

SOP Code COVID # 006	Standard Operating Protocol for the Detection of SARS-CoV-2 using CBNAAT
Version-I – (10/2021)	

### 1. Scope & Application:

Xpert® Xpress SARS-CoV-2

### 2. Responsibility:

Lab Technician, Consultants

### 3. Purpose of Examination:

The Xpert Xpress SARS-CoV-2 test is a molecular in vitro diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

### 4. Principle & method of the procedure used for examination:

The Xpert Xpress SARS-CoV-2 test is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems. 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 SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate 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 nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube containing 3 mL transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred

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to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, Xpert Xpress SARS-CoV-2 5 automated sample processing, and real-time RT-PCR for detection of viral RNA.

**5. Equipment / Reagents Used for Examination:** Cepheid CB NAAT, 1X10 tests kit per box,

## REAGENTS

### Materials Provided

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

<b>Xpert Xpress SARS-CoV-2 Cartridges with Integrated Reaction Tubes</b>	<b>10</b>
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	<b>1 of each per cartridge</b>
• Lysis Reagent	<b>1.5 mL per cartridge</b>
• Binding Reagent	<b>1.5 mL per cartridge</b>
• Elution Reagent	<b>3.0 mL per cartridge</b>
<b>Disposable Transfer Pipettes</b>	<b>10-12 per kit</b>
<b>CD</b>	<b>1 per kit</b>
• Assay Definition File (ADF)	
• Instructions to import ADF into GeneXpert software	
<b>Flyer</b>	<b>1 per kit</b>
• Directions to locate the Product Insert on <a href="http://www.cepheid.com">www.cepheid.com</a>	

**Note** SafetyData Sheets (SDS) are available at [www.cepheid.com](http://www.cepheid.com) or [www.cepheidinternational.com](http://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.

### 5.1 Storage and Handling

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

### 5.2 Materials Required but Not Provided

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- GeneXpert Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual.

For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher

For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

## 6. Calibration Procedures (Metrological Traceability):

- Equipment calibration: Once a year

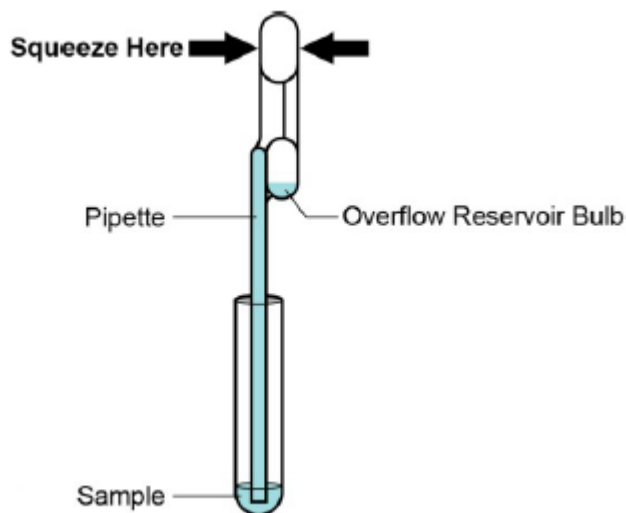
## 7. Procedural Steps:

### Preparing the Specimen

#### 7.1 Preparing the Cartridge

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

- Remove a cartridge from the package.
- Check the specimen transport tube is closed.
- Mix specimen by rapidly inverting the specimen transport tube 5 times. Open cap on the specimen transport tube.
- Open the cartridge lid.
- Remove the transfer pipette from the wrapper.
- Squeeze the top bulb of the transfer pipette completely and then place the pipette

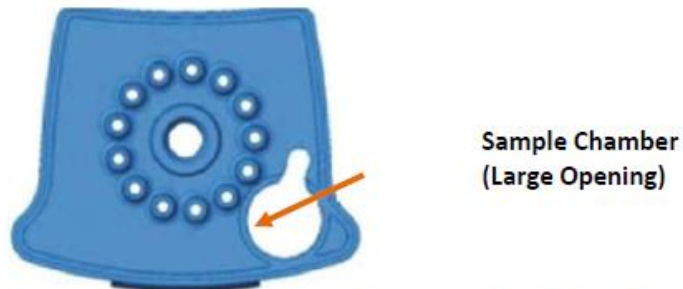


**Figure 5. Transfer Pipette**

- 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 5). Check that the pipette does not contain bubbles.

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- To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 6. Dispose of the used pipette.



**Figure 6. Xpert Xpress SARS-CoV-2 Cartridge (Top View)**

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

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- Close the cartridge lid.
- Load the cartridge into the GeneXpert Dx instrument or Infinity system.

## 7.2 Starting the Test

This section lists the basic steps for running the test.

