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Xpert® Xpress SARS-CoV-2 plus Assay

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this procedure is to provide testing steps and criteria for the Xpert® Xpress SARS-CoV-2 *plus* Assay.

II. CLINICAL SIGNIFICANCE:

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 (www.who.int). Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently the United States. Cases of severe illness and some deaths have been reported. The International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.

III. PRINCIPLE:

The Cepheid Xpert Xpress SARS-CoV-2 *plus* Assay is an automated, multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) assay intended for the *in vitro* qualitative detection of nucleic acid from SARS-CoV-2 from nasopharyngeal swab specimens. Results are for the detection of SARS-CoV-2 RNA. 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-dependant RNA polymerase (RdRP) genes of the SARS CoV-2 virus genome.The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab specimens and/or nasal wash/ aspirate specimens during the acute phase of infection. Positive results are indicative of active or recent infection with SARS-CoV-2; 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.

IV. SPECIMEN COLLECTION AND HANDLING:

A. Specimen Collection
    1. Refer to the Laboratory Test Directory (LTD) for detailed specimen collection information

B. Specimen
    1. Nasopharyngeal (preferred) or nasal swab in viral transport media (i.e. M4-RT, M5, UVT, UTM).
        a. Use only swabs with a synthetic tip (e.g. Dacron, nylon, or rayon) and an aluminum or plastic shaft.
        b. Do not use calcium alginate swabs, as they may contain substances that inhibit PCR testing.

C. Specimen Stability
    1. Room temperature for 8 hours after collection.
    2. Refrigerated for 7 days (2-8°C) after collection.

D. Rejection Criteria
    1. Dry swabs
    2. Wooden swabs
    3. Specimens in non-sterile containers.
    4. Specimens subjected to repeated freeze thaw cycles.

V. REAGENTS AND SUPPLIES:

A. Cepheid Xpert Xpress SARS-CoV-2 *plus* Cartridges (2-28 ºC) containing the following components:
    1. Bead 1, 2 and 3 (freeze-dried)
    2. Lysis Reagent
    3. Binding Reagent
    4. Elution Reagent

B. Controls (2-8 ºC)
    1. External controls in the form of inactivated virus(es) are available from ZeptoMetrix (Buffalo, NY)

External Positive Control: Catalog #NATFRC-6C (NATtrol Flu/RSV/SARS-CoV-2)
External Negative Control: Catalog #NATCV9-6C (Coxsackievirus CVA9) Supplies

2. Alternative External Controls
    a. Previously tested patient samples
    b. CAP proficiency samples

C. Supplies
    1. Disposable 300mcL Transfer Pipette (Included in box with cartridges)
    2. Cepheid Xpert Xpress SARS-CoV-2 *plus* Assay CD (included in box with cartridges)
    3. 3mL UTM or UVT for collection of samples

VI. EQUIPMENT:

A. Cepheid GeneXpert or Infinity Instrument system
B. Bar code scanner
C. Printer
D. Vortex mixer
E. Refrigerator 2-8ºC
F. Biological Safety Cabinet (BSC) or protective barrier (ie. dead air box or face shield)

VII. QUALITY CONTROL

A. **Internal Controls -** Each test includes a Sample Processing Control (SPC) and a Probe Check Control (PCC).
    1. **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
    2. **Probe Check Control (PCC, QC1, QC2)** — 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.

B. **External Controls -** To run a control using the Xpert Xpress SARS-CoV-2 *plus* test, perform the following steps:
    1. Mix control by vortexing tube for 5 seconds. Open cap on external control tube.
    2. Open the cartridge lid.
    3. 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 1.

4. Close cartridge lid.
5. Run test following testing procedure as listed above.

VIII. PROCEDURE:

A. **Preparing the Cartridge**

1. Decontaminate work area with 10% bleach solution, followed by 70% ethanol solution, or approved laboratory disinfectant.
2. Obtain acceptable specimen (see above).
3. Remove a cartridge and pipet from the package.
4. Mix the sample by inverting 5 times or vortex for 5-10 seconds. Allow specimen to settle before opening container.
5. Open the cartridge lid. Using a clean 300mcL transfer pipette (supplied in kit), transfer 300mcL (one draw) of the specimen from the transport medium tube to the sample chamber with the large opening (figure 1)
6. Close the cartridge lid. The cartridge must be loaded onto the instrument within 30 minutes of adding the specimen to the cartridge.
7. Remove or change gloves and proceed to the instrument.

