WHO Emergency Use Assessment SARS-CoV-2 IVDs
PUBLIC REPORT

Product: Xpert Xpress CoV-2 *plus*
Manufacturer: Cepheid AB
EUL Number: EUL 0720-070-00
Outcome: Accepted

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- Product Dossier Review: assessment of the documentary evidence of safety and performance.

Xpert Xpress CoV-2 plus code XP3SARS-COV2-10, CE-mark regulatory version, manufactured by Cepheid AB, 940 Caribbean Drive, Sunnyvale, California, 94089-1189, United States of America was listed on 31 August 2023.

Intended use:

According to the claim of intended use from Cepheid AB, "*The Xpert Xpress CoV-2 plus test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swab or anterior nasal swab specimen obtained from individuals meeting COVID-19 clinical and/or epidemiological criteria, as well as individuals without symptoms or other reasons to suspect COVID-19 infection. Results are for the identification of SARS-CoV-2 RNA.*
Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.
Negative results do not preclude SARS-CoV-2 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 epidemiological information.
The Xpert Xpress CoV-2 plus test is intended to be performed by trained users in both laboratory and near patient testing settings."

Specimen type that was validated:

Nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimens.

Test kit contents:

Component	**10 tests (product code XP3SARS-COV2-10)**
Xpert Xpress CoV-2 plus Cartridges with Integrated Reaction Tubes	10
Disposable Transfer Pipettes	10-12 per kit
Flyer	1 per kit
Quick Reference Instructions	2 per kit

Items required but not provided:

- Nylon flocked swab (Copan P/N 502CS01, 503CS01) or equivalent
- 3 mL Viral transport medium
- 0.85 – 0.9% (w/v) saline, 3 mL
- Sample Collection Kit for Viruses (Cepheid P/N SWAB/B-100, SWAB/F-100) (Copan P/N 305C, 346C) or equivalent
- GeneXpert Dx System or GeneXpert Infinity System (catalogue number varies by configuration): GeneXpert instrument, computer, barcode scanner, and 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.

Storage:

Store all reagents Xpert Xpress CoV-2 plus cartridges at 2-28°C.

Shelf-life upon manufacture:

12 months.

Warnings/limitations:

Refer to the instructions for use (IFU)

Product dossier assessment

Cepheid AB submitted a product dossier for the Xpert Xpress CoV-2 plus for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347 version 4)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Post listing Commitments for EUL:

As commitments to listing, the manufacturer is required to:

1. Submit the Xpert Xpress CoV-2 *plus*'s stability report within 1 month of study completion.
2. Amend the IFU to clarify that specimens not transported or stored according to section 12 of the IFU must be excluded from testing and correct minor typographical errors.

The risk-benefit assessment conclusion is acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Cepheid AB was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff, it was established that Cepheid AB provided sufficient information to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_347)*".

The quality management documentation assessment conclusion is acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL listing status:
1. Notification to WHO of any planned changes to an EUL product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and

2. Post-market surveillance activities, in accordance with *"Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics*" (ISBN 978-92-4-001531-9).

Cepheid AB is also required to submit an annual report that details sales data and all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensuring that post-emergency use listing safety, quality and performance monitoring activities are in place in accordance with WHO guidance "Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics".[1]

Scope and duration of procurement eligibility

Xpert Xpress CoV-2 *plus* code XP3SARS-COV2-10 manufactured by Cepheid AB is eligible for WHO procurement 12 months from the day of listing. The assay may detect the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO-prequalified.

As part of the ongoing requirements for listing as eligible for WHO procurement, Cepheid AB must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Cepheid AB is required to notify WHO of any complaints, including adverse events related to the use of the product, within 7 days.

WHO reserves the right to rescind eligibility for WHO procurement if additional information on the safety, quality, and performance during post-market surveillance activities and if new data becomes available to WHO that changes the risk-benefit balance.

