GeneXpert
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Xpert® Xpress CoV-2 *plus*

REF **XPRS-COV2-10**

CLIA Complexity: Moderate

Instructions for Use

For Use with GeneXpert® Dx System or GeneXpert Infinity System

IVD

Cepheid

In Vitro Diagnostic Medical Device

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Trademark, Patents, and Copyright Statements

See Section 27, Revision History for a description of changes.

Xpert® Xpress CoV-2 *plus*

For *In Vitro* Diagnostic Use

℞only

1 Proprietary Name

Xpert® **Xpress** CoV-2 ***plus***

2 Common or Usual Name

Xpert Xpress CoV-2 *plus*

3 Intended Use

The Xpert Xpress CoV-2 *plus* test, performed on the GeneXpert Dx and GeneXpert Infinity Systems, is a rapid real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal and anterior nasal swab specimens collected from individuals with signs and symptoms of respiratory tract infection.

The Xpert Xpress CoV-2 *plus* test is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiologic, and laboratory findings. Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out bacterial infection or co-infection with other pathogens.

Negative results do not preclude SARS-CoV-2 infection. The results of this test should not be used as the sole basis for diagnosis and patient management decisions.

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]

The Xpert Xpress CoV-2 *plus* is a RT-PCR *in vitro* diagnostic test that detects SARS-CoV-2 RNA and aids in the diagnosis of COVID-19 when used in conjunction with other clinical and epidemiological information and laboratory findings. The Xpert Xpress CoV-2 *plus* test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in nasopharyngeal swab and anterior nasal swab specimens.

5 Principle of the Procedure

The Xpert Xpress CoV-2 *plus* test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress CoV-2 *plus* test is performed on GeneXpert Instrument Systems (Dx and Infinity systems). The primers and probes in the Xpert Xpress CoV-2 *plus* test are designed to amplify and detect sequences in the nucleocapsid (N), envelope (E) and RNA-dependent RNA polymerase (RdRP) genes of the SARS-CoV-2 virus genome.

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 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 RT-PCR reagents and host the 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 *plus* test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal swab (NPS) or anterior nasal swab (NS) 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 viral transport tube containing 3 mL viral transport medium (VTM)/Universal Transport Medium (UTM) or 2 mL 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 *plus* cartridge. The GeneXpert cartridge is loaded onto the GeneXpert instrument, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Materials Provided

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

Xpert Xpress CoV-2 *plus* Cartridges with Integrated Reaction Tubes	**10**
Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
Lysis Reagent (Guanidinium Thiocyanate)	1.0 mL per cartridge
Binding Reagent	1.0 mL per cartridge
Elution Reagent	2.0 mL per cartridge
Wash Reagent	0.5 mL per cartridge
Disposable Transfer Pipettes	**12 per kit**
Flyer (with instructions to web location) for:	**1 per kit**

- Assay Definition File (ADF)
- Instructions to import ADF into GeneXpert software
- Instructions for Use

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 *plus* test cartridges at 2–28 °C.
- Do not open the 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

Sample Collection Swabs and Transport Media

Nylon flocked swabs, viral transport medium (VTM), Universal Transport Medium (UTM) and eNAT Molecular Transport Medium are compatible for use with the Xpert Xpress CoV-2 *plus* test.

The following materials are examples of those that are compatible with the Xpert Xpress CoV-2 *plus* test:

- **Nasopharyngeal Sample Collection Kit for Viruses**
  - Copan UTM® 3C057N (Flexible Minitip Flocked Swab with UTM Medium w/o Beads)
  - Becton Dickinson Universal Viral Transport Kit P/N 220531 (Flexible Minitip Flocked Swab with UVT Medium)
  - Copan eNAT Molecular Collection and Preservation Medium P/N 6U074S01 (Flexible Minitip Flocked Swab with eNAT Medium)
- **Nasal Sample Collection Kit for Viruses**
  - Copan UTM 3C064N (Regular Flocked Swab with UTM Medium w/o Beads)
  - Copan eNAT Molecular Collection and Preservation Medium P/N 6U073S01 (Regular Flocked Swab with eNAT Medium)
- **Alternatively, swabs and transport media can be obtained separately:**
  - Nylon flocked swab (Copan P/N 502CS01, 503CS01)
  - Viral transport medium, 3 mL (Copan P/N 3C047N, Remel M4RT, Remel M5)

GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, and appropriate GeneXpert System 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

ZeptoMetrix® External Controls – External controls in the form of inactivated virus are available from ZeptoMetrix (Buffalo, NY) for optional use with the Xpert Xpress CoV-2 *plus*.

- NATtrol SARS-Related Coronavirus 2 Positive Control, Catalog# NATSARS(COV2)-ERC-IVD
- NATtrol SARS-Related Coronavirus 2 Negative Control, Catalog# NATSARS(COV2)-NEG-IVD

CD – available upon request

- ADF
- Import Instructions for ADF

10 Warnings and Precautions

10.1 General

- For *in vitro* Diagnostic Use.
- For prescription use only
- Positive results are indicative of presence of SARS-CoV-2 RNA.
- Positive results for SARS-CoV-2 should be reported to state, local, or federal health departments according to local reporting requirements.
- 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 treated using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[3] and the Clinical and Laboratory Standards Institute.[4]
- 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.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.
- 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, dispose biological specimens, and used cartridges per WHO [World Health Organization] medical waste handling and disposal guidelines.
- Used cartridges may contain potentially infectious materials, as well as PCR amplicons. Do not open or attempt to alter any part of the used cartridge for disposal.
- NPS and NS specimens should be collected with appropriate infection control precautions. Refer to the CDC Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing for more information. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.
- Do not attempt viral culture in cases of positive results for SARS-CoV-2 and/or any similar microbial agents unless a facility with an appropriate level of laboratory biosafety (e.g., BSL 3 or higher) is available to receive and culture specimens.
- Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

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 *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 expiration date.
- Each single-use Xpert Xpress CoV-2 *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 collected in UTM/VTM 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.
- In the event of spill of specimens collected in Copan eNAT, refer to Copan eNAT Package Insert for proper handling of a spill.

11 Chemical Hazards[5,6]

- 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.

Nasopharyngeal swab and anterior nasal swab specimens can be stored at room temperature (15–30 °C) for up to 48 hours in VTM/UTM or eNAT medium until testing is performed on the GeneXpert Instrument Systems. Alternatively, nasopharyngeal swab and anterior nasal swab specimens can be stored refrigerated (2–8 °C) for up to seven days in VTM/UTM or eNAT medium until testing is performed on the GeneXpert Instrument Systems.

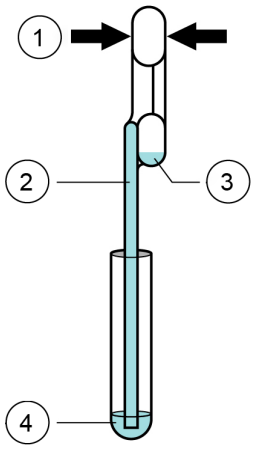
Nasopharyngeal and anterior nasal swab specimens collected in VTM/UTM or eNAT can be frozen at -80 °C and undergo 1 freeze/thaw cycle.

13 Procedure

13.1 Preparing the Cartridge

Note **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 and place the pipette tip in the specimen transport tube (see Figure 1).



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

Figure 1. Transfer Pipette

7. Slowly release the top bulb of the pipette to fill the pipette before removing from the tube. After filling pipette, excess sample may be seen in the overflow reservoir bulb of the pipette (see Figure 1). Check that the pipette does not contain bubbles.
8. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) of the cartridge shown in Figure 2. Dispose of the used pipette.

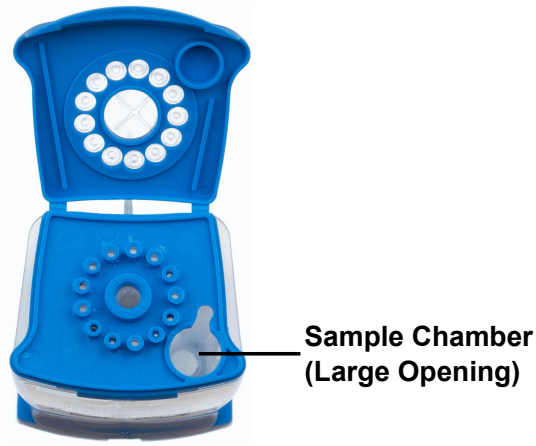


Figure 2. Xpert Xpress CoV-2 *plus* Cartridge (Top View)

Note 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 may be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert Xpress CoV-2 *plus* test:

1. Mix control by rapidly inverting the external control tube 5 times.
2. Open the cap on the external control tube.
3. Open the cartridge lid.

