infinity System 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 <i>plus</i> Cartridges with Integrated Reaction Tubes | 10 |
| <ul style="list-style-type: none"> • Bead 1, Bead 2, and Bead 3 (freeze-dried) • Lysis Reagent • Binding Reagent • Elution Reagent • Wash Reagent | 1 of each per cartridge 1.0 mL per cartridge 1.0 mL per cartridge 3.0 mL per cartridge 0.4 mL per cartridge |
| Disposable Transfer Pipettes | 10-12 per kit |
| Flyer | 1 per kit |
| <ul style="list-style-type: none"> • Instructions to locate (and import) the ADF and documentation such as the Product Insert on www.cepheid.com. | |
| Quick Reference Instructions | 2 per kit |
| (For use with the GeneXpert Xpress System only) | |

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

Note The bovine serum albumin (BSA) 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 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 Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual.
- For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher
- For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

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 (NATrol Flu/RSV/SARS-CoV-2)
- External Negative Control: Catalog #NATCV9-6C (NATrol 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 Prevention⁹ 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-\(covid-19\)](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.

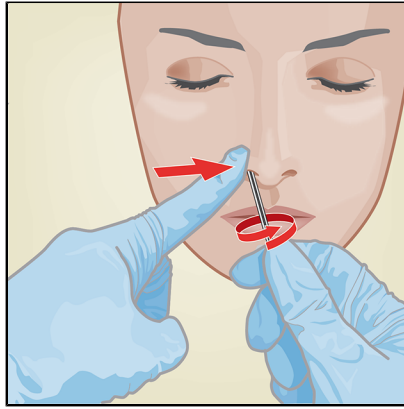


Figure 3. Nasal Swab Collection for Second Nostril

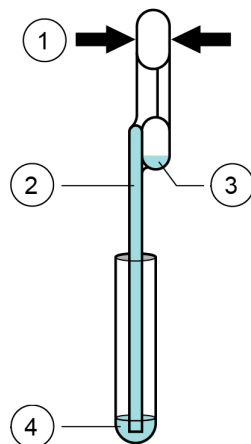
3. Remove and place the swab into the tube containing 3 mL of viral transport medium, 3 mL of saline, or 2 mL of eNAT. Break swab at the indicated break line and cap the specimen collection tube tightly.

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 μ L) 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.

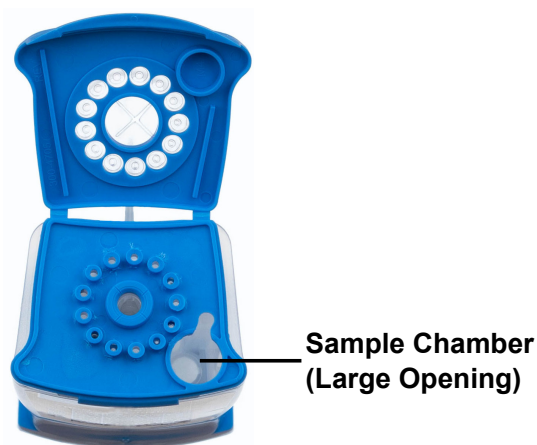


Figure 5. Xpert Xpress CoV-2/Flu/RSV plus Cartridge (Top View)

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 μ L) into the large opening (Sample Chamber) in the cartridge shown in Figure 5.
4. Close cartridge lid.

13.3 Starting the Test

Note Before you start the test, make sure that the system contains modules with GeneXpert Dx software version 4.7b or higher or Infinity Xpertise software 6.4b or higher, and that the Xpert Xpress CoV-2/Flu/RSV plus Assay Definition File (ADF) is imported into the software.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

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 Instrument System:
 - **GeneXpert Dx:**

If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double-clicking on the GeneXpert Dx shortcut icon on the Windows® desktop.

or
 - **GeneXpert Infinity System:**

If using the GeneXpert Infinity instrument, power up the instrument by turning the power switch clockwise to the **ON** position. On the Windows desktop, double-click the Xpertise Software shortcut icon to launch the software.
2. Log on to the System software. The login screen appears. Type your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** followed by **Order Test** (Infinity).

4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result.
6. Scan the barcode on the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date, and Selected Assay.

Note If the barcode on the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity) if Auto-Submit is not enabled. In the dialog box that appears, type your password, if required.

For the GeneXpert Dx Instrument:

- a. Locate the module with the blinking green light, open the instrument module door and load the cartridge.
- b. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.
- c. Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

or

For the GeneXpert Infinity System:

- a. After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click OK to continue. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed onto the waste shelf for disposal.
- b. When all samples are loaded, click on the **End Order Test** icon.

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

14 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

15 Quality Control

15.1 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.

