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| GeneXpert Xpress SARS-CoV-2 Assay | | | | | |
| **Purpose** | This procedure provides instructions for performing the Xpert Xpress SARS-CoV-2 assay on the Cepheid GeneXpert system. | | | | |
| **Policy Statements** | This procedure applies to all technical staff performing testing on the GeneXpert. | | | | |
| **Principle and Clinical Significance** | The Xpert Xpress SARS-CoV-2 Assay is intended to aid in the diagnosis of novel Coronavirus-19 (COVID-19) virus in patients with signs and symptoms of respiratory infection. The results are intended to be used in conjunction with clinical and epidemiological risk factors.  In December, 2019 the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously temporarily named 2019 novel corona virus (2019-nCov) disease (COVID-19) caused a large global outbreak and is a major public health issue.  The RNA virus is spread by human-to human transmission via droplets or direct contact, and infection has been estimated to have a mean incubation period of 6.4 days and a basic reproduction number of 2.24 to 3.58. Transmission primarily occurs after days of illness and is associated with viral loads in the respiratory tract early in the illness, with viral loads peaking approximately 10 days after symptom onset.  Among patients with pneumonia caused by SARS-CoV-2 fever was the most common symptom followed by cough.1-4  The Xpert Xpress SARS-CoV-2 test is an automated in vitro diagnostic test for qualitative detection of nucleic acids from SARS-CoV-2. The test targets portions of the N2 and E genes.  The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process.  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 is collected and placed into a viral transport tube containing 3 mL transport medium. The specimen is briefly mixed by vortexing collection tube for 10 seconds. 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. | | | | |
| **Test Code** | **COVC** | | | | |
| **Sample** | 1. **Acceptable specimens:**  * Preferred: Nasopharyngeal (NP) swabs: Mini-tip flocked swabs placed in 3mL UTM or sterile saline (0.9% - 0.85%) * Alternative: Nasal swabs: regular flocked swabs placed in 3 mL UTM or sterile saline (0.9% - 0.85%)  1. **SDES codes/Specimen type:**  * **NP** – Nasopharyngeal Swab * **Nasal** – Nasal Swab  1. **Specimen Collection and Transport:**  * Refer to *Lab Test Directory* on StarNet  1. **Specimen assessment:**  * Refer to the policy MCVI 2.1 *Specimen Rejection Criteria*  1. **Specimen Storage**  * Specimens should be refrigerated (2–8 °C) up to seven days until testing is performed * Specimens can be stored at room temperature (15–30 °C) for up to 8 hours if required | | | | |
| **Special Safety Precautions** | **Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology* and *Virology Policy Manual*:**   1. ***Biohazard Containment*** 2. ***Safety in the Microbiology/Virology Laboratory***  * ***Biohazardous Spills*** | | | | |
| **Materials** | |  |  |  | | --- | --- | --- | | Reagents | Supplies | Equipment | | * 10% bleach * 70% ethanol | * Xpert Xpress SARS-CoV-2 Assay cartridges * Transfer pipettes * Sample racks * Cartridge transfer tray * Gloves   Store kits at 2-28°C. Kits are stable until the expiration date printed on the outer box. | * Biosafety Hood * Cepheid GeneXpert Instrument and computer * Printer | | | | | |
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| **Calibration** | Annual “Xpert Check Kit” calibration performed by Cepheid. | | | | |
| **Quality Control** | **Daily Quality Control:**  Once an Xpert cartridge has been loaded and before the sample processing steps begin, the software checks the optics, the readiness of the module’s mechanical components, and the ambient temperature of the module to assure proper performance of PCR, and the physical integrity of the cartridge.  Each test includes a Sample Processing Control (SPC) and a Probe Check Control (PCC).   * **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. * **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.   + The PCC passes if it meets the validated acceptance criteria. If any of the PCC criteria fail, the test results in an **ERROR**.   **External Quality Control:**   * Perform QC using external positive and negative controls every 30 days and with new lot/shipments. * Record results in the SARS-CoV-2 assay QC binder. * See Quality Control Procedure.   **New Lot/Shipment Quality control:**   * Perform QC using external positive and negative controls with each new lot or shipment before putting into service. Record results in the SARS-CoV-2 assay QC binder. * See Quality Control Procedure.   **Wipe testing control:**   * Perform wipe testing every 30 days to monitor for contamination. Record results in the SARS CoV-2 assay QC binder. * See Quality Control Procedure.   **NOTE:** External quality control may be performed on an as needed basis if certain circumstances arise. Examples include:   * Drift in results (e.g., increasing/decreasing positivity rates) * Potential contamination (negative control) * After drastic system maintenance | | | | |