4. Using a clean transfer pipette, transfer one draw of the external control sample into the large opening (Sample Chamber) in the cartridge shown in Figure 2.
5. Close cartridge lid and start the test.

14 Running the Test

- For the GeneXpert Dx System, see Section 14.1.
- For the GeneXpert Infinity System, see Section 14.2.

14.1 GeneXpert Dx System

14.1.1 Starting the Test

Before you start the test, make sure that:

Important

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

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual*.

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

1. Turn on the GeneXpert Dx System, then turn on the computer and log on. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on using your username and password.
3. In the **GeneXpert System** window, click **Create Test**.
   The **Create Test** window displays. The **Scan Patient ID barcode** dialog box displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly.
   The Patient ID is associated with the test results and displays in the **View Results** window and all the reports. The **Scan Sample ID barcode** dialog box displays.
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 associated with the test results and displays in the **View Results** window and all the reports. The **Scan Cartridge Barcode** dialog box displays.
6. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

7. Click **Start Test**. In the dialog box that displays, type your password, if required.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking.
   When the test is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door, then remove the cartridge.
11. Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

Note Do not open or attempt to alter any part of the used cartridge for disposal. Do not turn off or unplug the instrument while a test is in progress. Turning off or unplugging the instrument or computer will stop the test.

14.1.2 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual*.

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.

14.2 GeneXpert Infinity System

14.2.1 Starting the Test

Before you start the test, make sure that:

Important

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

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Infinity System Operator Manual*.

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

1. Power up the instrument. The Xpertise software will launch automatically. If it does not, double-click the Xpertise software shortcut icon on the Windows® desktop.
2. Log on to the computer, then log on to the GeneXpert Xpertise software using your user name and password.
3. In the **Xpertise Software Home** workspace, click **Orders** and in the **Orders** workspace, click **Order Test**. The **Order Test - Patient ID** workspace displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and displays in the **View Results** window and all the reports.
5. Enter any additional information required by your institution, and click the **CONTINUE** button. The **Order Test - Sample ID** workspace displays.
6. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and displays in the **View Results** window and all the reports.
7. Click the **CONTINUE** button. The **Order Test - Assay** workspace displays.
8. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

After the cartridge is scanned, the **Order Test - Test Information** workspace displays.

9. Verify that the information is correct, and click **Submit**. In the dialog box that displays, type your password, if required.
10. Place the cartridge on the conveyor belt.

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

The cartridge automatically loads, the test runs, and the used cartridge are placed into the waste container.

14.2.2 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Infinity System Operator Manual*.

1. In the **Xpertise Software Home** workspace, click the **RESULTS** icon. The Results menu displays.
2. In the Results menu, select the **VIEW RESULTS** button. The **View Results** workspace displays showing the test results.
3. Click the **REPORT** button to view and/or generate a PDF report file.

15 Quality Controls

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 Instrument 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

The external quality control materials provided by the specified vendor in Section 9 are an optional source. All external controls must be used in accordance with local, state and/or federal regulations or accreditation requirements, as applicable.

16 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. Xpert Xpress CoV-2 *plus* test provides test results based on the detection of three gene targets according to the algorithms shown in Table 1.

Table 1. Xpert Xpress CoV-2 *plus* Possible Results

Result Text	N2	E	RdRP	SPC
SARS-CoV-2 POSITIVE	POS	POS	POS	Not Applicable
SARS-CoV-2 POSITIVE	POS	POS/NEG	POS/NEG	Not Applicable
SARS-CoV-2 POSITIVE	POS/NEG	POS	POS/NEG	Not Applicable
SARS-CoV-2 POSITIVE	POS/NEG	POS/NEG	POS	Not Applicable
SARS-CoV-2 NEGATIVE	NEG	NEG	NEG	PASS
INVALID[a]	INVALID	INVALID	INVALID	FAIL

[a] If any gene target is **INVALID**, the Xpert Xpress CoV-2 *plus* test result is reported as **INVALID** regardless of the SPC result.

See Table 2 to interpret test result statements for the Xpert Xpress CoV-2 *plus* test.

Table 2. Xpert Xpress CoV-2 *plus* Test Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	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 for one or more nucleic acidtargets (N2, E, RdRP). ● SPC: NA (Not Applicable); SPC is ignored because coronavirus target amplification occurred. ● Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE	SARS-CoV-2 target RNA is not detected. ● The SARS-CoV-2 signal for nucleic acid targets (N2, E and RdRP) do not have 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.
INVALID	SPC does not meet acceptance criteria. Presence or absence of SARS-CoV-2 nucleic acids cannot be determined. Repeat test according to Section 17.2. ● SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct with invalid range and endpoint below minimum setting. ● Amplification curve(s) for one or more target gene (E, N2, or RdRP) does not meet acceptance criteria. ● Probe Check: PASS; all probe check results pass.
ERROR	Presence or absence of SARS-CoV-2 cannot be determined. A system component failed, the maximum pressure was reached, or the probe check failed. Repeat test according to Section 17.2. ● SARS-CoV-2: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL[a]; all or one of the probe check results fail.
NO RESULT	Presence or absence of SARS-CoV-2 cannot be determined. Repeat test according to Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, or the run was aborted due to a system component failure. ● SARS-CoV-2: NO RESULT ● SPC: NO RESULT ● Probe Check: NA (not applicable).

[a] If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.

The Xpert Xpress CoV-2 *plus* test includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens if the signal from the target nucleic acid reaches a predetermined threshold before all PCR cycles have been completed. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC and/or other target amplification curves may not be seen and their results may not be reported.

17 Retests

17.1 Reasons to Repeat the Assay

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

- An **INVALID** result indicates that the control SPC failed or amplification curve(s) for one or more target gene (E, N2, or RdRP) does not meet acceptance criteria. 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, 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 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 *plus* cartridge and a new transfer pipette.
2. Confirm that 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

- For prescription use only.
- A negative test result does not exclude the possibility of viral or bacterial infection.
- Negative results do not preclude SARS-CoV-2 and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Xpress CoV-2 *plus* test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- The performance of this device has not been assessed in individuals without signs or symptoms of respiratory infection.
- Performance of the Xpert Xpress CoV-2 *plus* test has only been established in nasopharyngeal swab and anterior nasal swab specimens. Use of the Xpert Xpress CoV-2 *plus* test with other specimen types has not been assessed and performance characteristics are unknown.
- The performance of the Xpert Xpress CoV-2 *plus* test has not been specifically evaluated for nasopharyngeal swab and anterior nasal swab specimens from immunocompromised individuals.
- Clinical performance has not been established with 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.
- Use of this test is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- As with any molecular test, mutations within the target regions of Xpert Xpress CoV-2 *plus* could affect primer and/or probe binding and result in failure to detect the presence of the target virus or newly emerging variants.
- Positive and negative predictive values are highly dependent on prevalence. The likelihood of a negative result being false is higher during peak activity when prevalence of disease is high. The likelihood of a positive result being false is higher during periods when prevalence is moderate to low.

- 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 the target virus is present at levels below the analytical limit of detection.
- 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.
- The Xpert Xpress CoV-2 *plus* test is a qualitative test that reports Ct values for individuals who test positive for SARS-CoV-2. These Ct values should not be interpreted as a measure of viral levels.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- This test has not been established for screening of blood, blood products, or post-mortem specimens for the presence of SARS-CoV-2.
- 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.
- Mucin (Type I-S) and Fluticasone Propionate may interfere with COVID-19 detection at levels greater than 1.25 mg/mL and 2.5 µg/mL, respectively.
- Anterior nasal swab and nasopharyngeal swab specimens collected in 2 mL Copan eNAT, Remel M4RT, and Remel M5 are compatible for use with the Xpert Xpress CoV-2 *plus* test. Performance of the Xpert Xpress CoV-2 *plus* test with specimens collected in Copan eNAT, Remel M4RT and Remel M5 has been established in analytical studies, however, clinical performance of the test in these media types was not established.
- Performance has not been established with media containing guanidine thiocyanate (GTC) other than eNAT.
- Cross-reactivity with respiratory tract organisms other than those described herein may lead to erroneous results.
- The E gene targeted by the Xpert Xpress CoV-2 *plus* test can detect, in addition to SARS-CoV-2, other coronavirus species within the *Sarbecovirus* subgenus.

