Xpert® Xpress CoV-2 *plus* EUA* Verification Protocol

Disclaimer: This protocol was developed by Cepheid Medical & Scientific Affairs to provide assistance to customers who are performing verification procedures of the Xpert Xpress CoV-2 plus test. It contains one aspect of the verification process, which is testing of known positive and negative samples. It is the laboratory's responsibility to ensure that a complete and adequate verification process is performed in accordance with federal, state and local laws.

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

For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.

1 Intended Use

The Xpert **Xpress** CoV-2 ***plus*** test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, oropharyngeal swabs or nasal wash/aspirate specimens obtained from individuals suspected of COVID-19 by a healthcare provider as well as anterior nasal swab specimens from any individual, including individuals without symptoms or other reasons to suspect COVID-19.

Testing of nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, oropharyngeal swabs or nasal wash/aspirate specimens using the Xpert **Xpress** CoV-2 ***plus*** test run on the GeneXpert® and GeneXpert Infinity systems is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests.

Testing of nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, using the Xpert **Xpress** CoV-2 ***plus*** test run on the GeneXpert **Xpress** system (tablet and hub configurations) is limited to laboratories certified under CLIA that meet requirements to perform high, moderate, or waived complexity tests. Testing of these specimens is authorized for use at the point of care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation. Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection.

* About Emergency Use Authorization Status
This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by authorized laboratories. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
This bulletin applies to the EUA version of Xpert **Xpress** CoV-2 ***plus*** test.

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 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** CoV-2 ***plus*** test is intended for use by trained operators who are proficient in performing tests using either GeneXpert, GeneXpert Infinity and/or GeneXpert **Xpress** systems. The Xpert **Xpress** CoV-2 ***plus*** test is only for use under the Food and Drug Administration's Emergency Use Authorization.

2 Required Materials

- ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Control, Catalog# NATSARS(COV2)-ERC1
- ZeptoMetrix SARS Associated Coronavirus 2 (SARS-CoV-2) Negative Control, Catalog# NATSARS(COV2)-NEG
- 5 negative clinical swab specimens in viral transport medium from patients not suspected of containing SARS-CoV-2 (~ 3 mL each). These can be residual specimens from other diagnostic testing.
- 24 Xpert **Xpress** CoV-2 ***plus*** cartridges (excluding the cartridges used for external control testing – at least 2 additional cartridges)

Other laboratory supplies

- 3 sterile, screw-capped test tubes; approximately 2 mL capacity and test tube rack
- Sterile disposable pipet tips and adjustable volume pipette capable of delivering 0.25 mL and 0.3 mL of samples

3 Precautions

The ZeptoMetrix reference material, the NATtrol™ SARS-CoV-2 External Run Controls, are formulated with purified, intact viral particles (positive control) and human A549 cells (negative control). The virus particles have been chemically modified to render them non-infectious and refrigerator stable. 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.

4 External Controls Testing

4.1. Test one positive and one negative external control listed in Section 13.2 of the Xpert **Xpress** CoV-2 ***plus*** instructions for use (IFU).

4.2. 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

5.1. Label 20 of the Xpert **Xpress** CoV-2 ***plus*** cartridges #1 to #20.

5.2. Label three sterile, screw-capped test tubes A, B, and C.

5.3. Create a 1:2 dilution of the positive NATSARS(COV2)-ERC1 reference material (DILUTED positive) in a test tube by taking 1.0 ml (one vial) of the NATtrol 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.

5.4. Using pipet provided in the Xpert **Xpress** CoV-2 ***plus*** test 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).

5.5. Next, prepare a pool of NATtrol UNDILUTED positive reference material by pipetting out 1.0 mL from each of two NATtrol positive reference material vials to a separate test tube. Label this test tube B.

5.6. Using pipet provided in the Xpert **Xpress** CoV-2 ***plus*** test kit, add 0.3 mL of UNDILUTED positive reference material (test tube B) to cartridges #1 to #5. (Note that there will be approximately 0.5 mL volume remaining in test tube B.)

5.7. Next, prepare a pool of NATtrol NEGATIVE reference material by pipetting out 0.5 mL from each of four NATtrol negative reference material vials to a separate test tube. Label this test tube C.

5.8. Using pipet provided in the Xpert **Xpress** CoV-2 ***plus*** test kit, add 0.3 mL of NATtrol 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).

5.9. Using pipet provided in the Xpert **Xpress** CoV-2 ***plus*** test kit, add 0.3 mL of negative patient swab samples in viral transport medium matrix (5 clinical specimens) to cartridges #16 to #20 (each clinical specimen added to different cartridge).

5.10. Run the Xpert **Xpress** CoV-2 ***plus*** cartridges as per the IFU.

5.11. Fill out the results in *Table 1* next page.

Expected Results

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

Acceptance criteria for the testing are:

- 100% of tests results should be in agreement with the expected results for the undiluted positive samples and negative samples
- ≥ 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.

Table 1:
% 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		

6 Verification Procedure: Reproducibility

6.1. Select two operators to repeat the Xpert **Xpress** CoV-2 ***plus*** test on selected vials of NATtrol positive reference material and NATtrol negative reference material.

6.2. Using pipet provided, operator #1 should add 0.3 mL of NATtrol positive reference material to cartridge #21. Operator #2 should add 0.3 mL of NATtrol positive to cartridge #22.

6.3. Using pipet provided, Operator #1 should add 0.3 mL of NATtrol negative reference material to cartridge #23. Operator #2 should add 0.3 mL of NATtrol negative to cartridge #24.

6.4. Fill out results in *Table 2* on next page.

Table 2:
Reproducibility

Cartridge #/Date	SARS-CoV-2 Positive Sample Testing Result	% Agreement (Acceptance criteria – 100%)	Cartridge #/Date	SARS-CoV-2 Negative Sample Testing Result	% 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.

7 References for Additional Information on Verification/Validation Testing

7.1. Xpert **Xpress** CoV-2 ***plus*** Instructions for Use. GeneXpert Xpress System. 302-7069 Rev A.

7.2. Xpert **Xpress** CoV-2 ***plus*** Instructions for Use. GeneXpert System. 302-7070 Rev A.

CORPORATE HEADQUARTERS
904 Caribbean Drive
Sunnyvale, CA 94089 USA
TOLL FREE **1.888.336.2743**
PHONE **1.408.541.4191**
FAX **1.408.541.4192**

EUROPEAN HEADQUARTERS
Vira Solelh
81470 Maurens-Scopont France
PHONE **33.563.82.53.00**
FAX **33.563.82.53.01**
EMAIL **cepheid@cepheideurope.fr**

www.**Cepheid**.com