15.2 External Controls

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

16 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 | <p>The SARS-CoV-2 target RNA is detected.</p> <ul style="list-style-type: none"> 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 | <p>The Flu A target RNA is detected.</p> <ul style="list-style-type: none"> 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: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting Probe Check: PASS; all probe check results pass |
| Flu B POSITIVE | <p>The Flu B target RNA is detected.</p> <ul style="list-style-type: none"> The Flu B signal has a Ct within the valid range and endpoint above the minimum setting SPC: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting Probe Check: PASS; all probe check results pass |
| RSV POSITIVE | <p>The RSV target RNA is detected.</p> <ul style="list-style-type: none"> The RSV signal has a Ct within the valid range and endpoint above the minimum setting SPC: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting Probe Check: PASS; all probe check results pass |
| SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE | <p>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.</p> <ul style="list-style-type: none"> 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 | <p>SPC does not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting Probe Check: PASS; all probe check results pass |

| Result | Interpretation |
|------------------|--|
| ERROR | <p>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 17.2 of the IFU.</p> <ul style="list-style-type: none"> ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● RSV: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL¹; all or one of the probe check results fail <p>¹If 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.</p> |
| NO RESULT | <p>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 17.2 of the IFU. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.</p> <ul style="list-style-type: none"> ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● RSV: NO RESULT ● SPC: NO RESULT ● Probe Check: NA |

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another FDA cleared, approved, or authorized test, 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 | <p>The SARS-CoV-2 target RNA is detected.</p> <ul style="list-style-type: none"> ● 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 | <p>The Flu A target RNA is detected.</p> <ul style="list-style-type: none"> ● 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: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass |
| Flu B POSITIVE | <p>The Flu B target RNA is detected.</p> <ul style="list-style-type: none"> ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass |
| SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE | <p>SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected.</p> <ul style="list-style-type: none"> ● 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 | <p>SPC does not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> ● SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting. ● Probe Check: PASS; all probe check results pass |
| ERROR | <p>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 17.2 of the IFU.</p> <ul style="list-style-type: none"> ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL¹; all or one of the probe check results fail <p>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.</p> |

| Result | Interpretation |
|------------------|---|
| NO RESULT | <p>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 17.2 of the IFU. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.</p> <ul style="list-style-type: none"> • SARS-CoV-2: NO RESULT • Flu A: NO RESULT • Flu B: NO RESULT • SPC: NO RESULT • Probe Check: NA |

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another FDA cleared, approved, or authorized test, 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 | <p>The SARS-CoV-2 target RNA is detected.</p> <ul style="list-style-type: none"> • 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 | <p>SARS-CoV-2 target RNA is not detected.</p> <ul style="list-style-type: none"> • 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 | <p>SPC does not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> • SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting • Probe Check: PASS; all probe check results pass |
| ERROR | <p>Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> • SPC: NO RESULT • Probe Check: FAIL¹; all or one of the probe check results fail <p>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.</p> |
| NO RESULT | <p>Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.</p> <ul style="list-style-type: none"> • 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.

17 Retests

17.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2, Retest Procedure.

- 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.
- 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.

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

17.2 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.

18 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.
- 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.

19 Performance Characteristics

19.1 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 1.4% (4/279). On repeat testing, all four (4) 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 2.1% (5/240). Four (4) of the five (5) 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%) |

TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval

19.2 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 (NATrol 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 |

| Virus/Strain | LoD Concentration |
|-------------------------------|-----------------------------|
| 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 |

19.3 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 RSV A 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 | NATrol 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 |

| Virus | Strain | Tested Titer | SARS-CoV-2 | Flu A | Flu B | RSV |
|------------------------------------|---|-------------------------------|------------|-------|-------|-----|
| | 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 |
| 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 |

| Virus | Strain | Tested Titer | SARS-CoV-2 | Flu A | Flu B | RSV |
|--------------------------------|--|------------------------------|------------|-------|-------|-----|
| | 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 |
| | 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 |

| Virus | Strain | Tested Titer | SARS-CoV-2 | Flu A | Flu B | RSV |
|------------------------------|----------------------------------|------------------------------|------------|-------|-------|-----|
| 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 |
| | 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.

19.4 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 |
| | <i>Chlamydia pneumoniae</i> |
| | <i>Haemophilus influenzae</i> |
| | <i>Legionella pneumophila</i> |
| | <i>Mycobacterium tuberculosis</i> |
| | <i>Streptococcus pneumoniae</i> |
| | <i>Streptococcus pyogenes</i> |
| | <i>Bordetella pertussis</i> |
| | <i>Mycoplasma pneumoniae</i> |
| | <i>Pneumocystis jirovecii</i> (PJP) |
| | <i>Parechovirus</i> |
| | <i>Candida albicans</i> |
| | <i>Corynebacterium diphtheriae</i> |
| | <i>Legionella non-pneumophila</i> |
| | <i>Bacillus anthracis</i> (Anthrax) |
| | <i>Moraxella catarrhalis</i> |
| | <i>Neisseria elongata</i> and <i>N. meningitidis</i> |
| | <i>Pseudomonas aeruginosa</i> |
| | <i>Staphylococcus epidermidis</i> |
| | <i>Streptococcus salivarius</i> |
| | <i>Leptospira</i> |
| | <i>Chlamydia psittaci</i> |
| | <i>Coxiella burnetii</i> (Q-Fever) |

| Microorganisms from the Same Genetic Family | High Priority Organisms |
|---|------------------------------|
| | <i>Staphylococcus aureus</i> |

