JULY 6, 2020

STANDARD OPERATING PROTOCOL FOR XPERT XPRESS SARS COV-2 TEST FOR DIAGNOSIS OF COVID-19, NEPAL

VERSION 1 – JULY 2020

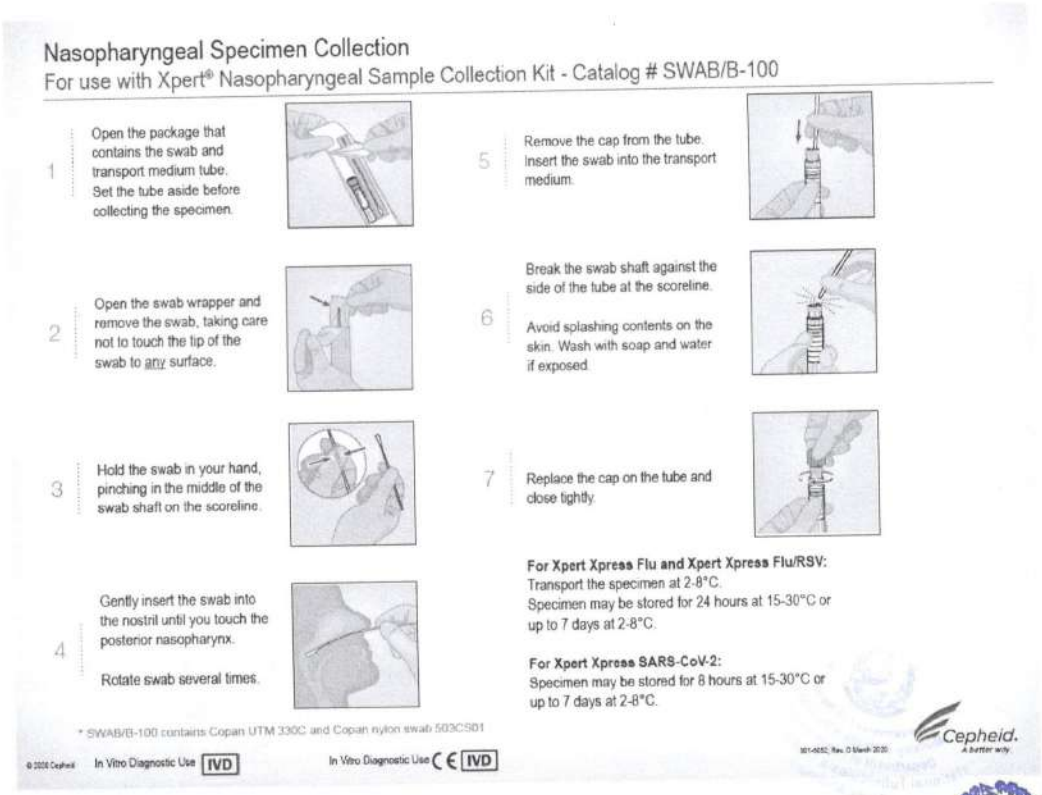
Joint collaboration with National Tuberculosis Control Center and
National Public Health Laboratory
DEPARTMENT OF HEATH SERVICES
Ministry of Health and Population. Nepal

1. **Purpose**
   The purpose of the Xpert Xpress SARS-CoV-2 Test is to detect SARS-CoV-2 RNA in individuals eligible for this test according to the predetermined criteria for using this test for diagnosis of COVID-19 in Nepal *(please see attached document)*.

2. **Reference**
   Xpert Xpress SARS-CoV-2 instructions for use.

3. **Responsibility**
   Laboratory personnel with adequate training or orientation on use of GeneXpert machine for Xpert Xpress SARS-CoV-2 Test.

4. **Specimen used and collection methods:**
   Nasopharyngeal swab collected following the guidelines provided by National Public Health Laboratory (https://youtu.be/8Hh8bUplwP0). Nasopharyngeal swab is preferred, though nasal wash/aspirate oropharyngeal, nasal, or mid-turbinate swab are acceptable too. The specimen is preferably collected and placed into a viral transport tube containing 3 mL transport medium. The specimens can be stored at room temperature (15–30 °C) for up to 8 hours and refrigerated (2–8 °C) up to seven days until testing is performed on the GeneXpert Instrument Systems.



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5. Materials/Equipment used

a. Class II Biosafety cabinet
b. GeneXpert instrument, computer, barcode scanner, operator manual
c. GeneXpert Dx software version 4.7b or higher
d. Category I Personnel Protective Equipment (Gown, N95 respirator, safety goggles or face shield and gloves)
e. Xpert Xpress SARS-CoV-2 Cartridges
f. Disposable Transfer Pipettes
g. CD - Assay Definition File (ADF) and Instructions to import ADF into GeneXpert software

6. Storage and Handling of Cartridges

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

7. Principle:

Gene Xpert Xpress SARS-CoV-2 test is a rapid, real time RT- PCR test intended for the automated and qualitative detection of ribonucleic acid (RNA) from SARS-CoV-2 in either nasopharyngeal swab and/or/nasal wash/aspirate specimens collected from individuals suspected of COVID-19.

The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems which automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in 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. The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets (N2 and E genes).