19 Expected Values

Expected values as determined by Xpert Xpress CoV-2 *plus* are presented for fresh versus frozen specimens, stratified by nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimen types (Table 3) and by age group (Table 4).

Table 3. Positivity Rate Stratified by Specimen Type for Fresh and Frozen Specimens

Specimen Category	Total	Specimen Type	
		NPS	NS
Fresh (Category I)	16.6% (612/3697)	16.8% (312/1852)	16.3% (300/1845)
Frozen (Category II)	32.1% (17/53)	29.6% (8/27)	34.6% (9/26)
Overall	16.8% (629/3750)	17% (320/1879)	16.5% (309/1871)

Table 4. Positivity Rate Stratified by Age Group for Fresh and Frozen Specimens

Specimen Category	Total	Age Group (Years)			
		≤ 5	6-21	22-59	≥ 60
Fresh (Category I)	16.6% (612/3697)	6.9% (5/72)	9.9% (75/754)	18.7% (443/2365)	17.6% (89/506)
Frozen (Category II)	32.1% (17/53)	NA	33.3% (5/15)	36.7% (11/30)	12.5% (1/8)
Overall	16.8% (629/3750)	6.9% (5/72)	10.4% (80/769)	19.0% (454/2395)	17.5% (90/514)

20 Clinical Performance

The clinical performance of the Xpert Xpress CoV-2 *plus* test was evaluated in a multi-site, observational and method comparison study that included 32 geographically diverse sites in the United States (US). Of the 32 sites, 5 sites participated in specimen collection only, 26 sites performed Xpert testing and specimen collection, and 1 site performed Xpert testing as well as comparator and discrepant testing.

The performance of the Xpert Xpress CoV-2 *plus* test was evaluated using prospectively collected fresh (98.6%) and frozen (1.4%) clinical nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimens in viral transport medium or universal transport medium. These specimens were collected in 2022 from individuals with signs and symptoms of respiratory tract infection and tested using Xpert Xpress CoV-2 *plus* side-by-side with a U.S. FDA-cleared molecular respiratory panel that includes SARS-CoV-2, in a randomized and blinded fashion. A total of 4047 specimens (2029 NPS and 2018 NS) were evaluated from individuals with signs and symptoms of respiratory infection. Of these, 60 specimens yielded non-determinate results (**INVALID**, **ERROR**, and **NO RESULT**) with Xpert Xpress CoV-2 *plus*. Additionally, 237 specimens either yielded non-determinate result, or were not tested per comparator package insert. Therefore, 297 (60 + 237) specimens were excluded and a total of 3750 (1879 NPS and 1871 NS) specimens that yielded valid results by both Xpert Xpress CoV-2 *plus* and the U.S. FDA-cleared molecular respiratory panel were included in the clinical performance evaluation. Available demographic data collected from study participants are presented in Table 5.

Table 5. Demographic Data Summary of Participants Symptomatic of Respiratory Infection

	NPS (N=1879)	NS (N=1871)	Overall (N=3750)
Gender			
Female	1107 (58.9%)	1171 (62.6%)	2278 (60.7%)
Male	772 (41.1%)	700 (37.4%)	1472 (39.3%)
Age Group (Years)			
≤5	8 (0.4%)	64 (3.4%)	72 (1.9%)
6-21	389 (20.7%)	380 (20.3%)	769 (20.5%)
22-59	1228 (65.4%)	1167 (62.4%)	2395 (63.9%)
≥60	254 (13.5%)	260 (13.9%)	514 (13.7%)
Race			
American Indian or Alaska Native	4 (0.2%)	4 (0.2%)	8 (0.2%)
Asian	44 (2.3%)	44 (2.4%)	88 (2.3%)
Black or African American	525 (27.9%)	524 (28.0%)	1049 (28.0%)
White	1236 (65.8%)	1222 (65.3%)	2458 (65.5%)

	NPS (N=1879)	NS (N=1871)	Overall (N=3750)
Native Hawaiian or Other Pacific Islander	4 (0.2%)	0 (0%)	4 (0.1%)
Black or African American, White	7 (0.4%)	8 (0.4%)	15 (0.4%)
Other Mixed (each N≤5)	7 (0.4%)	2 (0.1%)	9 (0.2%)
Participant declined to answer, or unknown	52 (2.8%)	67 (3.6%)	119 (3.2%)
Ethnicity			
Hispanic	170 (9.0%)	165 (8.8%)	335 (8.9%)
Non-Hispanic	1681 (89.5%)	1668 (89.2%)	3349 (89.3%)
Participant declined to answer, or unknown	28 (1.5%)	38 (2.0%)	66 (1.8%)
Specimen Testing			
Fresh	1852 (98.6%)	1845 (98.6%)	3697 (98.6%)
Frozen	27 (1.4%)	26 (1.4%)	53 (1.4%)
Testing Environment			
Laboratory/NPT	996 (53.0%)	971 (51.9%)	1967 (52.5%)
CW	883 (47.0%)	900 (48.1%)	1783 (47.5%)
Vaccine Status			
Vaccinated	1336 (71.1%)	1344 (71.8%)	2680 (71.5%)
Not Vaccinated	523 (27.8%)	508 (27.2%)	1031 (27.5%)
Unknown	20 (1.1%)	19 (1.0%)	39 (1.0%)

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of the Xpert Xpress CoV-2 *plus* test relative to the results of a U.S. FDA-cleared molecular respiratory panel for the SARS-CoV-2 target. Based on the specimens that yielded non-determinate results (**INVALID**, **ERROR**, and **NO RESULT**) with Xpert Xpress CoV-2 *plus*, the non-determinate rate was 1.6% (32/2029) with NPS and 1.4% (28/2018) with NS specimens.

A total of 3750 (1879 NPS and 1871 NS) specimens that yielded valid results by both Xpert Xpress CoV-2 *plus* and comparator were used to evaluate the clinical performance. Xpert Xpress CoV-2 *plus* demonstrated an overall PPA and NPA of 98.1% and 98.3% for SARS-CoV-2, respectively (Table 6).

Table 6. Xpert Xpress CoV-2 *plus* Performance Results in Symptomatic Individuals

		Comparator								
		Overall			Nasopharyngeal Swab Specimen			Anterior Nasal Swab Specimen		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Xpert Xpress CoV-2 *plus*	Positive	574	55	629	292	28[a]	320	282	27[b]	309
	Negative	11	3110	3121	9[c]	1550	1559	2[d]	1560	1562
	Total	585	3165	3750	301	1578	1879	284	1587	1871
Positive Percent Agreement		98.1% (95% CI: 96.7% - 98.9%)			97.0% (95% CI: 94.4% - 98.4%)			99.3% (95% CI: 97.5% - 99.8%)		
Negative Percent Agreement		98.3% (95% CI: 97.7% - 98.7%)			98.2% (95% CI: 97.4% - 98.8%)			98.3% (95% CI: 97.5% - 98.8%)		

[a] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 8/28 SARS-CoV-2 positive; 19/28 SARS-CoV-2 negative; 1/28 invalid.
[b] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 8/27 SARS-CoV-2 positive; 18/27 SARS-CoV-2 negative; 1/27 invalid.
[c] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 7/9 SARS-CoV-2 negative; 1/7 SARS-CoV-2 positive; 1/9 invalid.
[d] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 2/2 SARS-CoV-2 negative.