| **Procedure** | **Cartridge preparation:**   1. Clean hood with10% bleach (made daily) followed by 70% ethanol. 2. Change gloves. 3. Obtain an Assay cartridge, transfer pipette, and sample transport tube to be tested.   **NOTE:** Do not use cartridge if it appears wet or has leaked, the lid seal has been broken or the reaction tube has been damaged. Collect damaged cartridges so they can be credited back to our account.   1. Label the side of the cartridge with a bar-coded foot-label that contains container ID (CID). 2. Open the cartridge lid. 3. Mix the sample by vortexing for 10 seconds. 4. Open the sample transport tube and draw up specimen into the transfer pipette by completely squeezing the fill bulb (bulb closer to the shaft of the pipette) and releasing. Ensure there are no air bubbles. 5. Insert the pipette to the bottom of the sample chamber of cartridge (see **Figure 1**)and empty entire pipette’s contents.      1. Close the cartridge lid, and set onto the transfer tray. 2. Change gloves and proceed to prepare additional samples or start the test.   **NOTES:**  -Hood surfaces must be cleaned between samples with 10% bleach followed with 70% ethanol if there were any splashes, spills, or uncertainty of cleanliness.  -\*\*Start the test within 30 minutes of adding the sample to the cartridge  **Starting the test:**   1. Ensure clean gloves are on before stepping to the computer work space. 2. If instrument and computer are turned off: start up the instrument by flipping the power switch located in the back of the instrument. Turn on the computer next. 3. Log onto the appropriate Windows account:    1. User: Cepheid-Admin    2. Password: cphd 4. The GeneXpert software will launch automatically. If it doesn’t double-click the GeneXpert Dx software shortcut icon on the desktop. 5. Log onto the software using own unique username and password. 6. In the GeneXpert System window, click **Create Test.** 7. When **Scan** **Sample ID Barcode** box appears, scan or manually enter Container ID (CID) from cartridge. 8. When **Scan Cartridge Barcode** box appears, scan the barcode on the cartridge.   **NOTE:** if the barcode on the cartridge does not scan, then repeat the test with a new cartridge.   1. Verify appropriate assay (**SARS-CoV2**) is chosen under the **Select Assay** field. 2. Enter specimen source under the **Other Sample Type** field. 3. Enter additional information under the **Notes** field (day of QC, collect date, etc.) if needed. 4. Click **Start Test**. 5. Enter your username and password, if requested. 6. Open the instrument module door with the blinking green light.   **NOTE:** when setting up for testing you may opt to use any available module.   1. With the barcode facing towards you, set the cartridge into the module and close the door. 2. Wait for the test to start and the light to stop blinking. The test will run for approximately 45 minutes.   **NOTE:** early assay positive result call out may happen as early as 30 minutes into sample processing   1. Ensure printer is on. 2. Once testing is complete (the light will be off and the system will release the door lock), remove the used cartridge and dispose of into biohazard bag. Place biohazard bag into biohazard sharps bin. 3. Clean any equipment used (pipettes, racks, transfer tray, etc.), hood, and counters (including keyboard, scanner, and mouse) at the end of the day.   **NOTE:** Sample processing, testing, and cleaning should follow a unidirectional work-flow to avoid contamination. | | | | |