8. Procedures

8.1. Preparing the cartridge

IMPORTANT: The following procedures (from 2 to 10) should be carried out inside a Class II Biosafety cabinet. Category I PPE (*Guidelines for use of PPE in relation to COVID-19,MoHP*) to be donned while handling specimen and hand hygiene practiced. Change gloves between processing each sample.

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Note: Perform the process inside the biosafety cabinet only

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 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 then place the pipette tip in the specimen transport tube (see Figure 1).
7. Ensure that one pipette is to be used for one specimen

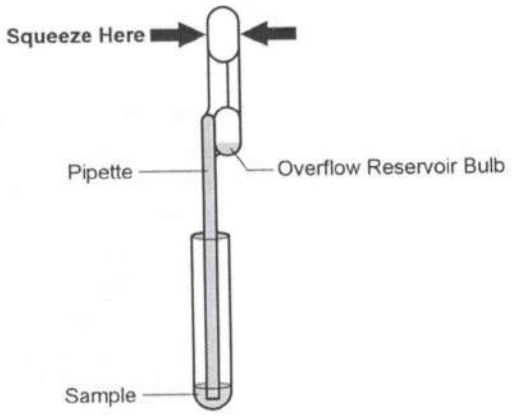

Figure 1: Transfer Pipette

8. 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 1). Check that the pipette does not contain bubbles.
9. 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) in the cartridge shown in Figure 2. 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.
10. Dispose of the used pipette in a container containing freshly prepared 1% Hypochlorite solution.

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

11. Close the cartridge lid

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

8.2. Starting the Test

IMPORTANT: Prior to initiating the test, ensure availability of Gene Xpert Dx software version 4.7b or higher to recognize Xpress SARS-CoV-2 assay file

1. Turn on the GeneXpert 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.
2. Log on to the System software. The login screen appears. Type your username and password.
3. In the GeneXpert System window, click Create Test
4. Type in the Patient ID and other relevant details manually.
5. Scan the barcode on the Xpert Xpress SARS-CoV-2 cartridge.

Note: If the barcode on the Xpert Xpress SARS-CoV-2 cartridge does not scan, then repeat the test with a new cartridge.

1. Click Start Test. In the dialog box that appears, type your password, if required.
a. Locate the module with the blinking green light, open the instrument module door and load the cartridge.
b. 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.
c. Dispose of used cartridges in the appropriate sample waste containers containing freshly prepared 1% Hypochlorite solution.

Note: Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

8.3. Viewing and Printing Results

1. Select VIEW RESULTS from Title bar on the screen to view the entire list of testing performed in the system.
2. In case single result to be viewed, select VIEW TEST icon at the bottom of the screen.
3. Select the test result to be viewed.

9. Quality Control

9.1. 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.

10. Interpretation and report

The results are interpreted automatically by the GeneXpert System and are clearly shown in the View Results window. Interpret test result statements for the Xpert Xpress SARS-CoV-2 test as shown in the table below.

Result	Interpretation	Remarks
SARS-CoV-2 POSITIVE	The 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.- REPORT as SARS-COV-2 POSITIVE
SARS-CoV-2 PRESUMPTIVE POSITIVE	The SARS-CoV-2 nucleic acids may be present.	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. **Sample should be retested**. For samples with a repeated Presumptive Positive result, additional confirmatory testing at NPHL or other COVID-19 RT-PCR laboratory will need to be conducted. REPEAT TEST using left over sample. If not sample left, ask for fresh sample
SARS-CoV-2 NEGATIVE	The SARS-CoV-2 target nucleic acids are **not detected.**	REPORT as SARS-COV-2 NEGATIVE
INVALID	Presence or absence of the SARS-CoV-2 nucleic acids cannot be determined.	**Repeat the test** using left over sample. If not sample left, ask for fresh sample.

ERROR	Presence or absence of the SARS-CoV-2 nucleic acids cannot be determined.	**Repeat the test** using left over sample. If not sample left, ask for fresh sample.
NO RESULT	Presence or absence of the SARS-CoV-2 nucleic acids cannot be determined.	**Repeat the test** using left over sample. If not sample left, ask for fresh sample.

11. Procedure for repeating the test

1. Use the leftover sample from the original specimen transport medium tube and process according to the procedure for testing mentioned above in the SOP.
2. If left over sample is not available, then request for fresh sample is to be raised and efforts must be taken to track and obtain the sample.

12. Limitations of the test

1. **Performance of the Xpert Xpress SARS-CoV-2 has only been established in nasopharyngeal swab specimens.**
2. Oropharyngeal, 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.
3. 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.
4. 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.
5. This test cannot rule out diseases caused by other bacterial or viral pathogens.

13. Warning and precautions

1. Do not open the Xpert Xpress SARS-CoV-2 cartridge lid except when adding specimen.
2. Do not use a cartridge that has been dropped after removing it from the packaging.
3. Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
4. Do not place the sample ID label on the cartridge lid or on the barcode label on the cartridge.
5. Do not use a cartridge with a damaged barcode label.
6. Do not use a cartridge that has a damaged reaction tube.
7. Each single-use Xpert Xpress SARS-CoV-2 cartridge is used to process one test. Do not reuse processed cartridges.
8. Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
9. Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
10. **Change gloves between the handling of each specimen**.
11. 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.

12. **Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents.**

Approved By

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Acknowledgement of having read this SOP

№	Name	Position	Signature

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