CI: 95% two-sided Confidence Interval

21 Analytical Performance

21.1 Analytical Sensitivity (Limit of Detection)

Analytical Sensitivity (Limit of Detection) – NATtrol SARS-CoV-2 Virus in Clinical NPS-UTM/VTM matrix

The analytical sensitivity of the Xpert Xpress CoV-2 *plus* test was first estimated by testing limiting dilutions of a NATtrol inactivated SARS-CoV-2 virus in pooled negative clinical NPS-UTM/VTM matrix, using two reagent lots and following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. LoD was estimated by considering each target gene (E, N2, and RdRP) in addition to the overall positivity rate for the Xpert Xpress CoV-2 *plus* test. The estimated LoD value as determined by Probit regression analysis was based on the weakest target gene (N2) and verified using two lots of Xpert Xpress CoV-2 *plus* reagents in replicates of 20 per lot for two clinical NPS matrices (UTM/VTM and eNAT). The concentration level with observed hit rates greater than or equal to 95% in the LoD determination study were 403, 200 and 70 copies/mL for the N2 target, RdRP target and E target, respectively. The claimed LoD is 403 copies/mL (Table 7 and Table 8).

Table 7. Xpert Xpress CoV-2 *plus* Limit of Detection – Verification of NATtrol SARS-CoV-2 LoD in NPS-UTM/VTM Matrix

Virus Strain	Reagent Lot	LoD (copies/mL)	Positives out of # Valid Replicates	% Positive	Mean E Ct	Mean N2 Ct	Mean RdRP Ct
NATtrol™ SARS-CoV-2	Reagent Lot 1	403	20/20	100%	34.3	38.3	36.6
	Reagent Lot 2	403	20/20	100%	34.1	37.7	36.5

Table 8. Xpert Xpress CoV-2 *plus* Limit of Detection – Verification of NATtrol SARS-CoV-2 LoD in NPS-eNAT Matrix

Virus Strain	Reagent Lot	LoD (copies/ mL)	Positives out of # Valid Replicates	% Positive	Mean E Ct	Mean N2 Ct	Mean RdRP Ct
NATtrol SARS-CoV-2	Reagent Lot 1	403	20/20[a]	100%	33.4	36.8	35.5
	Reagent Lot 2	403	20/20	100%	33.5	36.8	35.5

[a] One of 20 replicates tested reported **INVALID**. The run was successfully repeated to obtain 20 valid replicates.

Analytical Sensitivity (Limit of Detection) – NATtrol SARS-CoV-2 Virus in Clinical NS-UTM/VTM Matrix

The analytical sensitivity of the Xpert Xpress CoV-2 *plus* test was first estimated by testing limiting dilutions of a NATtrol inactivated SARS-CoV-2 virus in pooled negative clinical NS-UTM/VTM matrix, using two reagent lots and following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. LoD was estimated by considering each target gene (E, N2, and RdRP) in addition to the overall positivity rate for the Xpert Xpress CoV-2 *plus* test. The LoD point estimates and 95% upper and lower confidence intervals (CI) for individual SARS-CoV-2 targets (E, N2 and RdRP) in clinical NS-UTM/VTM matrix were determined using Probit regression analysis and the point estimated LoD values were 97, 462 and 157 copies/mL for the E target, N2 target and RdRP target, respectively. The estimated LoD value of the weakest target gene (N2) was verified using two lots of Xpert Xpress CoV-2 *plus* reagents in replicates of 20 per lot for clinical NS-UTM/VTM matrix. The claimed LoD is 462 copies/mL (Table 9).

Table 9. Xpert Xpress CoV-2 *plus* Limit of Detection – Verification of NATtrol SARS-CoV-2 LoD in NS-UTM/VTM Matrix

Virus Strain	Reagent Lot	LoD (copies/ mL)	Positives out of # Valid Replicates	% Positivity Rate	Mean E Ct	Mean N2 Ct	Mean RdRP Ct
NATtrol SARS-CoV-2	Reagent Lot 1	462	20/20	100%	34.4	38.9	36.8
	Reagent Lot 2	462	20/20	100%	34.1	37.6	36.1

Analytical Sensitivity (Limit of Detection)- First WHO International Standard SARS-CoV-2 RNA in Clinical NS-UTM/VTM Matrix

The analytical sensitivity of the Xpert Xpress CoV-2 *plus* test was estimated by testing limiting dilutions of the First WHO International Standard for SARS-CoV-2 RNA in pooled negative clinical NS-UTM/VTM matrix, using one reagent lot. The study was performed in a random and blinded fashion.

The lowest concentration level of the First WHO International Standard for SARS-CoV-2 RNA that generated >95% positive results (19/20 positive results) for the weakest target gene (N2) by the Xpert Xpress CoV-2 *plus* test was used to confirm the analytical sensitivity or Limit of Detection (LoD). The verified LoD for the First WHO International Standard for SARS-CoV-2 RNA tested in NS-UTM/VTM matrix with the Xpert Xpress CoV-2 *plus* test was 1000 IU/mL. The positivity rate for the overall SARS-CoV-2 results and the analyte target, including the mean Ct values for E, N2 and RdRP at the verified LoD are presented in Table 10.

Table 10. LoD Concentration, Positivity Rate and Mean Ct Values for SARS-CoV-2 for the First WHO International Standard for SARS-CoV-2 RNA in Clinical NS-UTM/VTM Matrix

Virus	LoD Concentration	SARS-CoV-2 Positive Results / Test Replicates	E Target		N2 Target		RdRP Target	
			Positive Results / Test Replicates	Mean Ct	Positive Results / Test Replicates	Mean Ct	Positive Results / Test Replicates	Mean Ct
1st WHO International Standard for SARS-CoV-2 RNA Virus	1000 IU/mL	20/20	20/20	34.3	19/20	39.5	20/20	36.5

21.2 Analytical Reactivity (Inclusivity)

SARS-CoV-2 *in silico* Analyses

The inclusivity of Xpert Xpress CoV-2 *plus* primers was evaluated on June 30, 2022 using in silico analysis of the assay amplicons in relation to 11,650,640 SARS-CoV-2 sequences available in the GISAID gene database for three targets, E, N2 and RdRP. The 11,650,640 SARS-CoV-2 sequences were separated into the lineages of interest based on the Pango Lineage assigned to each genome by GISAID, and those with ambiguous nucleotides were removed. Thus, the following inclusivity analyses focuses on the combined, non-ambiguous sequences from the variants of interest and variants of concern as of June 30, 2022. These constituted 10,469,612 sequences for the E target, 10,587,381 sequences for the N2 target and 10,333,656 sequences for the RdRP target. Table 11 summarizes the effective predicted inclusivity for E, N2 and RdRP amplicons for the variants of interests and concern.

Table 11. Predicted Inclusivity for E, N2 and RdRP Amplicons for SARS-CoV-2 Variants of Interests and Concern

SARS-CoV-2 Target Amplicon	Exact Match	1 Mismatch[a]	2 or More Mismatches	Predicted Inclusivity
E	10,420,248 of 10,469,612 total (99.5%)	48,562 (0.5%)	802 (0.01%)	100%
N2	10,386,068 of 10,587,381 total (98.1%)	196,336 (1.9%)	4,977 (0.05%)	99.95%
RdRP	10,247,146 of 10,333,656 total (99.2%)	85,373 (0.8%)	1,137 (0.01%)	100%

[a] Single-nucleotide mismatches are predicted to not impact the performance of the test.

The *in silico* inclusivity of the Xpert Xpress CoV-2 *plus* probe oligonucleotides for E, N2 and RdRP were also assessed against the top 20 most frequent matches in the GISAID EpiCoV sequence database as of June 15, 2022, which constituted 10,310, 839 for the E target, 10,428,014 for the N2 target and 10,178,602 for the RdRP target. For each of the probe oligonucleotides used in the Xpert Xpress CoV-2 *plus* test, Table 12 summarizes the number sequences as well as the corresponding percentage of sequences from this dataset with exact match, 1 mismatch/insertion, and 2 or more mismatches/ insertions in the alignment.

Table 12. Predicted Inclusivity for E, N2 and RdRP Probes for SARS-CoV-2 Variants of Interests and Concern

SARS-CoV-2 Target Probe	Exact Match	1 Mismatch/Insertion[a]	2 or More Mismatches/ Insertions	Predicted Inclusivity
E	10,300,688 of 10,310,839 total (99.9%)	9,853 (0.1%)	22 (0.0002%)	100%
N2	10,351,581 of 10,428,014 total (99.3%)	72,957 (0.7%)	0 (0%)	100%
RdRP	0	10,140,254 of 10,178,602 total (99.6%)	37,492 (0.4%)	99.6%

[a] Single-nucleotide mismatches/insertions are predicted to not impact the performance of the test.