[1] 2 https://www.who.int/publications/i/item/9789240015319

Labelling

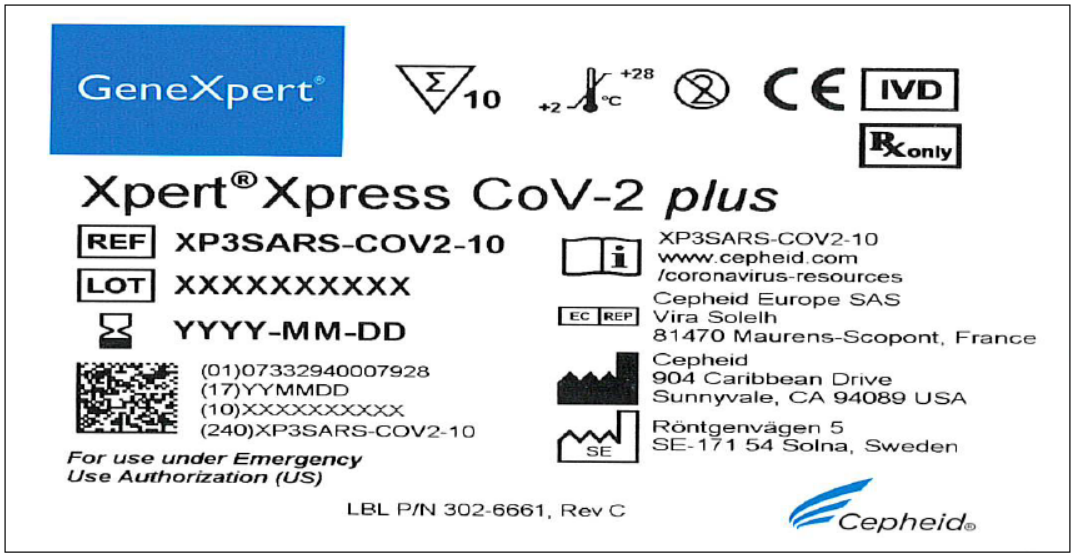
1.0 Labels

2.0 Instructions for Use (IFU)

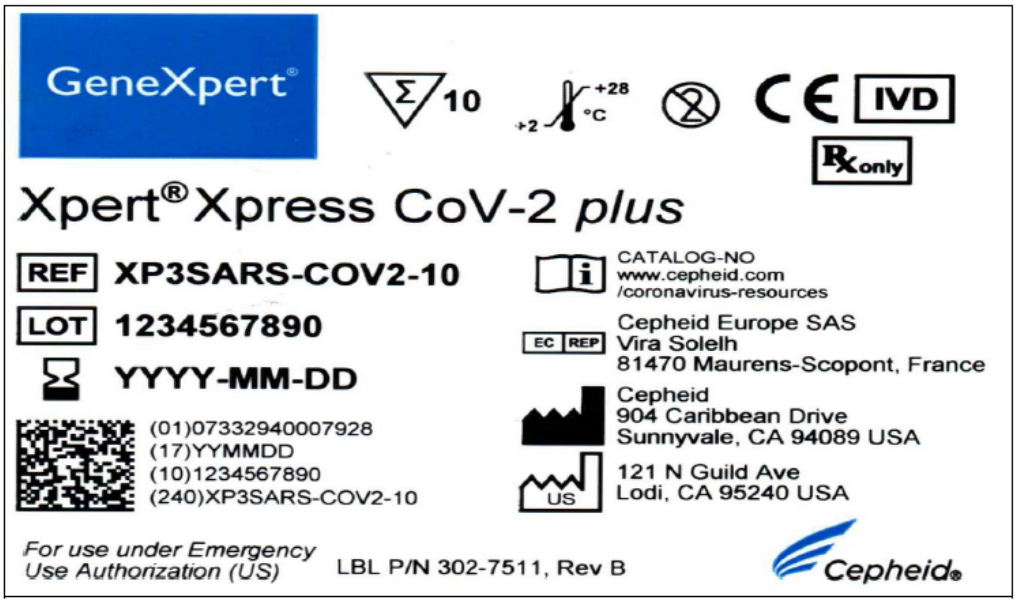
1.0 Product labels

1.1 Xpert Xpress CoV-2 *plus* kit carton, top panel labels

Sunnyvale 10-test kit label

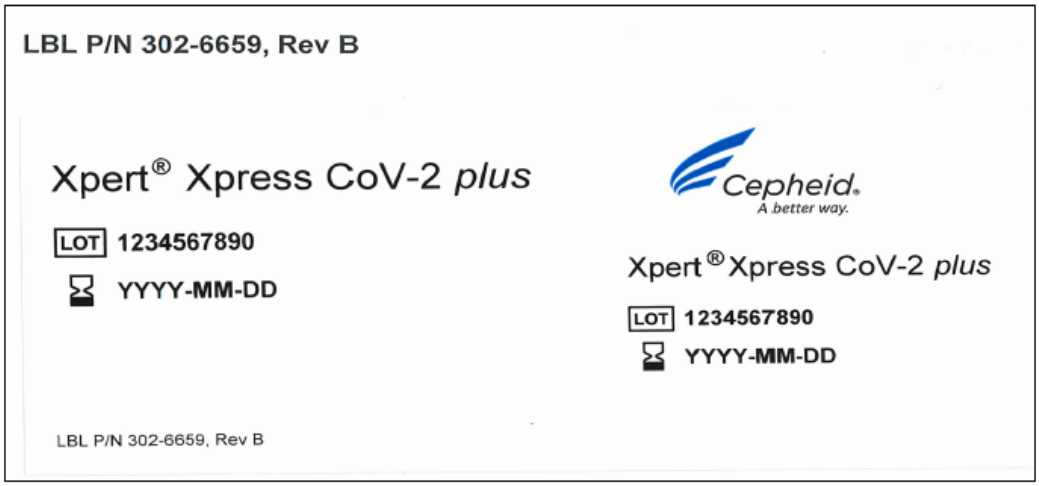


Solna 10-test kit label

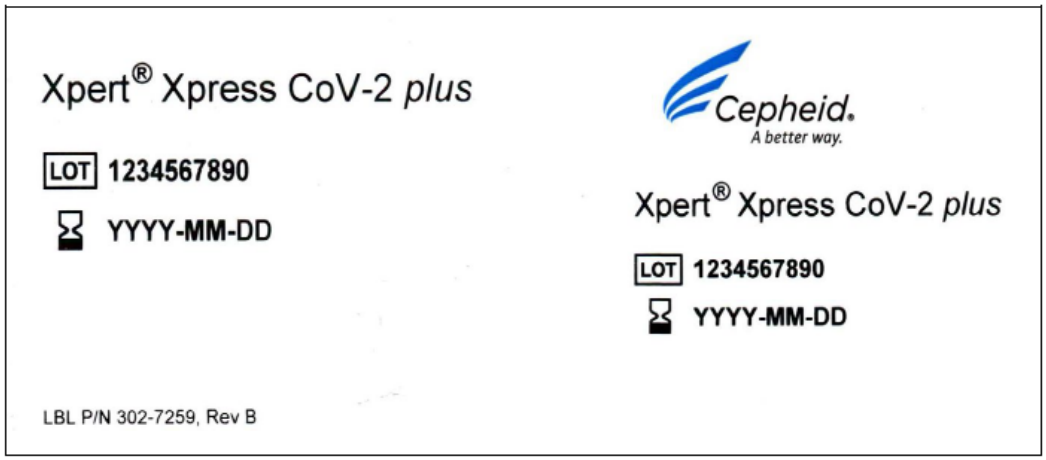


Lodi 10-test kit Label

1.2 Xpert Xpress CoV-2 *plus* kit carton, side panel



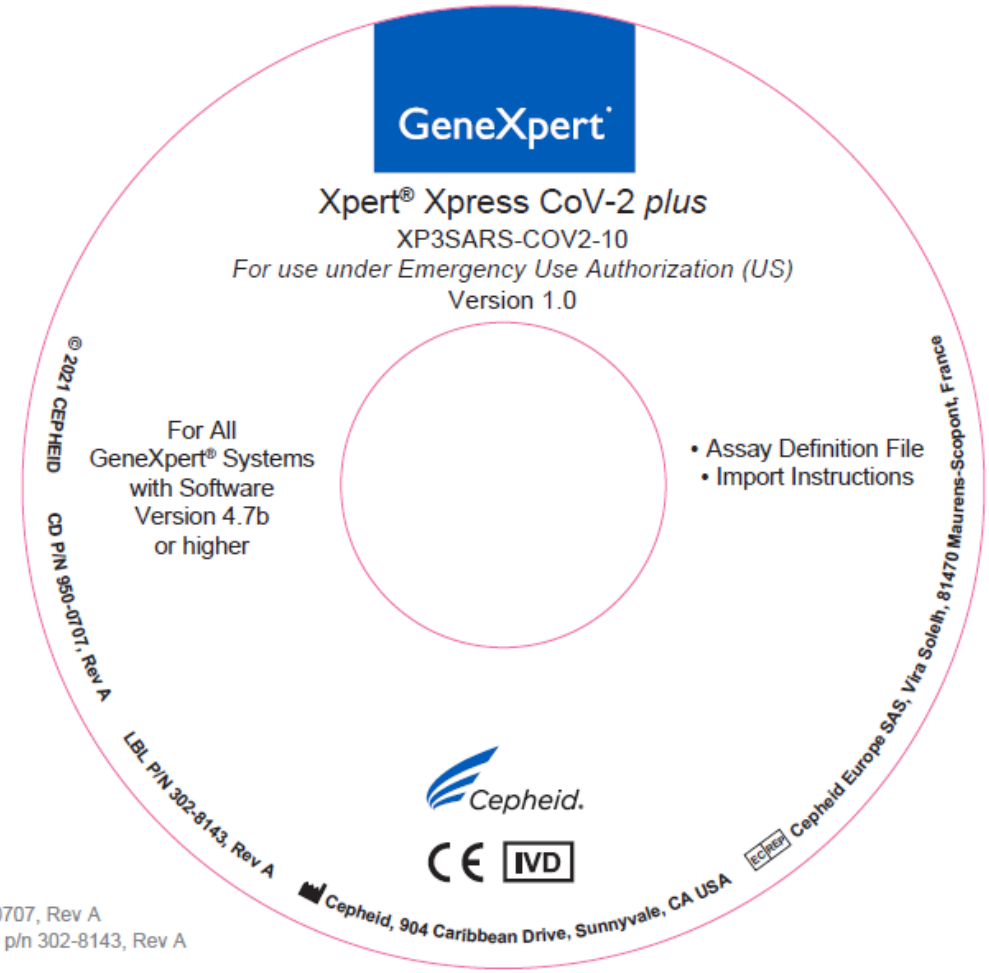
Sunnyvale and Solna



Lodi

1.3 Cartridge Label

1.4 CD label



CD Label
CD p/n 950-0707, Rev A
Imprint Label p/n 302-8143, Rev A

2.0 Instructions for use[2]

[2] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Xpress CoV-2 *plus*

REF **XP3SARS-COV2-10**

Instructions for Use

For Use with GeneXpert® Dx System or GeneXpert Infinity System

CE IVD

Cepheid

In Vitro Diagnostic Medical Device

302-7342, Rev. D
May 2023

Trademark, Patents, and Copyright Statements

See Revision History for a description of changes.

Xpert® Xpress CoV-2 *plus*

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 is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swab or anterior nasal swab specimen obtained from individuals meeting COVID-19 clinical and/or epidemiological criteria, as well as individuals without symptoms or other reasons to suspect COVID-19 infection. Results are for the identification of SARS-CoV-2 RNA.

Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2 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 epidemiological information.

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

4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections that have spread globally, resulting in a pandemic of coronavirus disease 2019 (COVID-19). Cases of severe illness and some deaths have been reported. The International Committee on Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2] 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 Xpert Xpress CoV-2 *plus* is a molecular in vitro diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and is based on widely used nucleic acid amplification technology. 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 specimens and/or anterior nasal swab specimens.