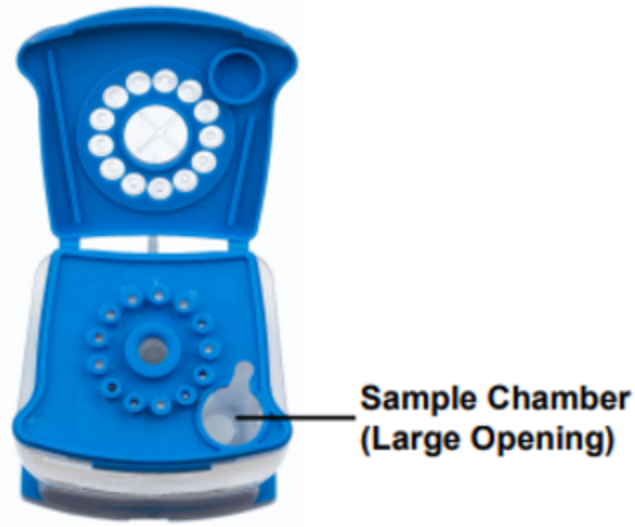

Figure 1. Cepheid Xpert Xpress SARS CoV2 *plus* Cartridge (Top View)

B. Before starting the test, check the current Cepheid Xpert Xpress SARS-CoV-2 *plus* Assay definition file (ADF) is imported into the software. If a new version is received inside (found inside the testing kit box), import the new ADF into the software.

C. **GeneXpert Infinity (Performed Only at Royal Oak And Dearborn Microbiology Laboratory)**

1. If not already turned on, turn on the Cepheid instrument:
    a. First turn on the instrument. Wait a full two minutes. The Windows software will launch automatically.

b. Log on to Windows using the user name and password.

c. Open the GeneXpert software by double-clicking the Xpertise software icon on the Windows® desktop.

2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (GeneXpert Infinity).The "Create Test" window opens.
4. Scan (or type in) the Sample ID (order number). If typing the Sample ID, review the Sample ID to ensure it is typed correctly. Scan (or type in) the Patient ID (MRN) (optional). The Sample ID is shown on the left side of the View Results window and is associated with the test results.
5. Press the "Continue" button.
6. Scan the Xpert Xpress SARS-CoV-2 *plus* Assay cartridge barcode. Using the barcode information, the software automatically fills in the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.
7. Review the patient information and order number (sample ID) and click "Submit Test"
8. Place the cartridge on the conveyor belt with the XPRESS cartridge barcode facing forward (reaction "tail" towards the rear of the instrument). The cartridge must be facing forward, otherwise it will not be able to enter the instrument and will cause a spill.
9. Observe the cartridge as it enters the instrument and is loaded by the automated gantry arm.
10. Repeat steps 4 to 9 above for consecutive specimens.
11. Once loading is complete, exit the Order Test window and return to the Home Screen.

D. **GeneXpert DX (Performed at Farmington Hills, Grosse Pointe, Trenton, Taylor, Troy, and Wayne Laboratories)**

1. If not already on, turn on the GeneXpert Instrument System.
    a. First turn on the instrument, and then turn on the computer
    b. Enter the password: (campus specific)
    c. Log on to the GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software icon on the Windows® desktop.
    d. Enter the user name: (campus specific)
    e. Enter the password: (campus specific)
2. Click on **Create Test** and follow the prompts
3. Scan the patient label barcode, if you are unable to scan, you may manually enter the order number.

4. Scan the Xpert Xpress SARS-CoV-2 *plus* cartridge barcode. Once the barcode is scanned the software automatically fills in the boxes for the following fields. If unable to scan, you may manually enter the number.
    a. Reagent Lot ID
    b. Cartridge SN
    c. Expiration Date.
5. Click on **Start Test**
6. Open the instrument module door with the blinking green light and load the cartridge.
7. When the green light turns to solid green, close the module door
8. When the test is finished, the instrument module light turns off.
9. Wait until the system releases the door lock before opening the module door and removing the cartridge.
10. Discard cartridge in biohazard container.

IX. INTERPRETATION:

Possible Test Results

Result Test	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	-	-	-	-

Instrument Interpretations

Result	Interpretation
SARS-CoV-2 POSITIVE	SARS CoV-2 target RNA is detected. • The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting for one or more nucleic acid targets (N2, E, RdRP). • SPC: NA; SPC is ignored because coronavirus target amplification occurred. Probe Check: PASS; all probe check results pass.
SARS-CoV-2	SARS CoV-2 target RNA is not detected.