The in silico inclusivity of the Xpert Xpress CoV-2 *plus* primer/probe sequences for E, N2 and RdRP was assessed against SAR-CoV-2 sequences submitted to the GISAID EpiCoV database up to 180 days prior to and including July 31, 2023 for SARS-CoV-2 isolates currently circulating in the United States. There were no instances where a specific primer/probe sequence was observed with greater than 0.5% frequency of mismatch during this 180-day period. The analysis predicted that the Xpert Xpress CoV-2 *plus* test will detect all currently circulating variants/lineages of SARS-CoV-2.

Moreover, based on the built-in redundancy of the Xpert Xpress CoV-2 *plus* test's SARS-CoV-2 amplification system (i.e., 3 independent targets, only 1 of 3 must be detected to assign a positive result), it is not anticipated that any of the evaluated sequences would be missed by the Xpert Xpress CoV-2 *plus* test.

SARS-CoV-2 Wet-Testing

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 *plus* test was evaluated by bench testing against multiple strains of SARS-CoV-2 at levels near the analytical LoD (~3x LoD). A total of 61 strains comprised of 23 intact SARS-CoV-2 viral particles (18 inactivated viral cultures and 5 BSL-3 live viral cultures), and 38 SARS-CoV-2 *in vitro* RNA transcripts representing variant strains were tested in this study with the Xpert Xpress CoV-2 *plus* test. Three replicates were tested for each strain. All SARS-CoV-2 strains tested positive in all three replicates. Results are shown in Table 13.

Table 13. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2 *plus* Test

Count No.	SARS-CoV-2 Strain	Tested Concentration	SARS-CoV-2 Test Results	Number of Positive Results Obtained out of the Total Number of Valid Replicates		
				E	N2	RdRP
1	2019-nCoV/Italy-INMI1[a]	5 $TCID_{50}$/mL	POS	3/3	3/3	3/3
2	England/204820464/2020[a]	0.5 $TCID_{50}$/mL	POS[b]	3/3	3/3	3/3
3	Hong Kong/VM20001061/2020[a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3

Count No.	SARS-CoV-2 Strain	Tested Concentration	SARS-CoV-2 Test Results	Number of Positive Results Obtained out of the Total Number of Valid Replicates		
				E	N2	RdRP
4	South Africa/KRISP-K005325/2020[a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
5	USA/CA_CDC_5574/2020[a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
6	USA/MD-HP01542/2021[a]	1.3e3 genome equivalents/mL	POS	3/3	3/3	3/3
7	USA/GA-EHC-2811C/2021[a]	1.3e3 genome equivalents/mL	POS	3/3	3/3	3/3
8	NY-Wadsworth-103677-01/2020[a]	0.15 $TCID_{50}$/mL	POS	3/3	3/3	3/3
9	NY-Wadsworth-21006055-01/2021[a]	0.04 $TCID_{50}$/mL	POS	3/3	3/3	3/3
10	NY-Wadsworth-21025952-01/2021[a] (Isolate 1)	0.625 $TCID_{50}$/mL	POS	3/3	3/3	3/3
11	NY-Wadsworth-21018781-01/2021[a] (Isolate 2)	0.625 $TCID_{50}$/mL	POS	3/3	3/3	3/3
12	NY-Wadsworth-21033899-01/2021[a]	1.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
13	NY-Wadsworth-33126-01/2020 [a]	0.15 $TCID_{50}$/mL	POS	3/3	3/3	3/3
14	USA/CA-Stanford-15_S02/2021[a]	0.04 $TCID_{50}$/mL	POS	3/3	3/3	3/3
15	USA/PHC658/2021[a]	25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
16	Japan/TY7-503/2021[a]	0.75 $TCID_{50}$/mL	POS	3/3	3/3	3/3
17	hCoV-19/USA/MD-HP30386/2022[a]	27.5 copies/mL	POS	3/3	3/3	3/3
18	hCoV-19/USA/COR-22-063113/2022[a]	19 copies/mL	POS	3/3	3/3	3/3
19	Australia/VIC01/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
20	Belgium/ULG/10004/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
21	England/205041766/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
22	England/MILK-9E05B3/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
23	England/SHEF-C05B2/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
24	France/HF2393/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
25	Hong Kong/HKU-211129-001/2021[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
26	Iceland/5/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3

Count No.	SARS-CoV-2 Strain	Tested Concentration	SARS-CoV-2 Test Results	Number of Positive Results Obtained out of the Total Number of Valid Replicates		
				E	N2	RdRP
27	India/CT-ILSGS00361/2021[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
28	India/MH-NCCS-P1162000182735/2021[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
29	India/MH-SEQ-221_S66_R1_001/2021[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
30	Japan/Hu_DP_Kng_19-020/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
31	Japan/IC-0564/2021[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
32	Portugal/PT9543/2021[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
33	South Africa/KRISP-EC-K005299/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
34	Taiwan/NTU02/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
35	USA/CA9/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
36	USA/CA-PC101P/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
37	USA/CA-CZB-12943/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
38	USA/MN2-MDH2/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
39	USA/NY-MSHSPSP-PV24650/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
40	USA/TX1/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
41	USA/WA2/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
42	USA/WA-CDC-UW21061750277/2021[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
43	Wuhan-Hu-1[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
44	Germany/BavPat1/2020[d]	1.2e3 genome copies/mL	POS	3/3	3/3	3/3
45	Singapore/2/2020[d]	300 genome equivalents/mL	POS	3/3	3/3	3/3
46	USA/AZ1/2020[d]	300 genome equivalents/mL	POS	3/3	3/3	3/3
47	USA/CA1/2020[d]	1.2e3 genome equivalents/mL	POS	3/3	3/3	3/3
48	USA/CA2/2020[d]	1.2e3 genome equivalents/mL	POS	3/3	3/3	3/3
49	USA/CA3/2020[d]	400 genome equivalents/mL	POS	3/3	3/3	3/3
50	USA/CA4/2020[d]	1.2e3 genome equivalents/mL	POS	3/3	3/3	3/3

Count No.	SARS-CoV-2 Strain	Tested Concentration	SARS-CoV-2 Test Results	Number of Positive Results Obtained out of the Total Number of Valid Replicates		
				E	N2	RdRP
51	USA/IL1/2020[d]	150 genome equivalents/mL	POS	3/3	3/3	3/3
52	USA/New York-PV08001/2020[d]	1.2e3 genome copies/mL	POS	3/3	3/3	3/3
53	USA/New York-PV08410/2020[d]	300 genome equivalents/mL	POS	3/3	3/3	3/3
54	USA/New York-PV08449/2020[d]	300 genome equivalents/mL	POS	3/3	3/3	3/3
55	USA/New York-PV09158/2020[d]	300 genome equivalents/mL	POS	3/3	3/3	3/3
56	USA/WI1/2020[d]	1.2e3 genome equivalents/mL	POS	3/3	3/3	3/3
57	hCoV-19/USA/MD-HP38861/2022[e]	0.29 $TCID_{50}$/mL	POS	3/3	3/3	3/3
58	hCoV-19/USA/MD-HP38960/2022[e]	0.043 $TCID_{50}$/mL	POS	3/3	3/3	3/3
59	hCoV-19/USA/CO-CDPHE-2102544747/2021[e]	0.028 $TCID_{50}$/mL	POS	3/3	3/3	3/3
60	hCoV-19/USA/MD-HP38288/2022[e]	0.24 $TCID_{50}$/mL	POS	3/3	3/3	3/3
61	hCoV-19/USA/MD-HP40900/2022[e]	0.72 $TCID_{50}$/mL	POS	3/3	3/3	3/3

[a] Inactivated viral culture fluid (intact viral particles)

[b] One of 3 replicates reported **ERROR**. The run was successfully repeated to obtain 3 valid replicates.