The term “qualified laboratories” refers to laboratories in which all users, analysts, and any person reporting results from use of this device are proficient in performing real-time RT-PCR assays.

5 Principle of the Procedure

The Xpert Xpress CoV-2 *plus* test is an automated *in vitro* diagnostic test for qualitative detection of SARS-CoV-2 viral RNA. 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 unique 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 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 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 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 viral transport tube containing 3 mL viral transport medium, 3 mL saline 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 System platform, 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	**10–12 per kit**
Flyer	**1 per kit**

- Instructions to locate 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 *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

- Nylon flocked swab (Copan P/N 502CS01, 503CS01) or equivalent
- 3 mL Viral transport medium
- 0.85–0.9% (w/v) saline, 3 mL
- Sample Collection Kit for Viruses (Cepheid P/N SWAB/B-100, SWAB/F-100) (Copan P/N 305C, 346C) or equivalent
- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, and 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

- SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Control, Catalog# NATSARS(COV2)-ERC
- SARS Associated Coronavirus 2 (SARS-CoV-2) Negative Control, Catalog# NATSARS(COV2)-NEG

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

- eNAT Molecular Collection and Preservation Medium, 2mL medium in tube + Copan Minitip FLOQSwab in peel pouch Copan Catalog # 6U074S01
- eNAT Molecular Collection and Preservation Medium, 2mL medium in tube + Copan Regular FLOQSwab in peel pouch Copan Catalog # 6U073S01

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- Positive results are indicative of presence of SARS-CoV-2 RNA.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[4] and the Clinical and Laboratory Standards Institute.[5]
- 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. 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.

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 expiry 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 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[6,7]

- Signal Word: WARNING
- **UN GHS Hazard Statements:**
  - Harmful if swallowed.
  - May be harmful in contact with skin.
  - Causes eye irritation.
- **UN GHS Hazard 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 swab and anterior nasal swab can be stored at room temperature (15–30 °C) for up to 48 hours in viral transport medium, saline, 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) up to seven days in viral transport medium, saline, or eNAT medium until testing is performed on the GeneXpert Instrument Systems.

Nasopharyngeal and anterior nasal swab samples collected into saline and eNAT should not be frozen. Refer to the WHO Laboratory Biosafety Guidance Related to the 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).

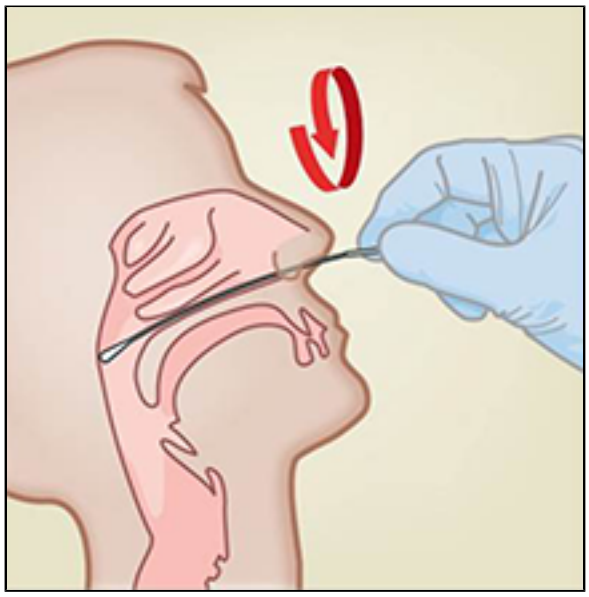


Figure 1. Nasopharyngeal Swab Collection

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 saline or 2 mL eNAT.
4. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.2 Anterior 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).

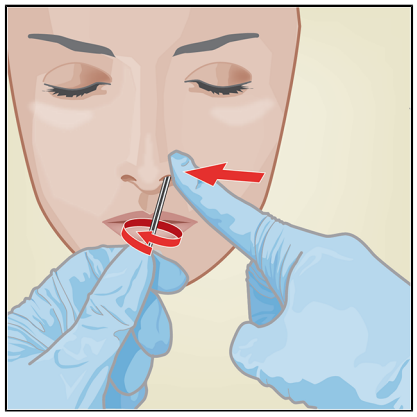


Figure 2. Anterior Nasal Swab Collection for First Nostril

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. Anterior Nasal Swab Collection for Second Nostril

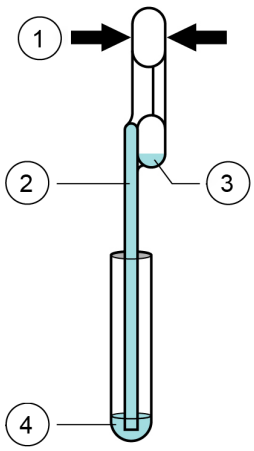
3. Remove and place the swab into the tube containing 3 mL of viral transport medium, 3 mL saline or 2mL eNAT. Break swab at the indicated break line and cap the specimen collection tube tightly.

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 4).



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

Figure 4. 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 will be seen in the overflow reservoir bulb of the pipette (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 transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) of the cartridge shown in Figure 5. Dispose of the used pipette.