- Turn on the GeneXpert instrument:
- Log on to the GeneXpert Instrument System software using your user name and password.
- In the GeneXpert System window, click **Create Test** (GeneXpert Dx).
- Type the Patient LRN, make sure the Patient LRN is typed correctly. The Patient LRN is associated with the test results and is shown in the View Results window.
- 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 is shown in the View Results window and all reports. The Scan Cartridge dialog box appears.
- Scan the barcode on the Xpert Xpress SARS-CoV-2 Assay cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
- Click **Start Test** (GeneXpert Dx). Enter your password, if requested.
- For the GeneXpert Dx Instrument:
  - Open the instrument module door with the blinking green light and load the cartridge.
  - Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.

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- Wait until the system releases the door lock before opening the module door and removing the cartridge.
- The used cartridges should be disposed in the appropriate specimen waste containers according to your institution’s standard practices.

## 8. Viewing and Printing Results

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

## 9. Quality Control Procedures:

### 9.1 Internal Controls

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

**9.2 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.

**9.3 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.

**9,5 External Controls** – External controls, not available in the kit, should be used in accordance with local, state, and federal accrediting organizations’ requirements as applicable.

## 10. Interpretation of Results

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**Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation**

<b>Result</b>	<b>Interpretation</b>
<b>SARS-CoV-2 POSITIVE</b>	<p>The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected.</p> <ul style="list-style-type: none"> <li>• The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting</li> <li>• SPC: NA; SPC is ignored because coronavirus target amplification occurred</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>
<b>SARS-CoV-2 PRESUMPTIVE POS</b>	<p>The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested according to the Retest Procedure in Section 17.2. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.</p> <ul style="list-style-type: none"> <li>• The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting</li> <li>• SPC: NA; SPC is ignored because a target amplification has occurred.</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>
<b>SARS-CoV-2 NEGATIVE</b>	<p>The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected.</p> <ul style="list-style-type: none"> <li>• The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting</li> <li>• SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>

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Result	Interpretation
<b>INVALID</b>	<p>SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2.</p> <ul style="list-style-type: none"> <li>• SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting</li> <li>• Probe Check – PASS; all probe check results pass</li> </ul>
<b>ERROR</b>	<p>Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2.</p> <ul style="list-style-type: none"> <li>• SARS-CoV-2: NO RESULT</li> <li>• SPC: NO RESULT</li> <li>• Probe Check: FAIL<sup>1</sup>; all or one of the probe check results fail</li> </ul> <p><sup>1</sup> If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.</p>
<b>NO RESULT</b>	<p>Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.</p> <ul style="list-style-type: none"> <li>• SARS-CoV-2: NO RESULT</li> <li>• SPC: NO RESULT</li> <li>• Probe Check: NA (not applicable)</li> </ul>

The Xpert Xpress SARS-CoV-2 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 amplification curve may not be seen and its results may not be reported.

## 11. Retests

### Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to the instructions in Section 14 (Table 2, Retest Procedure).

- An **INVALID** result indicates one or more of the following:
  - The sample was not properly processed or PCR was inhibited.
- An **ERROR** result indicates that the assay was aborted. Possible causes include: insufficient volume of sample was added, the reaction tube was filled improperly, a reagent probe integrity problem was detected, or the maximum pressure limit was exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, or a power failure occurred.

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## 12. Laboratory clinical Interpretation:

The performance of the Xpert Xpress SARS-CoV-2 test was evaluated using archived clinical nasopharyngeal (NP) swab specimens in viral transport medium. A total of 45 SARS-CoV-2 positive and 45 SARS-CoV-2 negative NP swab specimens were tested with Xpert Xpress SARS-CoV-2 in a randomized and blinded fashion.

All the 45 SARS-CoV-2 positive specimens and 30 of the 45 SARS-CoV-2 negative specimens were collected during COVID-19 pandemic in the US and had previously been characterized as positive or negative for SARS-CoV-2 by an EUA RT-PCR test. Fifteen of the 45 SARS-CoV-2 negative NP swab specimens were collected before December 2019 and are expected to be negative for SARS-CoV-2.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of the Xpert Xpress SARS-CoV-2 test relative to the expected results. Results of these 90 archived clinical NP swab specimens are shown in Table 3. The PPA

## 13. References:

1. Latest Kit Insert
2. Centers for Disease Control and Prevention. <https://www.cdc.gov/coronavirus/2019-ncov/index.html>. Accessed February 9, 2020.
3. bioRxiv. (<https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1>). Accessed March 3, 2020.
4. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical laboratories* (refer to latest edition). <http://www.cdc.gov/biosafety/publications/>
5. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline*. Document M29 (refer to latest edition).
6. 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).
7. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

### SOP CHANGE HISTORY:

New Version # (Date)	New Version # (Date)	No of Changes	Description of Changes	Source of Change Request

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