[c] Synthetic, *in vitro* RNA transcript, TWIST controls

[d] Genomic RNA

[e] BSL-3 live viral culture fluid (intact viral particles)

21.3 Analytical Specificity (Exclusivity)

In silico Analyses

The analytical specificity/cross-reactivity of the Xpert Xpress CoV-2 *plus* plan included evaluation of the SARS-CoV-2 test primer and probes with potentially cross-reactive microorganisms by *in silico* analysis. The analysis was conducted by mapping the primers and probes of Xpert Xpress CoV-2 *plus* individually to the microorganism sequences downloaded from the NCBI 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 14 is expected based on the *in silico* analysis.

Table 14. 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	Cytomegalovirus
Human coronavirus HKU1	Enterovirus (e.g., EV68)
Human coronavirus NL63	Epstein-Barr virus
SARS-coronavirus	Human Metapneumovirus (hMPV)
MERS-coronavirus	Influenza A & B
Bat coronavirus	Measles
	Mumps
	Parainfluenza virus 1-4
	Parechovirus
	Respiratory syncytial virus
	Rhinovirus
	Bacillus anthracis (Anthrax)
	Bordetella pertussis
	Bordetella parapertussis
	Chlamydia pneumoniae
	Chlamydia psittaci
	Corynebacterium diphtheriae
	Coxiella burnetii (Q-Fever)
	Escherichia coli
	Fusobacterium necrophorum
	Haemophilus influenzae
	Lactobacillus sp.
	Legionella non-*pneumophila*
	Legionella pneumophila
	Leptospira
	Moraxella catarrhalis
	Mycobacterium tuberculosis
	Mycoplasma genitalium
	Mycoplasma pneumoniae
	Neisseria elongata
	Neisseria meningitidis
	Pneumocystis jirovecii (PJP)
	Pseudomonas aeruginosa
	Staphylococcus aureus

Microorganisms from the Same Genetic Family	High Priority Organisms
	Staphylococcus epidermidis
	Staphylococcus salivarius
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Aspergillus sp
	Candida albicans

Wet-Testing

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 *plus* test was evaluated by bench-testing a panel of 62 microorganisms comprising (4) human coronaviruses, (1) MERS-coronavirus, (1) SARS-coronavirus, (20) other respiratory viruses, (30) respiratory bacteria, (2) yeast strains, (3) fungal strains, and 1 human nasal wash fluid representing a diverse microbial flora in the human respiratory tract.

The intact viruses were tested at concentrations of $\geq 10^5$ $TCID_{50}$/mL, $\geq 10^5$ $CEID_{50}$/mL, and $\geq 10^5$ copies/mL. Bacteria and yeast were tested at $\geq 10^6$ CFU/mL. The bacteria *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were tested at concentrations of $\geq 10^6$ IFU/mL and $\geq 10^6$ CCU/mL, respectively. The live strains of *Coxiella burnetii*, *Lactobacillus reuteri*, and *Neisseria meningitides* were not available, therefore genomic DNA at $\geq 10^6$ genome equivalent copies/mL was used. The *in vitro* transcribed (IVT) RNA sample was tested at $\geq 10^6$ genome equivalent/mL for human Coronavirus HKU1, and the genomic RNA sample was tested at $\geq 10^6$ genome equivalent/mL for SARS-coronavirus Urbani.

The microorganisms being evaluated for cross-reactivity were tested in groups or individually and spiked into negative simulated NPS/NS background matrix for testing. If a grouped microorganisms produced a **SARS-CoV-2 POSITIVE** result, then each member of the group was tested separately. If the individual non-target organism yielded a positive result, retesting was performed at lower concentrations until a concentration that no longer produce a false positive result was identified.

The results of the analytical specificity/exclusivity study demonstrate that the primer/probe sets included in the Xpert Xpress CoV-2 *plus* does not cross-react with the nucleic acids from non-intended respiratory microorganisms and phylogenetically related human coronavirus species. The one exception was the SARS-coronavirus, Urbani which yielded the expected test result of **SARS-CoV-2 POSITIVE**. We expected cross-reactivity for the E gene target with the SARS-coronavirus Urbani strain which has the highly conserved E gene target shared among coronaviruses in the B lineage *Betacoronavirus*. Results are shown in Table 15.

Table 15. Analytical Specificity (Exclusivity) of the Xpert Xpress CoV-2 *plus* Test

Respiratory Microorganisms	Test Group	Tested Concentration	Final SARS-CoV-2 Result Call-Out	Number of Positive Results Obtained from Total Number of Replicates Tested		
				E	N2	RdRP
Human coronavirus, 229E	G1	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Human coronavirus, OC43		1.1e5 $TCID_{50}$/mL				
MERS-coronavirus		1.1e5 $TCID_{50}$/mL				
Human coronavirus, NL63	G2	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Human coronavirus, HKU1[a]	G3	1.1e6 genome cp/mL	NEG	0/3	0/3	0/3
SARS-coronavirus, Urbani [a]	G4	1.1e6 genome cp/mL	POS	3/3	0/3	0/3
Influenza A H1N1 (pdm2009), Michigan/272/2017	G5	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3

Respiratory Microorganisms	Test Group	Tested Concentration	Final SARS-CoV-2 Result Call-Out	Number of Positive Results Obtained from Total Number of Replicates Tested		
				E	N2	RdRP
Influenza B (Victoria Lineage), Hawaii/01/2018 (NA D197N)		1.1e5 $TCID_{50}$/mL				
RSV-A, Strain: 4/2015 Isolate #1		1.1e5 TCID50/mL				
Adenovirus Type 1	G6	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Adenovirus Type 7A		1.1e5 $TCID_{50}$/mL				
Cytomegalovirus		1.1e5 $TCID_{50}$/mL				
Echovirus	G7	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Enterovirus, D68 strain US/KY/14-18953		1.1e5 $TCID_{50}$/mL				
Epstein Barr Virus (Human Herpes Virus 4 [Hhv-4])		1.1e5 cp/mL				
Herpes Simplex Virus (HSV) type 1		1.1e5 $TCID_{50}$/mL				
Human metapneumovirus (hMPV-5, type B1)		1.1e5 $TCID_{50}$/mL				
Measles		1.1e5 $TCID_{50}$/mL				
Mumps virus		1.1e5 $TCID_{50}$/mL				
Human parainfluenza Type 1	G8	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Human parainfluenza Type 2		1.1e5 $TCID_{50}$/mL				
Human parainfluenza Type 3		1.1e5 $TCID_{50}$/mL				
Human parainfluenza Type 4		1.1e5 $TCID_{50}$/mL				
Rhinovirus, Type 1A[b]		4.5e4 $TCID_{50}$/mL				
Acinetobacter baumannii	G9	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Burkholderia cepacia		1.1e6 CFU/mL				
Candida albicans		1.1e6 CFU/mL				
Candida parapsilosis		1.1e6 CFU/mL				
Bordetella pertussis		1.1e6 CFU/mL				
Chlamydia pneumoniae		1.1e6 IFU/mL				
Citrobacter freundii		1.1e6 CFU/mL				
Corynebacterium xerosis	G10	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Escherichia coli		1.1e6 CFU/mL				
Enterococcus faecalis		1.1e6 CFU/mL				
Hemophilus influenzae		1.1e6 CFU/mL				
Legionella spp. [c]		1.1e6 CFU/mL				
Moraxella catarrhalis		1.1e6 CFU/mL				