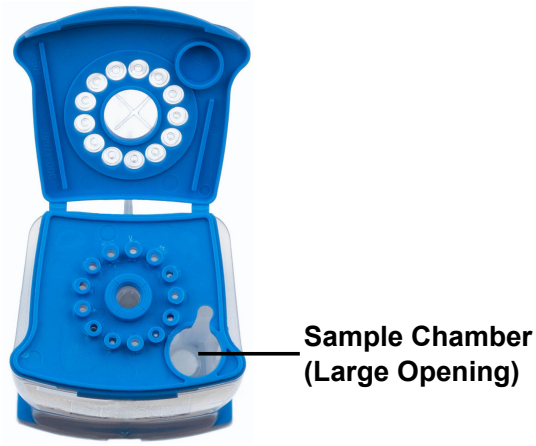


Figure 5. Xpert Xpress CoV-2 *plus* Cartridge (Top View)

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, perform the following steps:

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 5.
5. Close cartridge lid.

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

Important

Before you start the test, make sure that:

- The system is running the correct 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.

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

Important

Before you start the test, make sure that:

- The system is running the correct 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.
    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 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 may be used in accordance with local, state and federal accrediting organizations as applicable.

Cepheid recommends that all laboratories perform external QC with each new lot and shipment of reagents, at a minimum, while running the Xpert Xpress CoV-2 *plus* test.

If the expected results for the external control materials are not obtained, repeat the external controls, prior to releasing patient results. If the expected results for the external control material are not obtained upon repeat, contact Cepheid Technical Support.

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	+	+	+	+/-
SARS-CoV-2 POSITIVE	+	+/-	+/-	+/-
SARS-CoV-2 POSITIVE	+/-	+	+/-	+/-
SARS-CoV-2 POSITIVE	+/-	+/-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	-	+
INVALID	-	-	-	-

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. ● One or more SARS-CoV-2 nucleic acid targets (N2, E, or RdRP) has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because coronavirus target amplification might have 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 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 nucleic acid targets do not have a Ct within valid 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. 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.

Result	Interpretation
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. • 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 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 the full 45 PCR cycles have been completed. When SARS CoV-2 titers are high enough to initiate the EAT function, the SPC and/or additional target amplification curve 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

- Performance of the Xpert Xpress CoV-2 *plus* has only been established in nasopharyngeal swab and anterior nasal swab specimens. Specimen types other than nasopharyngeal swab and anterior nasal swab have 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 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.

- The performance of this device has not been assessed in a population vaccinated against COVID-19 or treated with COVID 19 therapies.
- Negative results do not preclude SARS-CoV-2 and should not be used as the sole basis for treatment or other patient management decisions.
- False negative results may occur if virus is present at levels below the analytical limit of detection.
- 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.
- 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 virus.
- 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.
- 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 monitoring treatment of infection.
- This test has not been evaluated for screening of blood or blood products 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.
- 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 can lead to erroneous results.

19 Clinical Performance

19.1 Clinical Evaluation–Performance of Xpert Xpress CoV-2 *plus* Test on NPS and NS Specimens

The performance of the Xpert Xpress CoV-2 *plus* test was evaluated using archived clinical nasopharyngeal (NP) swab and anterior 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 164 NP swab and 111 NS specimens were tested with Xpert Xpress CoV-2 *plus* side by side with a CE-marked SARS-CoV-2 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 *plus* test relative to the results of a SARS-CoV-2 CE-marked RT-PCR test for the SARS-CoV-2 target.

For the NPS specimens, Xpert Xpress CoV-2 *plus* demonstrated a PPA and NPA of 100.0% and 96.5% for SARS-CoV-2, respectively (Section 19.1). The initial non-determinate rate for the Xpert Xpress CoV-2 *plus* test was 1.8% (3/164). On repeat testing, all three (3) specimens yielded valid results. The final non-determinate rate for the Xpert Xpress CoV-2 *plus* test was 0% (0/164).

Table 3. Xpert Xpress CoV-2 *plus* Performance Results Using NPS Specimens

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	164	79	3	82	0	100.0% (95.4% - 100.0%)	96.5% (90.1% - 98.8%)

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

For the NS specimens, Xpert Xpress CoV-2 *plus* demonstrated a PPA and NPA of 100.0% and 100.0% for SARS-CoV-2, respectively (Table 4). The initial non-determinate rate for the Xpert Xpress CoV-2 plus test with NS specimens was 2.7% (3/111). On repeat testing, all three (3) specimens yielded valid results. The final non-determinate rate for the Xpert Xpress CoV-2 *plus* test was 0% (0/111).

Table 4. Xpert Xpress CoV-2 *plus* Performance Results Using NS Specimens

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	111	46	0	65	0	100.0% (92.3% - 100.0%)	100.0% (94.4% - 100.0%)

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

Performance in Specimens with N2 Mutations

Table 5 shows the analysis comparing the results of the Xpert Xpress CoV-2 *plus* test relative to the results of the Xpert Xpress SARS-CoV-2 test for the specimens with N2 mutations.

Table 5. Xpert Xpress CoV-2 *plus* Test Performance Results on Specimens with N2 Mutations

Specimen	Mutation	Xpert Xpress SARS-CoV-2			Xpert Xpress CoV-2 *plus*			
		Test Result	E	N2	Test Result	E	N2	RdRP
1	C29200T	SARS-CoV-2 Presumptive Positive[a]	+	-	SARS-CoV-2 Positive	+	+	+
2	C29200T	SARS-CoV-2 Presumptive Positive[a]	+	-	SARS-CoV-2 Positive	+	+	+
3	C29200T	SARS-CoV-2 Presumptive Positive[a]	+	-	SARS-CoV-2 Positive	+	+	+
4	C29200T	SARS-CoV-2 Positive	+	+	SARS-CoV-2 Positive	+	+	+
5	C29197T	SARS-CoV-2 Presumptive Positive[a]	+	-	SARS-CoV-2 Positive	+	+	+
6	C29197T	SARS-CoV-2 Presumptive Positive[a]	+	-	SARS-CoV-2 Positive	+	+	+

[a] Presumptive positive with the Xpert Xpress SARS-CoV-2 test is included as positive in the final data analysis.

