**PROCEDURE: Xpert® Xpress SARS-CoV-2**

1. **PRINCIPLE**

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 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 nasopharyngeal swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube medium. 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 SARS-CoV-2 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.

1. **AVAILABILITY**
	1. Daily:
		1. All shifts at RIH
		2. All shifts at TMH
		3. All shifts at NH
2. **TEST CODE**
	1. Soft – COV19
	2. Epic – COVID-19, PCR
3. **SPECIMEN**
	1. Nasopharyngeal specimen collected in 1ml Universal Transport Medium (UTM).
	2. The flocked swab must be in the UTM when received in the microbiology laboratory.
	3. Store specimen at 2-8°C once received in lab. The specimen is stable for up to 7 days when stored at 2-8°C.
	4. Specimens are only good for 24 hours when stored at 9-30°C.
4. **MATERIALS AND EQUIPMENT**
5. MATERIALS:
	1. Xpert**®** Xpress SARS-CO-V-2 assay kit - Enough reagents to process 10 specimens or quality control samples
		1. Xpert**®** Xpress SARS-Co-V-2 cartridges with integrated reaction tubes – 10
			1. Bead 1, Bead 2, and Bead 3 (freeze dried) 1 of each per cartridge
			2. Lysis Reagent 1.5ml per cartridge
			3. Binding Reagent 1.5 ml per cartridge
			4. Elution reagent 3.0ml per cartridge
		2. CD – 1 per kit
			1. Assay definition files (ADF)
			2. Instructions to import ADF into GeneXpert software
6. MATERIALS AVAILABLE BUT NOT PROVIDED
	1. Specimen collection kit: BD UTM 1 ml vial / flocked swab.
	2. External Controls
7. SeraCare AccuPlexTm  Reference Material Kit, catalog number 0505-0125 ( Order Code Cepheid)
	1. Materials to properly clean the hood at the beginning of each shift, between testing or any time it is needed. To be used in this order:
		1. 10% bleach
		2. DI H2O
		3. 70% Ethanol
8. EQUIPMENT
	1. GeneXpert Dx System (software version 4.7 or higher) or Infinity (software version 6.4 or higher)
	2. Barcode scanner
	3. Printer
9. **STORAGE AND HANDLING**
	1. Store the Xpert**®** Xpress SARS-Co-V-2 Assay cartridges and reagents at 2-28º C.
	2. Do not use UTM collection kits or cartridges that have passed the expiration date.
	3. Do not open a cartridge lid except when adding sample.
	4. Do not use a cartridge that has been shaken, dropped or damaged.
	5. Do not reuse spent cartridges.
	6. Do not use cartridges that appear wet or if the lid’s seal appears broken.
	7. Start the test within 30 minutes of adding the sample to the cartridge.
10. **QUALITY CONTROL**
	1. Maintenance
		1. Cleaning and maintenance of the instrument will be performed in accordance with the vendors Operator’s Manual. For further information, refer to the Dx or Infinity System’s Operator’s Manual.
	2. Each test includes two internal controls to validate the assay:
		1. Sample processing control (SPC) and probe check.
	3. Test samples are controlled according to the following procedures:
		1. **Sample processing control (SPC) -** The SPC ensures that that the sample was processed correctly and verifies that the sample 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.
		2. **Probe check –** Before the start of the PCR reaction, the GeneXpert system measures the fluorescence signal from the probes to monitor bead dehydration, reaction tube filling, probe integrity, and dye stability
	4. External quality control specimens are run on new shipments and/or every 30 days, whichever is more frequent. External controls are run after major system maintenance including software upgrade, annual PM, and if 3 or more modules are replaced at the same time. External controls are repeated if the controls are out of range or invalid. QC must be acceptable in order for the lot and instrument to be used for patient samples.
	5. Environmental “wipe” testing is performed monthly on the molecular hood in the Microbiology Lab. Any positive result will be brought up to a tech specialist. A thorough cleaning protocol using 10% bleach, deionized water, and 70% ethanol will be performed. Environmental wipe tests will be repeated. Environmental testing is acceptable when results for all targets are negative.
	6. Refer to IQCP for complete Quality Control procedure.
	7. Positivity Rate is monitored monthly.
11. **TEST PROCEDURE – For a Quick Reference Guide, Refer to: *Appendix 1***

*TEST WILL BE PERFORMED IN THE ASSIGNED MOLECULAR HOOD FOUND IN THE MICRO LAB*

**NOTE:** Be sure to load the cartridge into the GeneXpert Dx instrument and start the test within 30 minutes of adding the reagents.

1. Pre-analytical
	1. Clean designated molecular hood by spraying with 10% bleach; rinse with deionized water; clean with 70% ethanol. This cleaning procedure must be performed before and after each specimen.
	2. If multiple tests are ordered, split for molecular testing BEFORE it is processed for routine testing.
2. Preparing the Cartridge using NP flocked swab specimens – *Refer to Figure Below*
	1. Remove the cartridge from the package.
	2. Mix the specimen by INVERTING the UTM tube five times.

**NOTE**: DO NOT VORTEX

* 1. Open the cartridge lid. Using a clean 300uL transfer pipette (supplied with kit), transfer 300uL (one draw) of the suspension from the transport medium tube to the sample chamber with the large opening in the cartridge.
	2. Close the cartridge lid.



1. Starting the Test
	1. Turn on the GeneXpert Dx instrument and then turn on the computer.

# GeneXpert Dx:

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

# GeneXpert Infinity System:

If using the GeneXpert Infinity instrument, power up the instrument by turning the power switch clockwise to the **ON** position. On the Windows desktop, double-click the Xpertise Software shortcut icon to launch the software.