Respiratory Microorganisms	Test Group	Tested Concentration	Final SARS-CoV-2 Result Call-Out	Number of Positive Results Obtained from Total Number of Replicates Tested		
				E	N2	RdRP
Mycobacterium tuberculosis (avirulent)	G11	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Mycoplasma pneumoniae		1.1e6 CCU/mL				
Neisseria mucosa		1.1e6 CFU/mL				
Propionibacterium acnes (= Cutibacterium acnes) Z144		1.1e6 CFU/mL				
Pseudomonas aeruginosa, Z139		1.1e6 CFU/mL				
Staphylococcus aureus		1.1e6 CFU/mL				
Staphylococcus epidermidis	G12	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Staphyloccus haemolyticus		1.1e6 CFU/mL				
Streptococcus agalactiae		1.1e6 CFU/mL				
Streptococcus pneumoniae		1.1e6 CFU/mL				
Streptococcus pyogenes		1.1e6 CFU/mL				
Streptococcus salivarius		1.1e6 CFU/mL				
Streptococcus sanguinis		1.1e6 CFU/mL				
Pneumocystis jirovecii (PJP)		1.1e6 CFU/mL				
Lactobacillus reuteri, F275[d]	G13	1.1e6 genome cp/mL	NEG	0/3	0/3	0/3
Neisseria meningitides [d]		1.1e6 genome cp/mL				
Pooled human nasal wash	G14	N/A	NEG	0/3	0/3	0/3
Influenza C (Taylor/1233/1947)	G15	1.1e5 $CEID_{50}$/mL	NEG	0/3	0/3	0/3
Rhinovirus, Type 1A[b]	G16	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Aspergillus flavus	G17	1.35e7 CFU/mL	NEG	0/3	0/3	0/3
Aspergillus fumigatus	G18	3.67e6 CFU/mL	NEG	0/3	0/3	0/3
Coxiella burnetii [d]	G19	2.5e6 genome cp/mL	NEG	0/3	0/3	0/3
Leptospira broomii	G20	2.9e6 CFU/mL	NEG	0/3	0/3	0/3
Parechovirus type 1	G21	3.39e5 TCID50/mL	NEG	0/3	0/3	0/3
Fusobacterium necrophorum	G22	6.52e6 CFU/mL	NEG	0/3	0/3	0/3
Mycoplasma genitalium [d]	G23	3.6e6 genome cp/mL	NEG	0/3	0/3	0/3

[a] Microorganism in the form of genomic RNA were tested in Tris-EDTA+ ($[NH_4]_2SO_4$) buffer using an assay definition file (ADF) without sample preparation.

[b] Rhinovirus, Type 1A was initially tested at 4.5 e4 $TCID_{50}$/mL in test group G8 using virus lot 325725. It was re-tested individually (G16) at a higher concentration of 1.1e5 $TCID_{50}$/mL using virus lot 326527.

[c] *Legionella pneumophila* was tested in this study.

[d] Microorganisms in the form of genomic DNA were tested in simulated NPS/NS background matrix using the ADF with full sample preparation.

21.4 Microbial Interference

A microbial interference study was performed to assess the inhibitory effects of commensal microorganisms potentially encountered in upper respiratory tract specimens on the performance of the Xpert Xpress CoV-2 *plus* test. A panel of 18 commensal microorganisms, consisting of 15 viral strains and 3 bacterial strains was tested. Contrived samples consisted of SARS-CoV-2 virus seeded at 1.15x-3x LoD into simulated nasopharyngeal swab (NPS)/ anterior nasal swab (NS) matrix in the presence of fifteen (15) commensal virus strains and three (3) commensal bacterial strains (*Haemophilus influenzae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) spiked at their respective concentrations listed in Table 16.

Replicates of 8 positive samples were tested with SARS-CoV-2 virus and each potential microbial interference strain combination. All 8 of 8 positive replicate samples were correctly identified as SARS-CoV-2 POSITIVE using the Xpert Xpress CoV-2 *plus* test. No interference by the listed above commensal viral or bacterial strains was reported at the concentrations tested.

Table 16. Microbial Interference Study Results

Commensal Strain	Concentration Tested	Number of Correct Test Results/Number Tested
Adenovirus Type 1C[a]	$1x10^5$ $TCID_{50}$/mL	8/8
Adenovirus Type 2C[b]	$1x10^5$ $TCID_{50}$/mL	8/8[c]
Adenovirus Type 3B[b]	$1x10^5$ $TCID_{50}$/mL	8/8
Human Coronavirus-OC43[a]	$1x10^5$ copies/mL	8/8
Human Coronavirus-229E[b]	$1x10^5$ $TCID_{50}$/mL	8/8
Human Coronavirus-NL63[b]	$1x10^5$ $TCID_{50}$/mL	8/8
Human Coronavirus-HKU1[b]	$1x10^5$ copies/mL	8/8
Metapneumovirus5, Type B1[a]	$1x10^5$ $TCID_{50}$/mL	8/8
ParainfluenzaType 1[a]	$1x10^5$ $TCID_{50}$/mL	8/8
Parainfluenza Type 2[a]	$1x10^5$ $TCID_{50}$/mL	8/8
Parainfluenza Type 3[a]	$1x10^5$ $TCID_{50}$/mL	8/8
Rhinovirus Type 1A[a]	$1x10^5$ PFU/mL	8/8
Influenza A H1N1[b]	$1x10^5$ $CEID_{50}$/mL	8/8
Influenza B[b]	$1x10^5$ $TCID_{50}$/mL	8/8[d]
Respiratory Syncytial Virus A[b]	$1x10^5$ $TCID_{50}$/mL	8/8
Haemophilus influenzae[a]	$1x10^7$ CFU/mL	8/8
Staphylococcusaureus[a]	$1x10^7$ CFU/mL	8/8
Staphylococcusepidermidis[a]	$1x10^7$ CFU/mL	8/8

a These commensal strains were tested with SARS-CoV-2 at a concentration of 3x LoD.

b These commensal strains were tested with SARS-CoV-2 at a concentration of 1.15x LoD.

c Two of 8 replicates reported as **ERROR**. The runs were successfully repeated to obtain 8 valid replicates.

d One of 8 replicates reported as **ERROR**. The run was successfully repeated to obtain 8 valid replicates.

21.5 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 were evaluated with direct testing on the Xpert Xpress CoV-2 *plus* test. 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)/ anterior nasal swab (ANS) 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 SARS-CoV-2 virus spiked at 3x LoD. The controls were samples with and without SARS-CoV-2 virus spiked at 3x LoD into simulated NPS/ NS matrix containing no potentially interfering substance.

The 23 potentially substances, with active ingredients, that were evaluated are listed in Table 17. For substances that resulted in an **INVALID** test result, the concentration of the substance was reduced by half and re-tested. Interfering effects were observed for fluticasone propionate and mucin type I-S.

Table 17. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Substance/ Active Ingredient	Concentrations Tested
No substance	Control	Simulated NPS/NS Matrix	100% (v/v)
Afrin	Nasal Spray	Oxymetazoline (0.05%)	15% (v/v)
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/ mL)	0.83 mg/mL (equivalent to 1 dose per day)
BD Universal Transport Medium	Transport Media	BD Universal Transport Medium	100% (v/v)
Blood	Blood	Blood (Human)	2% (v/v)
Copan Swab M	Transport Media	Copan Swab M	100% (v/v)
FluMist	FluMist®	Live intranasal influenza virus vaccine	6.7% (v/v)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate	5 µg/mL, 2.5 µg/mL
Ibuprofen	Analgesic (nonsteroidal anti-inflammatory drugs (NSAID))	Ibuprofen	21.9 mg/dL
Leukocytes	Leukocytes	Leukocytes (Human)	1 x 10^6 cells/mL
Menthol	Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol	1.7 mg/mL
Mucin type II	Mucin	Purified Mucin protein (Porcine submaxillary gland, type II)	0.1% (w/v)
Mucin type I-S	Mucin	Purified Mucin protein (Bovine submaxillary gland, type I-S)	2.5 mg/mL, 1.25 mg/mL
Mupirocin	Antibiotic, nasal ointment	Mupirocin (2%)	10 mg/mL
Human peripheral blood mononuclear cells (PBMC)	Human peripheral blood mononuclear cells (PBMC)	Human peripheral blood mononuclear cells (PBMC)	1x10^3 cells/µL
PHNY	Nasal Drops	Phenylephrine (1%)	15% (v/v)
Remel M4RT	Transport Media	Remel M4RT	100% (v/v)

Substance ID	Substance/Class	Substance/ Active Ingredient	Concentrations Tested
Remel M5	Transport Media	Remel M5	100% (v/v)
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)	15% (v/v)
Snuff	Tobacco	Nicotine	1% (w/v)
Tamiflu	Anti-viral drugs	Zanamivir	7.5 mg/mL
Tobramycin	Antibacterial, systemic	Tobramycin	4 µg/mL
Zicam	Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur (0.05%)	15% (w/v)
Zinc	Zinc supplement	Zinc Gluconate	0.1 µg/mL

The results for the negative and positive samples are presented in Table 18.

