# Xpert Xpress SARS-CoV-2 Verification Protocol

Please consult the following guidance from CMS regarding Emergency Use Authorized diagnostic tests: https://www.cms.gov/Medicare/Provider-Enrollment-and-Certification/SurveyCertificationGenInfo/Policy-and-Memos-to-States-and-Regions-Items/QSO18-19-CLIA

Note: This guide is an example of verification and may be modified to meet site-specific criteria. The Laboratory Director is ultimately responsible for ensuring that verification procedures are in accordance with local, state, and federal accrediting organizations. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.

## 1 Intended Use

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in either nasopharyngeal swab and/or nasal wash/ aspirate specimens collected from individuals suspected of COVID-19 by their healthcare provider. The Xpert Xpress SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Testing of nasopharyngeal swab and nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Dx and GeneXpert Infinity systems is limited to qualified laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high and moderate complexity tests.

Testing of nasopharyngeal swab specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is authorized to be distributed and used in patient care settings outside of the clinical laboratory environment.

Results are for the detection of SARS-CoV-2 RNA. 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 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. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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.

Testing with the Xpert Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems.

## 2 Required Materials

- AccuPlex™ SARS-CoV-2 Reference Material kit. SeraCare Material Number: 0505-0126 (5 x 1.5 mL vials, positive reference material; 5 x 1.5 mL vials, negative reference material). The positive reference material consists of recombinant Sindbis virus particles with sequences from the SARS-CoV-2 genome including the E gene, RdRp (RNA dependent RNA polymerase) gene, ORF1a gene, and N gene.
- Five negative clinical nasopharyngeal swab specimens in viral transport medium from patients not suspected of COV (~ 3 mL each), which could be residual specimens from other diagnostic testing.
- Three sterile, screw-capped test tubes; approximately 2 mL capacity and test tube rack
- Sterile disposable pipet tips or pipetman capable of delivering 0.25 mL and 0.3 mL of samples
- 24 Xpert Xpress SARS-CoV-2 cartridges (excluding the cartridges used for external control testing – at least 2 additional cartridges)

## 3 Precautions

The AccuPlex SARS-CoV-2 Reference Material contains recombinant virus that is heat-treated and replication-defective. However, the reference material should be handled as infectious materials using standard precautions and in accordance with Good Laboratory Practices to avoid contamination of laboratory equipment and reagents that could cause false positive results.

Store verification materials at appropriate temperatures per the manufacturer's storage requirements and hold on ice when thawed.

## 4 External Controls Testing

1. Test one positive and one negative external control listed in Section 9 of the Xpert Xpress SARS-CoV-2 instructions for use (IFU) according to the retest procedure described in the IFU.
2. Once the correct results have been obtained for the external controls, proceed with the verification procedure. If external control test results fail to give expected results, contact Cepheid Technical Support.

## 5 Verification Procedure: Accuracy, Reportable, and Reference Ranges

1. Label 20 of the Xpert Xpress SARS-CoV-2 cartridges #1 to #20.
2. Label three sterile, screw-capped test tubes "A", "B", and "C".
   a. Create a 1:2 dilution of the positive AccuPlex reference material (**DILUTED positive**) in a test tube by taking 1.0 ml of the AccuPlex material and adding 1.0 mL of a negative patient specimen (can be pooled negative specimens). Label this test tube "A". Cap the test tube tightly. Mix thoroughly. The total volume in the test tube A will be 2.0 mL.

3. Using pipet provided in Xpert kit, add 0.3 mL from Test Tube A to cartridges #6 to #10 (note there will be approximately 0.5 mL remaining in Test Tube A).
4. Next, prepare a pool of AccuPlex **UNDILUTED positive** reference material by pipetting out 1 mL from each of two AccuPlex positive reference material vials to a separate test tube. Label this test tube "B".
5. Using pipet provided in Xpert kit, add 0.3 mL of **UNDILUTED positive** reference material (Test tube B) to cartridges #1 to #5. (note there will be remaining approximately 0.5 mL volume remaining in Test Tube B).
6. Next, prepare a pool of AccuPlex **NEGATIVE** reference material by pipetting out 1 mL from each of two AccuPlex negative reference material vials to a separate test tube. Label this test tube "C".
7. Using pipet provided in Xpert kit, add 0.3 mL of AccuPlex negative reference material (from Test Tube C) to cartridges #11 to #15 (note there will be approximately 0.5 mL volume remaining in test tube C).
8. Using pipet provided in Xpert kit, add 0.3 mL of negative NP sample in viral transport medium matrix (5 clinical specimens) to cartridges #16 to #20 (each clinical specimen added to different cartridge).
9. Run the Xpert cartridges as per the product IFU.
10. Fill out the results in the Table below.

| **% Overall Agreement** | | | | | |
|---|---|---|---|---|---|
| **Cartridge #/Date** | **SARS-CoV-2 Positive Sample Testing Result** | **% Agreement (Acceptance criteria – 100% for specimens 1-5; 80% for specimens 6-10)** | **Cartridge #/Date** | **SARS-COV-2 Negative Sample Testing Result** | **% Agreement (Acceptance criteria – 100%)** |
| #1/ | | | #11/ | | |
| #2/ | | | #12/ | | |
| #3/ | | | #13/ | | |
| #4/ | | | #14/ | | |
| #5/ | | | #15/ | | |
| #6/ | | | #16/ | | |
| #7/ | | | #17/ | | |
| #8/ | | | #18/ | | |

| | | | | | |
|---|---|---|---|---|---|
| #9/ | | | #19/ | | |
| #10/ | | | #20/ | | |
| Comments | | | Comments | | |

**Expected Results**

Undiluted and 1:2 diluted positive samples should be positive for SARS-CoV-2. Negative samples (AccuPlex reference material and patient specimens) should be negative for SARS-CoV-2.

Acceptance criteria for the testing are:

1) 100% of tests results should be in agreement with the expected results for the undiluted positive samples and negative samples.
2) ≥ 4/5 (80%) of test results should be in agreement with the expected results for the diluted positive samples.

If test results fail to meet expected results, contact Cepheid Technical Support.

## 6 Verification Procedure: Reproducibility

1. Select two operators to repeat the Xpert SARS-CoV-2 test on selected vials of AccuPlex positive reference material and AccuPlex negative reference material.
2. Using pipet provided, Operator #1 add 0.3 mL of AccuPlex positive reference material to cartridge #21. Operator #2 add 0.3 mL of AccuPlex positive to cartridge #22.
3. Using pipet provided, Operator #1 add 0.3 mL of AccuPlex negative reference material to cartridge #23. Operator #2 add 0.3 mL of AccuPlex negative to cartridge #24.
4. Fill out results in the Table below:

| **Reproducibility** | | | | | |
|---|---|---|---|---|---|
| **Cartridge #/Date** | **SARS-CoV-2 Positive Sample Testing Results** | **% Agreement (Acceptance criteria – 100%)** | **Cartridge #/Date** | **SARS-COV-2 Negative Sample Testing Results** | **% Agreement (Acceptance criteria – 100%)** |
| **Operator 1**: #21/ | | | **Operator 1**: #23/ | | |
| **Operator 2**: #22/ | | | **Operator 2**: #24/ | | |

**Expected Results**

Acceptance criteria for the testing are: All replicates of the positive samples should be 100% positive, and all replicates of the negative samples should be 100% negative.

If test results fail to meet expected results, contact Cepheid Technical Support.