* 1. Log on to the GeneXpert computer. Select “Cepheid-Admin” as the user. The password is: **CPHD1.**
	2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.
	3. Log on to the GeneXpert Dx System software using your unique user name and password.
	4. In the GeneXpert Dx System window, click **Create Test**. The “Scan Patient Information" dialog box appears.
	5. Scan (or manually type in) the Patient label that is attached to the cartridge.
	6. The “Scan Cartridge Barcode” dialog box appears.
	7. Scan the bar code on the Xpert**®** Xpress SARS-Co-V-2cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for Reagent Lot ID, Cartridge Serial Number, and Expiration date.

**NOTE:** Do not change any of the preprogrammed information.

* 1. Ensure the correct sample ID has been loaded. The sample ID is associated with the test results and is shown in the View Results window and on all the reports. Please do not include patient’s name.
	2. Click **Start Test**. Enter your username and password if requested in the dialog box.

# For the GeneXpert Dx Instrument

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

# For the GeneXpert Infinity System

1. After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click **OK** to continue. The cartridge will be automatically loaded, the test will run and the used cartridge will be placed onto the waste shelf for disposal.
2. When all samples are loaded, click on the **End Order Test** icon.
	1. The test will be completed in approximately 45 minutes. When the test is finished, the green light above the module turns off. The module door will unlock and open slightly. Remove the cartridge and discard in the biohazard sharps bucket.
3. Printing and Viewing Results
	1. The report is set up to automatically print when complete.
	2. Click **View Results** to view the results.
	3. The **Report** button will allow you to view the results and/or generate a PDF report file to print if needed.
4. **Table 1. Xpert Xpress SARS-CoV-2 Possible Results**
5. **INTERPRETATION**
	1. The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets according to the algorithms shown in Table 1.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Result Text** | **N2** | **E** | **SPC** |
| **SARS-CoV-2 POSITIVE** | **+** | **+** | **+/-** |
| **SARS-CoV-2 POSITIVE** | **+** | **-** | **+/-** |
| **SARS-CoV-2 PRESUMPTIVE POSITIVE** | **-** | **+** | **+/-** |
| **SARS-CoV-2 NEGATIVE** | **-** | **-** | **+** |
| **INVALID** | **-** | **-** | **-** |

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

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

| **Result** | **Interpretation** |
| --- | --- |
| **SARS-CoV-2 POSITIVE** | The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected.* 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
* SPC: NA; SPC is ignored because coronavirus target amplification occurred
* Probe Check: PASS; all probe check results pass
 |
| **SARS-CoV-2 PRESUMPTIVE POSITIVE** | The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present.Sample should be retested. 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.* 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
* SPC: NA; SPC is ignored because a target amplification has occurred.
* Probe Check: PASS; all probe check results pass
 |
| **SARS-CoV-2 NEGATIVE** | The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. * 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
* 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 the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. * SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting
* Probe Check – PASS; all probe check results pass
 |
| **ERROR** | Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in IFU (Section 17.2).* SARS-CoV-2: NO RESULT
* SPC: NO RESULT
* Probe Check: FAIL1; all or one of the probe check results fail

1 If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure. |
| **NO RESULT** | Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in IFU (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)
 |

# Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test once:

1. 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.
2. 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.

# Retest Procedure

To retest a non-determinate result **(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 SARS-CoV-2 cartridge and a new transfer pipette.
		2. Check the specimen transport tube or external control tube is closed.
		3. Mix the sample by rapidly invert 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.
1. **REPORTING RESULTS**
	1. Refer to Critical Call Policy. If result is called use the canned comment @CALM.
		1. **SARS-CoV-2 NOT DETECTED**
		2. **SARS-CoV-2 DETECTED**
	2. **INVALID or PRESUMPTIVE POSITIVE –** Result test as INVALID and test **will not** be repeated. Specimen will be sent to RIDOH for further testing. Notify a nurse or clinician about the delay in resulting for any inpatient specimens.
		* 1. **Prepare specimen to be sent to RIDOH:**
				1. Add-on order **DHCOR** if not already ordered.
				2. Add 1mL UTM to specimen.
				3. Parafilm specimen and put specimen and appropriate forms in biohazard specimen bag.
				4. Send specimen on next courier run to RIDOH.
2. **LIMITATIONS**
	1. Performance of the Xpert Xpress SARS-CoV-2 has only been established in nasopharyngeal swab specimens. Specimen types other than nasopharyngeal swab may give inaccurate results.
	2. A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
	3. As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
	4. This test cannot rule out diseases caused by other bacterial or viral pathogens.
3. **TECHNICAL SUPPORT**
	1. Contact Cepheid Technical Support At 888-838-3222 techsupport@cepheid.com
		1. Before contacting, collect the following information:
			1. Product name
			2. Lot number
			3. Serial number on instrument
			4. Error messages (if any)
			5. Software version
		2. Restarting the System:
			1. Make sure instrument is not processing a sample.
			2. Remove all cartridges from the modules.
			3. Quit GeneXpert Software (USER menu, click EXIT)
			4. Turn off the computer
			5. Turn off instrument
			6. Wait a few minutes
			7. Turn on the instrument
			8. Turn on the computer
			9. Start the GeneXpert software
4. **REFERENCES**
	1. Centers for Disease Control and Prevention. <https://www.cdc.gov/coronavirus/2019-ncov/index.html>. Accessed February 9, 2020.
	2. bioRxiv. (<https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1>). Accessed March 3, 2020.
	3. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical laboratories* (refer to latest edition). <http://www.cdc.gov/biosafety/publications/>
	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)