The six (6) SARS-CoV-2 specimens with an N2 mutation yielded SARS- CoV-2 positive results with Xpert Xpress CoV-2 *plus* test. When tested using the Xpert Xpress SARS-CoV-2 test (comparator), one (1) specimen yielded positive and five (5) yielded presumptive positive test results. The presumptive positive test results on the Xpert Xpress SARS-CoV-2 test were considered positive for analyses.

19.2 Clinical Evaluation– Performance of Xpert Xpress CoV-2 *plus* Test on Asymptomatic Screening Specimens

A total of 125 archived frozen de-identified clinical NS specimens from asymptomatic screening individuals. These specimens were selected consecutively by date and previously known analyte result. The specimens from the asymptomatic screening individuals were tested were tested with Xpert Xpress CoV-2 *plus* side by side with a CE-marked SARS-CoV-2 RT-PCR test in a randomized and blinded fashion. The Xpert Xpress CoV-2 *plus* demonstrated a PPA and NPA of 100.0% and 99.0% for SARS-CoV-2, respectively (Table 6). The non-determinate rate for the Xpert Xpress CoV-2 *plus* test was 0% (0/125).

Table 6. Xpert Xpress CoV-2 *plus* Performance Results Using NS Specimens from Asymptomatic Screening Individuals

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	125	20	1	104	0	100.0% (83.9% - 100.0%)	99.0% (94.8% - 99.8%)

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

20 Analytical Performance

20.1 Analytical Sensitivity (Limit of Detection) for Nasopharyngeal Swab

The analytical sensitivity of the Xpert Xpress CoV-2 *plus* test was first estimated using two reagent lots by testing limiting dilutions of one strain of NATtrol SARS-CoV-2 virus diluted into pooled negative clinical NPS matrix, 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 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 for two clinical NPS matrices (UTM/VTM, eNAT). The concentration level with observed hit rates greater than or equal to 95% in the estimated LoD determination study were 200 and 70 copies/mL for the RdRP target and E target, respectively. The verified SARS-CoV-2 virus LoD for respective clinical NPS matrices are summarized in Table 7

Table 7. Xpert Xpress CoV-2 *plus* Limit of Detection (Nasopharyngeal Swab)

Virus/Strain	NPS Matrix	N2 LoD Concentration
SARS-CoV-2 (USA-WA1/2020)	UTM/VTM	403 copies/mL
	eNAT	
	Saline	

20.2 Analytical Reactivity (Inclusivity)

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 8 summarizes the effective predicted inclusivity for E, N2 and RdRP amplicons for the variants of interests and concern.

Table 8. 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 9 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 9. 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.

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. A total of 25 strains comprised of 5 SARS-CoV-2 virus strains and 20 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 10.

Table 10. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2 *plus* Test

SARS-CoV-2 Strain	Tested Titer	Number of Positive Results Obtained out of the Total Number of Replicates Tested			
		SARS-CoV-2	E	N2	RdRP
2019-nCoV/Italy-INMI1[a]	5 $TCID_{50}$/mL	POS	3/3	3/3	3/3
England/204820464/2020[ab]	0.5 $TCID_{50}$/mL	POS	3/3	3/3	3/3
Hong Kong/VM20001061/2020[a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
South Africa/KRISP-K005325/2020[a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
USA/CA_CDC_5574/2020[a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
Australia/VIC01/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
Wuhan-Hu-1[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
Japan/Hu_DP_Kng_19-020/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
USA/TX1/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
USA/MN2-MDH2/2020[c]	1.2e3 copies/mL	POS	3/3	3/3	3/3
USA/CA9/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
France/HF2393/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
Taiwan/NTU02/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
USA/WA2/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
USA/CA-PC101P/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
Iceland/5/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
England/SHEF-C05B2/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
Belgium/ULG/10004/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
England/205041766/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
England/MILK-9E05B3/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
South Africa/KRISP-EC-K005299/2020[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
Japan/IC-0564/2021[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
India/CT-ILSGS00361/2021[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3
India/MH-NCCS-P1162000182735/2021 [c]	1.2e3 copies/ml	POS	3/3	3/3	3/3

SARS-CoV-2 Strain	Tested Titer	Number of Positive Results Obtained out of the Total Number of Replicates Tested			
		SARS-CoV-2	E	N2	RdRP
India/MH-SEQ-221_S66_R1_001/2021[c]	1.2e3 copies/ml	POS	3/3	3/3	3/3

[a] Heat-inactivated viral culture fluid

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

[c] *In vitro* RNA transcripts

20.3 Analytical Specificity (Exclusivity)

The analytical specificity/cross-reactivity of the Xpert Xpress CoV-2 *plus* 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 GISAID database. The E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. Other than that no potential unintended cross reactivity with other organisms listed in Table 11 is expected based on the *in silico* analysis.