The results from the SARS-CoV-2 negative samples showed that in the presence of fluticasone propionate nasal spray at 5 µg/mL and mucin type I-S at 2.5 mg/mL, 7 of 8 replicates correctly reported **SARS-CoV-2 NEGATIVE** test results, and 1 of 8 replicates reported **INVALID** test result for each substance. When the concentrations of fluticasone propionate nasal spray and mucin type I-S were reduced by half, 8/8 replicates provided valid test results and correctly reported **SARS-CoV-2 NEGATIVE** for each substance. No interference was observed at these lower concentrations.

The results from the SARS-CoV-2 positive samples showed that in the presence of fluticasone propionate nasal spray at 5 µg/mL, 7 of 8 replicates correctly reported **SARS-CoV-2 POSITIVE** test results and 1 of 8 replicate reported **INVALID** test result. When the concentration of the fluticasone propionate nasal spray was reduced to 2.5 µg/mL, 8/8 replicates provided valid test results and correctly reported **SARS-CoV-2 POSITIVE**. No interference was observed at this lower concentration.

Table 18. SARS-CoV-2 Negative and Positive Samples Tested in the Presence of Potentially Interfering Substances

Substance	Concentration Tested	Number SARS-CoV-2 Negative / Number of Valid Replicates Tested	Number SARS-CoV-2 Positive / Number of Valid Replicates Tested
SARS-CoV-2 Negative (No Substance) Control	**100% (v/v)**	**8/8**	**Not Applicable**
SARS-CoV-2 Positive (No Substance) Control	**100% (v/v)**	**Not Applicable**	**8/8**
Afrin	15% (v/v)	8/8	8/8
Albuterol Sulfate	0.83 mg/mL	8/8	8/8
BD Universal Transport Medium	100 (v/v)	8/8	8/8
Blood	2% (v/v)	8/8	8/8
Copan Swab M	100 (v/v)	8/8	8/8
FluMist®	6.7% (v/v)	8/8	8/8
Fluticasone Propionate Nasal Spray	5 µg/mL	7/8[a]	7/8[a]
	2.5 µg/mL	8/8	8/8

Substance	Concentration Tested	Number SARS-CoV-2 Negative / Number of Valid Replicates Tested	Number SARS-CoV-2 Positive / Number of Valid Replicates Tested
Ibuprofen	21.9 mg/dL	8/8	8/8
Leukocytes	1 x 10^6 cells/mL	8/8	8/8
Menthol	1.7 mg/mL	8/8	8/8
Mucin (Type II)	0.1% (w/v)	8/8	8/8
Mucin (Type I-S)	2.5 mg/mL	7/8[a]	8/8
	1.25 mg/mL	8/8	Not Applicable
Mupirocin	10 mg/mL	8/8	8/8
Human Peripheral Blood Mononuclear Cells (PBMC)	1 x 10^3 cells/µL	8/8	8/8
PHNY	15% (v/v)	8/8	8/8
Remel M4RT	100% (v/v)	8/8	8/8
Remel M5	100% (v/v)	8/8	8/8
Saline	15% (v/v)	8/8	8/8
Snuff	1% (w/v)	8/8	8/8
Tamiflu	7.5 mg/mL	8/8	8/8
Tobramycin	4 µg/mL	8/8	8/8
Zicam	15% (w/v)	8/8	8/8
Zinc	0.1 µg/mL	8/8	8/8

[a] One of 8 replicates reported **INVALID** test result, indicating interference from the substance. The substance was subsequently tested with 8 replicates at half the initial concentration.

21.6 Carry-Over Contamination

A study was conducted to assess whether the single-use, self-contained Xpert Xpress CoV-2 *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 SARS-CoV-2 virus concentration (inactivated SARS-CoV-2 USA-WA1/2020 at 5e4 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** and all 42 negative samples were correctly reported as **SARS-CoV-2 NEGATIVE** with the Xpert Xpress CoV-2 *plus* test. No specimen or amplicon carry-over contamination was observed in this study.

22 Reproducibility-Precision

The reproducibility of the Xpert Xpress CoV-2 *plus* test was established at 3 sites (2 external and 1 internal) using a 3-member panel including one negative sample, one low positive (~1.5x LoD) sample and one moderate positive (~3x LoD) sample. The negative sample consisted of simulated NS/NPS matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated matrix using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix).

Testing was conducted over 6 days, using 3 lots of Xpert Xpress CoV-2 *plus* cartridges at 3 participating sites each with 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 percent agreement of the qualitative results for SARS-CoV-2 detection for each panel member analyzed by each of the 6 operators and each site is shown in Table 19. In addition, the overall percent agreement for each sample (% total agreement) and the two-sided Wilson Score confidence intervals (CI) are presented in the last column.

Table 19. Summary of the Reproducibility Results - % Agreement

Panel Member	Site 1			Site 2			Site 3			% Total Agreement and 95% Confidence Interval by Panel Member
	Operator 1	Operator 2	Site	Operator 1	Operator 2	Site	Operator 1	Operator 2	Site	
Negative	100% (24/24)	95.8% (23/24)	97.9% (47/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (23/23)[a]	100% (47/47)	99.3% (142/143) [96.1% - 99.9%]
SARS-CoV-2 Low Positive	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%]
SARS-CoV-2 Moderate Positive	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%]

[a] One sample was non-determinate on both initial and retest and was excluded from the analyses.

The evaluation of reproducibility and within-laboratory precision of the underlying Ct values for E, N2 and RdRP obtained in the Xpert Xpress CoV-2 *plus* test was analyzed. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-operators, between-lots, between-days, between-runs and within-run for each panel member are presented in Table 20.

Table 20. Summary of Reproducibility Nested ANOVA by Coefficient of Variation

Sample	Analyte	N	Mean Ct	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	**SPC**	143	31.1	0.29	0.9	0.16	0.5	0.42	1.3	0	0	0	0	1.47	4.7	1.56	5.0
SARS-CoV-2 Low Positive	**E**	144	34.0	0.06	0.2	0.19	0.6	0	0	0.14	0.4	0	0	0.55	1.6	0.60	1.8
	N2	144	37.5	0.12	0.3	0	0	0.2	0.5	0.11	0.3	0	0	0.68	1.8	0.73	1.9
	RdRP	144	36.3	0.07	0.2	0	0	0.25	0.7	0.06	0.2	0	0	0.61	1.7	0.66	1.8
SARS-CoV-2 Moderate Positive	**E**	144	32.5	0.31	0.9	0.03	0.1	0	0	0.47	1.4	0	0	1.86	5.7	1.95	6.0
	N2	144	36.2	0.16	0.4	0	0	0.29	0.8	0.17	0.5	0	0	0.65	1.8	0.75	2.1
	RdRP	144	34.8	0.33	1.0	0	0	0	0	0.62	1.8	0	0	2.18	6.2	2.29	6.6

The precision of the Xpert Xpress CoV-2 *plus* test was established at a single site using a 3-member panel including one negative sample, one low positive (~1.5x LoD) sample and one moderate positive (~3x LoD) sample. The negative sample consisted of simulated NS/NPS matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated NS/NPS matrix using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix).

Testing was conducted over twenty (20) days, using one (1) lot of Xpert Xpress CoV-2 *plus* cartridges at a single site and with one (1) operator to yield a total of 80 observations per panel member (1 Site x 1 Operator x 1 Lot x 20 Days x 2 Runs x 2 Replicates = 80 observations/panel member). The results from the study are summarized in Table 21.

Table 21. Summary of the Precision Results - % Agreement

Panel Member	Agreement	% Total Agreement and 95% CI by Panel Member
Negative	80/80	100% (95.4%-100.0%)
SARS-CoV-2 Low Pos	80/80	100% (95.4%-100.0%)
SARS-CoV-2 Mod Pos	80/80	100% (95.4%-100.0%)

23 References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed December 15, 2022.
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3. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (6th edition). http://www.cdc.gov/biosafety/publications/ Accessed December 15, 2022.
4. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).
5. 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).
6. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

24 Cepheid Headquarters Locations

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25 Technical Assistance

Before Contacting Us

Collect the following information before contacting Cepheid Technical Support:

- 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.

26 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

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27 Revision History

Description of Changes: 302-8997, Rev. A to Rev B

Section	Description of Change
6	Updated contents of flyer.