| **Interpretation/ Results** | 1. Reports will print automatically after testing has been completed.    1. If report doesn’t print, check that printer is on.    2. To reprint reports: Click **View Results** from the top menu bar of GeneXpert Dx software, select **Report** from the bottom menu bar, select report you want to print, click **Preview PDF**, and click printer icon to print. 2. Place large patient label on results.   **Result Interpretation:**   1. The results reported are interpreted automatically by the GeneXpert Instrument System. 2. The Xpert Xpress SARS-CoV-2 assay provides results based on the detection of two gene targets (N2 and E). 3. **Table 1** below lists all the possible test results for SARS-CoV-2.   **Table 1: Possible SARS-CoV-2 Results**     1. **Table 2** below lists possible results and interpretations.   **Table 2: SARS-CoV-2 Instrument Results and Interpretations**         1. Review reports for results of INVALID, ERROR, NO RESULT or SARS-CoV-2 PRESUMPTIVE POS and repeat testing if necessary.   **Reasons to retest the original sample:**   1. An **INVALID** result (SPC failure). This may indicate:    1. The sample was not properly processed.    2. PCR was inhibited.    3. The sample was not properly collected. 2. An **ERROR** result. This may indicate:    1. Probe Check Control failure.    2. System component failure.    3. The maximum pressure limit was exceeded. 3. A **NO RESULT**:    1. This result indicated that insufficient data were collected (e.g. cartridge failed integrity test, test stopped while in progress or power failure occurred). 4. A **SARS-CoV-2** **PRESUMPTIVE POSITIVE** result. This may indicate:    1. SARS-CoV-2 nucleic acids may be present (likely near the limit of detection).    2. Human or Bat SARS-coronavirus nucleic acids may be present.   **NOTE:** Record any failures, errors, and repeat testing in the “GeneXpert Maintenance and Problem Logs” binder.  **Retesting procedure:**   1. Call provider to notify if result reporting will exceed the 60 minute turnaround time.   **NOTE:** Document who you called and the date/time in the problem log and on the results sheet.   1. Obtain the original sample and a new cartridge. 2. Retest the sample according to the instructions in this SOP. 3. Report results according to **Table 3** below.   **Table 3: Retesting results and interpretation**   |  |  |  | | --- | --- | --- | | **Initial result** | **Repeat Result** | **Report** | | **INVALID** | INVALID | Unresolved | | VALID | Valid results | | **ERROR** | ERROR or INVALID | Unresolved | | VALID | Valid results | | **NO RESULT** | NO RESULT, ERROR or INVALID | N/A – repeat testing | | **Presumptive Positive** | Presumptive Positive | SARS-CoV-2 Presumptive Pos; notify provider and send original sample to MDH for further identification. | | Positive | SARS-CoV-2 Positive | | Negative | SARS-CoV-2 Negative | | | | | |
| **Result Reporting** |  | | | | |
|  | **NOTE:** Negative results **will** auto file, Positive, Presumptive positive, and Invalid (Unresolved) will **not** auto file   1. Log into Sunquest Laboratory to release results. 2. Select **Result Entry** from menu options. 3. In the Configuration field select CGX from the dropdown box. 4. Click on the **Result** button located in the lower right corner to populate the transmitted results. 5. Review messages located on the top and results. Compare results to the GeneXpert report. 6. Record provider notification if reporting:    1. Positive/ Presumptive Positive (See Critical Results section)    2. Unresolved Results (See Reporting Invalid (unresolved) Results) 7. Check the release box. 8. Click **Save** button located on the lower left corner. Click **Accept** when the “Verify Release Destination” window opens.   **NOTE:** All samples will automatically have the following result comment attached: SARSC “The Xpert Xpress SARS-CoV-2 Assay was issued an Emergency Use Authorization (EUA) by the FDA on March 20, 2020.”     1. At the end of the shift call a completed worksheet for COVC, check results, and staple to GeneXpert Report. Place in the GeneXpert SARS-CoV-2 result binder. 2. Store samples in fridge:    1. Put in rack according to **day of the week**    2. **Mark positive samples with X** on the cap, and write results on the tube. 3. **Write results on a large patient label** and place in the bin by printer. Aliquots of positive samples will be sent to MDH.   **NOTE:** positive samples are to be sent to MDH with the reportable disease paper work   1. Discard old samples after 7 days. | | | | |
| **Alert Values** | 1. **All** results **Positive** and **Presumptive Positive** for SARS-CoV-2 on **patients** are to be called be called to the patient’s care provider.   **NOTE:** Positive and Presumptive Positive results on employees do NOT need to be relayed   1. The code SURE (Semi-urgent result) will automatically append. Add the code CAL, press tab, enter semi-colon, and record who the result was called to and the time/date.   **NOTE:** document the call to the care provider | | | | |