Table 11. 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
Batcoronavirus	Influenza B
	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

Microorganisms from the Same Genetic Family	High Priority Organisms
	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
	Staphylococcus epidermidis
	Streptococcus salivarius

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 55 microorganisms comprising 4 human coronaviruses, 1 MERS coronavirus, 1 SARS coronavirus, 19 other respiratory viruses, 26 respiratory bacteria, 2 yeast strains, 1 fungal strain, and 1 human nasal wash fluid representing a diverse microbial flora in the human respiratory tract. 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 1.1×10^6 IFU/mL and *Lactobacillus reuteri* which was tested at 1.1×10^6 copies/mL of genomic DNA. Viruses were tested at concentrations of $\geq 1 \times 10^5$ $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 12.

Table 12. Analytical Specificity (Exclusivity) of the Xpert Xpress CoV-2 *plus* Test

Viruses from the Same Genetic Family	Test Group	Tested Concentration	Number of Positive Results Obtained out of the Total Number of Replicates Tested			
			SARS-CoV-2	E	N2	RdRP
Human coronavirus, 229E	1	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	2	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Human coronavirus, HKU1[a]	3	1.1e6 genome copies/mL	NEG	0/3	0/3	0/3
SARS-coronavirus, Urbani[a]	4	1.1e6 genome copies/mL	POS	3/3	0/3	0/3
Influenza A H1N1 (pdm2009), Michigan/272/2017	5	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3

Viruses from the Same Genetic Family	Test Group	Tested Concentration	Number of Positive Results Obtained out of the Total Number of Replicates Tested			
			SARS-CoV-2	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 $TCID_{50}$/mL				
Adenovirus Type 1		1.1e5 $TCID_{50}$/mL				
Adenovirus Type 7A	6	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Cytomegalovirus		1.1e5 $TCID_{50}$/mL				
Echovirus		1.1e5 $TCID_{50}$/mL				
Enterovirus, D68 strain US/KY/14-18953		1.1e5 $TCID_{50}$/mL				
Epstein Barr Virus (Human Herpes Virus 4 [Hhv-4])		1.1e5 $TCID_{50}$/mL				
Herpes Simplex Virus (HSV) type 1	7	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
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		1.1e5 $TCID_{50}$/mL				
Human parainfluenza Type 2		1.1e5 $TCID_{50}$/mL				
Human parainfluenza Type 3	8	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Human parainfluenza Type 4		1.1e5 $TCID_{50}$/mL				
Rhinovirus, Type 1A		1.1e5 $TCID_{50}$/mL				
Acinetobacter baumannii		1.1e6 CFU/mL				
Burkholderia cepacia		1.1e6 CFU/mL				
Candida albicans		1.1e6 CFU/mL				
Candida parapsilosis	9	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Bordetella pertussis		1.1e6 CFU/mL				
Chlamydia pneumoniae		1.1e6 IFU/mL				
Citrobacter freundii		1.1e6 CFU/mL				
Corynebacterium xerosis		1.1e6 CFU/mL				
Escherichia coli		1.1e6 CFU/mL				
Enterococcus faecalis		1.1e6 CFU/mL				
Hemophilus influenzae	10	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Legionella spp.		1.1e6 CFU/mL				
Moraxella catarrhalis		1.1e6 CFU/mL				

Viruses from the Same Genetic Family	Test Group	Tested Concentration	Number of Positive Results Obtained out of the Total Number of Replicates Tested			
			SARS-CoV-2	E	N2	RdRP
Mycobacterium tuberculosis (avirulent)	11	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Mycoplasma pneumoniae		1.1e6 CFU/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	12	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[b]	13	1.1e6 genome copies/mL	NEG	0/3	0/3	0/3
Neisseria meningitides[b]		1.1e6 genome copies/mL				
Pooled human nasal wash	14	n/a	NEG	0/3	0/3	0/3
Influenza C	15	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3

[a] RNA specimens were tested in Tris-EDTA+ ($(NH_4)_2$)(SO_4) buffer in ADF without sample preparation.

[b] DNA specimens were tested in simulated NPS/NS background matrix using the full sample preparation ADF.

20.4 Microbial Interference

Microbial interference of the Xpert Xpress CoV-2 *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 virus seeded at 3x the Limit of Detection (LoD) into simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix in the presence of Adenovirus Type 1C, Human Coronavirus OC43, Rhinovirus Type 1A, Human metapneumovirus, Human parainfluenza Types 1, 2, and 3 (each seeded at $1x10^5$ units/mL), *Hemophilus influenzae*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (each seeded at $1x10^7$ CFU/mL).

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 commensal viral or bacterial strains was reported.

20.5 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*.

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 SARS-CoV-2 virus spiked at 3x the LoD. The controls were samples with SARS-CoV-2 virus 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 13.