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 $\geq 1 \times 10^6$ CFU/mL with the exception of *Chlamydia pneumoniae* which was tested at 1.2×10^6 IFU/mL and *Lactobacillus reuteri* which was tested at 5×10^7 copies/mL of genomic DNA. Viruses were tested at concentrations of $\geq 1 \times 10^5$ TCID₅₀/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 |
| 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 |
| <i>Acinetobacter baumannii</i> | 1.30e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Bordetella pertussis</i> | 6.40e7 CFU/mL | NEG | NEG | NEG | NEG |

| Strain | Tested Concentration | SARS-CoV-2 | Flu A | Flu B | RSV |
|---|----------------------|------------|-------|-------|-----|
| <i>Burkholderia cepacia</i> | 1.90e8 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Candida albicans</i> | 6.30e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Candida parapsilosis</i> | 1.45e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Citrobacter freundii</i> | 1.73e8 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Corynebacterium sp.</i> | 1.27e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Enterococcus faecalis</i> | 5.87e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Escherichia coli</i> | 1.55e8 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Hemophilus influenzae</i> | 6.62e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Lactobacillus reuteri</i> | 5.0e7 copies/mL | NEG | NEG | NEG | NEG |
| <i>Legionella spp.</i> | 1.42e8 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Moraxella catarrhalis</i> | 2.46e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Mycoplasma pneumoniae</i> | 2.7e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Neisseria meningitidis</i> | 4.2e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Neisseria mucosa</i> | 1.0e8 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Propionibacterium acnes</i> | 8.25e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Pseudomonas aeruginosa</i> | 1.05e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Staphylococcus haemolyticus</i> | 2.66e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Staphylococcus aureus</i> | 5.87e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Staphylococcus epidermidis</i> | 2.47e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Streptococcus agalactiae</i> | 1.75e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Streptococcus pneumoniae</i> | 2.26e7 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Streptococcus pyogenes</i> | 9.0e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Streptococcus salivarius</i> | 4.19e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Streptococcus sanguinis</i> | 8.67e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Chlamydia pneumoniae</i> | 1.20e6 CFU/mL | NEG | NEG | NEG | NEG |
| <i>Mycobacterium tuberculosis (avirulent)</i> | 1.20e6 CFU/mL | NEG | NEG | NEG | NEG |

19.5 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 1×10^5 units/mL), *Hemophilus influenzae* (seeded at 1×10^6 CFU/mL), *Staphylococcus aureus* or *Staphylococcus epidermidis* (each seeded at 1×10^7 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.

19.6 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 TCID₅₀/mL for Flu A/Idaho/072018, 38.7 CEID₅₀/mL for Flu B/Washington/2/2019; 0.99 TCID₅₀/mL for RSV A/2/Australia/61), and 1.11 TCID₅₀/mL for RSV B/9320/MA/77. The competitive strains were evaluated at 10^4 or higher titer units (copies/mL, TCID₅₀/mL, CEID₅₀/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 |
| 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.

19.7 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) |
| Anefrin | 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) |
| 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 operculata, 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 |
| Anefrin | 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 |
| 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.

19.8 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 TCID₅₀/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.

19.9 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] |

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21 Cepheid Headquarters Locations

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22 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

US



















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

France

Telephone: + 33 563 825 319
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Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en_US/support/contact-us.

23 Table of Symbols

| Symbol | Meaning |
|---|---|
|  | Catalog number |
|  | CE marking – European Conformity |
|  | <i>In vitro</i> diagnostic medical device |
|  | Do not reuse |
|  | Batch code |
|  | Consult instructions for use |
|  | Caution |
|  | Manufacturer |
|  | Country of manufacture |
|  | Contains sufficient for <i>n</i> tests |
|  | Control |
|  | Expiration date |
|  | Temperature limitation |
|  | Biological risks |
|  | For prescription use only |
|  | Authorized Representative in the European Community |
|  | Authorized Representative in Switzerland |
|  | Importer |



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24 Revision History

Description of Changes: 302-7085, Rev. C to Rev. D

Purpose: Addition of symbols and addresses.

| Section | Description of Change |
|---------|---|
| 23 | Addition of EC REP symbol, Swiss Importer symbol, CH REP symbol, and related addresses. |