| **Sending presumptive positive samples to MDH and Resulting reports** | 1. Results that give **SARS-CoV-2** **PRESUMPTIVE POSITIVE** twicewill be sent to MDH for further testing. MDH will send a final report back that needs to be resulted in computer. 2. Send a labeled Aliquot of the sample (~1.5 mL) and a clinical submission form requesting confirmation of COVID-19/2019-nCoV2 to MDH (save original patient printouts on shelf labeled “Pending MDH results” in rapid molecular). 3. When results are sent back: 4. Open SmarTerm and enter function “MEM”. 5. Return past worksheet (leave empty). 6. Under the “Test” prompt, enter test code COVC and accept. 7. Under the “Acc. No.” prompt, enter “M-accession number”. 8. Underneath result, add -SCAND and accept. 9. Make a copy of the MDH report to keep (attach to original patient printouts to be filed away) and put original in filing stack to be scanned into Cerner.   **NOTE:** St. Paul staff are to send samples to the Minneapolis Laboratory for form submission and packaging. | | | | |
| **Reporting Invalid (unresolved) Results** | If an INVALID/ERROR sample repeats:   1. The result will be reported as **unresolved** (UNRE) and the following code SIA will automatically append: “This sample is inhibitory to amplification and the results are inconclusive. Consider repeat collection if clinically indicated.” 2. Add the code CAL to one of the results, press tab, enter semi-colon record who the result was relayed to and the date/time. 3. Check the release box. 4. Click  button located on the lower left corner. Click  when the “Verify Release Destination” window opens. 5. Only release one of the two results. | | | | |
| **Correcting Results** | 1. Correcting incorrect results require notification to provider. 2. Open Result Entry, select the Manual resulting mode (top left corner), from the configuration drop down select the appropriate test. Click  in the lower right corner. 3. Enter the **Accession Number**, enter Tab and click Yes to modify the result. 4. Change the incorrect result. The corrected result comment will automatically append. Add the CAL comment, press tab, enter a semi-colon and record who was called and the time/date.      1. Click . Click  when the “Verify Release Destination” window opens. | | | | |
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| **Limitations** | * The Xpert Xpress SARS-CoV-2 Assay was issued an Emergency Use Authorization (EUA) by the FDA on March 20, 2020. * Performance of the Xpert Xpress SARS-CoV-2 has only been established in nasopharyngeal swab specimens. Use of the Xpert Xpress SARS-CoV-2 test with other specimen types has not been assessed and performance characteristics are unknown. * Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2 test but performance with these specimen types has not been established. * The effect of interfering substances has not been evaluated for this test. Because of assay similarities, refer to the Xpert Xpress Flu/RSV Assay package insert for a list substances tested. 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. * 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. * 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. * A presumptive positive result may indicate SARS-2-CoV-2 viral nucleic acids are present near the Limit of Detection or a cross-reaction with Human (SARS-CoV-1) or Bat SARS-coronavirus. * This test cannot rule out diseases caused by other bacterial or viral pathogens. | | | | |
| **Method Performance Specifications** | According to the manufacturer (per the package insert) – see **Table 4** Below.  **Table 4: Xpert SARS-CoV-2 Test Agreement with the Expected Results by Sample Concentration** | | | | |
| **References** | 1. Xpert Epress SARS-CoV-2 Package Insert, 3023562, Rev B, April 2020. In. Sunnyvale, CA: Cepheid. 2. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. 2020. 3. Xu X-W, Wu X-X, Jiang X-G, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. 2020;368. 4. Lai C-C, Shih T-P, Ko W-C, Tang H-J, Hsueh P-RJIjoaa. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. 2020:105924. | | | | |
| **Alternate Methods** | 1. Minnesota Department of Health: COVS 2. Mayo Clinic Laboratories: COVID 3. Quest Diagnostics: COVO | | | | |
| **Proficiency Testing** | CAP: COV2, 2 shipments with 3 samples | | | | |
| **Training Plan/ Competency Assessment** | **Training Plan** | | | **Initial Competency Assessment** | |
| 1. Employee must read the procedure. 2. Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer. | | | 1. Direct observation | |
| **Historical Record** |  |  |  | |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | | **Summary of Revisions** |
| 1 | Julie Laramie & Jamie Berg | 4/13/2020 | | Initial Version |
| 2 | Julie Laramie | 6/15/2020 | | -Added saline as transport media  -Changed QC to every 30 days and new lot/ship |
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| **Archived by:** |  | **Archived Date:** | |  |