NEGATIVE	• The SARS-CoV-2 signals for nucleic acid targets (N2, E, and RdRP) do not have a Ct within the valid range and endpoint above the minimum setting. • SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. Probe Check: PASS; all probe check results pass.
INVALID	SPC does not meet acceptance criteria. Presence or absence of SARS-CoV-2 nucleic acids cannot be determined. Repeat test according to the Retest Procedure. • 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 SARS-CoV-2 cannot be determined. Repeat test according to the Retest Procedure. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; 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 SARS-CoV-2 cannot be determined. Repeat test according to the Retest Procedure. 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. **Repeat Testing** - For retesting, use the original specimen and repeat test using a new cartridge. Repeat the test once if any of the test results below occur:

1. An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
2. 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.
3. 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.
4. If an External Control fails to perform as expected, repeat external control test and/

or contact Cepheid for assistance.

B. **Initial test run failure (i.e Error, No Result, Invalid, or power failure)**

1. Initial test result: Error, No Result, or Invalid
    a. No results will cross over into the laboratory information system (LIS)
    b. Repeat testing using new cartridge and original specimen
2. Upon Repeat testing: results are valid and acceptable for result reporting
    a. Result in the LIS
3. Upon Repeat testing: results remain Error, No Result, or Invalid
    a. Manually report all analytes as INVALID
    b. Result each test using the keypad
    c. FINAL status each test

X. REPORTING:

A. In the LIS, the follow result interpretations are available for reporting:

1. **Not Detected**
    a. For each analyte with a Negative result, the report will read: "Not Detected"
2. **DETECTED**
    a. For each analyte with a Positive result, report will read: "DETECTED"
    b. Significant (abnormal) flag will trigger automatically
3. **Invalid**
    a. Manually report all analytes as INVALID.
    b. When testing and retesting of patient specimen results in 'No Result' test results, report will read:
    c. "Inhibitory substances were present in this specimen and therefore results could not be obtained. Recollection is suggested if clinically indicated."
4. **Comments**
    a. Each order will also have a comment on report, noting the testing methodology: **"Test Methodology - Nucleic Acid Amplification.**

B. **Result Review – Download via Interface**

1. All results that are acceptable as outlined in above Retesting, Results, and Reporting sections will be auto-released.
2. Only the results that qualify for retesting as stated in the "Retesting" section must be

reviewed by the technologist prior to reporting.

C. **Result Review – Manual Entry** Please note: Most common occurrence for manual entry will be in situations in which the interface is not functional. A system for detecting clerical errors, significant analytical errors, and unusual laboratory results must be in place for all manual entries.

1. The technologist performing the test must review the results entered into the LIS workcard against the results printed on the instrument run log and the patient log sheet. Once verified, the technologist will save the result but will not status as final.
2. The preferred method is to have a separate Medical Technologist review all test results, one by one, that were saved by the preceding technologist. If a second technologist is not present the resulting technologist must go through the same review process themselves.
3. The technologist performing the review MUST access all of the following for accuracy:
    a. Instrument logs
    b. Result Review via LIS.
4. If any results are incorrectly transcribed to the patient logs from the instrument logs, or if incorrect results are entered into the LIS workcard:
    a. Correct the mistake, initial the correction and then status as preliminary or final as needed and release the report to chart.
5. If an error is discovered after this review:
    a. Immediately notify a manager . If unavailable, email all information to them.
    b. Correct the report. All corrected results must be called to floor or physician accordingly.
6. Manager/Director Review – All testing data will be reviewed periodically by the manager and/or Medical Director. It may be an indicator of: a) laboratory contamination b) deterioration of reagents, etc. (lower than normal percent positivity rate).

XI. LIMITATIONS:

A. Performance of the Xpert Xpress SARS-CoV-2 *plus* has only been established in nasopharyngeal swab specimens. Specimen types other than nasopharyngeal swab may give inaccurate results.

B. 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.

C. As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2 *plus* could affect primer and/or probe binding resulting in failure to detect the presence of virus.

D. This test cannot rule out diseases caused by other bacterial or viral pathogens.

XII. REFERENCES:

A. Cepheid, Xpert® Xpress SARS-CoV-2 *plus* product insert, Package Insert,302-7070, Rev. A June 2022.

B. World Health Organization website, www.who.int. Accessed 3/31/2020.

Approval Signatures

Step Description	Approver	Date
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	Ann Marie Blenc: System Med Dir, Hematopath	8/24/2022
	Vaishali Pansare: Chief, Pathology	8/23/2022
	Ryan Johnson: OUWB Clinical Faculty	8/22/2022
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Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	8/22/2022
	Daniel Ortiz: Technical Dir, Microbiology	8/22/2022
	Joyce Mitchell: Mgr Laboratory	8/11/2022
	Corey Webber: Mgr, Division Laboratory	8/10/2022
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