Table 13. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Substance/Active Ingredient
No substance	Control	Copan Universal Transport Medium (UTM)
Afrin	Nasal Spray	Oxymetazoline,0.05%
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/mL)
BD Universal Transport Medium	Transport Media	BD Universal Transport Medium
Blood	Blood	Blood (Human)
Copan 3U045N.PH (Cepheid Swab/M)	Transport Media	Copan 3U045N.PH (Cepheid Swab/M)
FluMist	FluMist®	Live intranasal vaccine
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate
Ibuprofen	Analgesic (nonsteroidal anti-inflammatory drug (NSAID))	Ibuprofen
Menthol	Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol
Mucin	Mucin	Purified Mucin protein (Bovine orporcine submaxillary gland)
Mucin	Mucin	Purified Mucin protein (Bovine submaxillary gland, type I-S)
Mupirocin	Antibiotic, nasal ointment	Mupirocin (20 mg/g=2%)
Human peripheral blood mononuclear cells (PBMC)	Human peripheral blood mononuclear cells (PBMC)	Human peripheral blood mononuclear cells (PBMC)
PHNY	Nasal Drops	Phenylephrine, 1%
Remel M4RT	Transport Media	Remel M4RT
Remel M5	Transport Media	Remel M5
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)
Snuff	Tobacco	Nicotine
Tamiflu	Anti-viral drugs	Zanamivir
Tobramycin	Antibacterial, systemic	Tobramycin
Zicam	Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur (0.05%)
Zinc	Zinc supplement	Zinc Gluconate

The results from the study (Table 14) show that for most cases, 8 out of 8 replicates reported positive results for each combination of SARS-CoV-2 virus and substance tested and no interference was observed. When Fluticasone Propionate nasal spray was tested at 5 µg/mL, one of 8 replicates reported **INVALID**.

Table 14. SARS-CoV-2 Virus Tested in the Presence of Potentially Interfering Substances

Substance	Concentration Tested	Number of Correct Results/Number Tested			
		SARS-CoV-2 (USA/WA/1/2020)	E	N2	RdRP
Control Simulated NPS/ NS Matrix (No substance)	100% (v/v)	8/8	8/8	8/8	8/8
Afrin	15% (v/v)	8/8	8/8	8/8	8/8
Albuterol Sulfate	0.83 mg/mL	8/8	8/8	8/8	8/8
BD Universal Transport Medium	N/A	8/8	8/8	8/8	8/8
Blood	2% (v/v)	8/8	8/8	8/8	8/8
Copan 3U045N.PH (Cepheid Swab/M)	N/A	8/8	8/8	8/8	8/8
FluMist	6.7% (v/v)	8/8	8/8	8/8	8/8
Fluticasone Propionate Nasal Spray	5 µg/mL	7/8[a]	7/8[a]	7/8[a]	7/8[a]
	2.5 µg/mL	8/8[b]	8/8[b]	8/8[b]	8/8[b]
Ibuprofen	21.9 mg/dL	8/8	8/8	8/8	8/8
Menthol	1.7 mg/mL	8/8	8/8	8/8	8/8
Mucin	0.1% (w/v)	8/8	8/8	8/8	8/8
Mucin	2.5 mg/mL	8/8	8/8	8/8	8/8
Mupirocin	10 mg/mL	8/8	8/8	8/8	8/8
Human peripheral blood mononuclear cells (PBMC)	$1x10^3$ cells/µL	8/8	8/8	8/8	8/8
PHNY	15% (v/v)	8/8	8/8	8/8	8/8
Remel M4RT	N/A	8/8	8/8	8/8	8/8
Remel M5	N/A	8/8	8/8	8/8	8/8
Saline	15% (v/v)	8/8	8/8	8/8	8/8
Snuff	1% (w/v)	8/8	8/8	8/8	8/8
Tamiflu	7.5 mg/mL	8/8	8/8	8/8	8/8
Tobramycin	4 µg/mL	8/8	8/8	8/8	8/8
Zicam	15% (w/v)	8/8	8/8	8/8	8/8
Zinc	0.1 µg/mL	8/8	8/8	8/8	8/8

[a] With 5 µg/mL of Fluticasone propionate nasal spray, one of 8 replicates reported **INVALID**. The target genes were assigned a Ct of 45 for statistical analysis. No clinically significant difference was observed between the control mean Ct for each target gene and the test mean Ct for each target gene.

[b] For the substance that reported **INVALID** (fluticasone propionate nasal spray), the concentration was decreased by half and no interference was observed.

20.6 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.

21 Reproducibility

The reproducibility of the Xpert Xpress CoV-2 *plus* test was established at three (3) sites 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 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 six (6) days, using three (3) lots of Xpert Xpress CoV-2 *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 15.

Table 15. Summary of the Reproducibility Results - % Agreement

Panel Member	Site 1			Site 2			Site 3			% Total Agreement and 95% CI by Panel Member
	Op1	Op2	Site	Op1	Op2	Site	Op1	Op2	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 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%]
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%]

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

22 References

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7. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

23 Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

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

European Headquarters

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300
Fax: + 33 563 825 301
www.cepheidinternational.com

24 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.

25 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Authorized Representative in the European Community
	CE marking – European Conformity
	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
	Authorized Representative in Switzerland
	Importer

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Phone: + 1 408 541 4191

Fax: + 1 408 541 4192

EC REP

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Phone:+ 33 563 825 300

Fax: + 33 563 825 301

CH REP

Cepheid Switzerland GmbH
Zürcherstrasse 66
Postfach 124, Thalwil
CH-8800
Switzerland

Cepheid Switzerland GmbH
Zürcherstrasse 66
Postfach 124, Thalwil
CH-8800
Switzerland

IVD CE

26 Revision History

Description of Changes: 302-7342, Rev. C to Rev. D

Purpose: Updates to analytical performance data

Section	Description of Change
20.2	Updated *in silico* inclusivity with data from analysis as of June 30, 2022.
20.3	Updated Table 11 to include additional high priority microorganisms analyzed by *in silico* exclusivity analysis.