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Dear Valued Customer:

The Xpert® **Xpress** CoV-2/Flu/RSV **plus*** test may be verified in your institution using an applicable Test Verification Guide (1) in which use of external control materials from ZeptoMetrix are referenced. It has come to Cepheid's attention that during the laboratory verification process whereby either simulated nasopharyngeal (NP) or nasal swabs are being created by dipping into the external control materials, that variable amounts of the liquid controls are being adsorbed on to the swab. This may lead to inconsistent positive results with the contrived samples made with the control in question. Thus, Cepheid is recommending the following procedure in the instance this occurs:

Vortex the NATtrol organism vial in question, and place in holder to keep the vial upright. Remove 300 ul from the NATtrol vial using a sterile pipette and deliver this volume into the previously inoculated collection device [3 mL Viral Transport Media (VTM), Saline or 2mL eNAT™]. Cap the tube and mix the specimen by rapid inversion 5 times. Add 300 µL of this "enhanced" sample (simulated NP or nasal swab specimen) using the transfer pipette supplied in the kit to the appropriately labeled cartridge. Perform the test as per the package insert instructions.

Please feel free to connect Cepheid Technical Support with additional questions.

Note that it is up to the laboratory director to decide the appropriate test verification process in conjunction with their local laboratory accreditation agency.

Yours sincerely,

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(1) Xpert® Xpress CoV-2/Flu/RSV *plus* Verification Guide

*For use under an Emergency Use Authorization in the United States

Xpert® Xpress CoV-2/Flu/RSV plus*

Verification Protocol

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

1 Objective

The objective of this protocol is to facilitate verification studies of the Xpert Xpress CoV-2/Flu/RSV plus test by describing how to use inactivated organisms from ZeptoMetrix (ZeptoMetrix, Buffalo, NY) to prepare mock or simulated nasopharyngeal (NP), nasal swabs (NS), and nasal aspirate/washes (NA/W) for verification testing.

2 Scope

ZeptoMetrix manufactures NATtrol inactivated organisms that will provide positive and negative results for verification studies with Xpert Xpress CoV-2/Flu/RSV plus. These materials are supplied in a stable liquid form as individual controls. The NATtrol inactivated viruses available are shown in [Table 1](#) and include strains of SARS-CoV-2, influenza A, influenza B, respiratory syncytial virus (RSV) B, and coxsackievirus, all in a purified protein matrix that mimics the composition of a clinical specimen. The NATFRC-6C control material is not used in the creation of simulated or contrived specimens, but may be used for future training or blinded proficiency investigations.

➤ **Table 1:**
NATtrol Available Control Materials (www.ZeptoMetrix.com)

Catalog #	Strain (Inactivated)	Expected Result			
		SARS-CoV-2	Flu A	Flu B	RSV
NATSARS(COV2)-ERC	SARS-CoV-2 (Isolate: USA-WA1/2020)	+	-	-	-
NATCV9-6C	Coxsackievirus A9	-	-	-	-
NATFRC-6C	Mixed virus positive control (Flu/RSV/SARS-CoV2, 4 targets)	+	+	+	+
NATFLUAB-6C	Mixed positive control (Influenza A/Brisbane/59/07 Influenza B/Florida/02/06, 2 targets)	-	+	+	-
NATRSV-6C	RSV B (CH93(18)-18)	-	-	-	+
NATFLURSV-6C	Mixed positive control, (Influenza A/Brisbane/59/07 Influenza B/Florida/02/06 RSV B, 3 targets)	-	+	+	+
NATCXVA9-6C	Coxsackievirus A9	-	-	-	-

*For Use Under an Emergency Use Authorization (EUA) only.
This bulletin applies to the Emergency Use Authorization (EUA) version of Xpert Xpress CoV-2/Flu/RSV plus.

Note: Before you start, please read through the procedure in its entirety and print out verification charts (1 and 2) Xpert **Xpress** CoV-2/Flu/RSV **plus** by clicking on the paperclip icon and selecting the PDF files (*Figure 1*). The charts contain specimen numbering/labeling information that is used throughout the procedure. Also, remember to change gloves between processing specimens.

➤ **Figure 1**
Verification Charts



3 Materials Required

- 3.1. 1 - Zeptomatrix verification panel, NATSARS(COV2)-ERC
- 3.2. 1 - ZeptoMetrix Flu A/B Positive control, NATFLUAB-6C
1 - ZeptoMetrix RSV Positive control, NATRSV-6C
1 - ZeptoMetrix Flu A/B/RSV Positive control, NATFLURSV-6C
1 - ZeptoMetrix Negative control, NATCV9-6C or NATCXVA9-6C (these may be used interchangeably during the protocol)
- 3.3. 22 - XP3COV2/FLU/RSV-10 cartridges (**Note:** Fewer may be required depending on specimen types to be verified, see procedure reference table)
- 3.4. 10 to 15 Collection devices (3 mL viral transport media [VTM], saline or 2mL eNAT)
- 3.5. 4 - Clinical Specimens

The four clinical specimens submitted for SARS-CoV-2 and Flu/RSV testing must have previously tested negative for all viruses by a verified laboratory method (preferably PCR). These specimens should have been stored in the appropriate transport medium for up to 24 hours at 2–30 °C, or up to seven days at 2–8 °C. Additionally, these specimens should have ≥300 µL residual volume.

- 3.6. Other laboratory supplies
 - 10 mL sterile saline
 - 30 - 300 µL transfer pipettes (supplied in the Xpert **Xpress** CoV-2/Flu/RSV **plus** kit)
 - Sterile test tubes and rack
 - Timer

Procedure

See [Table 2](#) below for a quick reference guide of the procedural sections. Some sections may be optional, depending on the specimen types to be verified. Fill out the supplied verification chart(s) after completion of each section of testing.

➤ **Table 2:**
Procedure Reference Table

Section Number	Description	Number of Xpert Cartridges Required	Specimen Labeling (according to protocol)	Specimens Required
4	Running positive and negative controls	2 or 3	According to label on control vial	Known positive and negative control samples
5	Preparation of simulated NP samples	5	SIM NP xxx series, see Verification Chart 1	Known positive and negative control samples
6	Preparation of simulated NS samples	5	SIM NS xxx series, see Verification Chart 1	Known positive and negative control samples
7	Procedure for spiking negative clinical specimens	4	CL xxx series, see Verification Chart 2	4 known negative NP, NS, or nasal aspirate/wash clinical (patient) specimens
8	Preparation of simulated nasal aspirate/wash specimens	5	SIM NA/W xxx series, see Verification Chart 2	Known positive and negative control samples

4 Run a Positive Control for SARS-CoV-2, Influenza A, Influenza B, RSV, and a Negative Control

Note: Combinational positive controls may be used (e.g. NATFLURSV-6C or NATFRC-6C)

- 4.1. Add 300 µL of each designated control to a separate Xpert **Xpress** CoV-2/Flu/RSV **plus** cartridge.
- 4.2. Test the cartridges on a GeneXpert® System as per the package insert.
- 4.3. Once you have obtained the correct results, proceed with the protocol.

5 Preparing Simulated NP Samples with NATtrol-Inactivated Influenza A/B, Influenza A/B/RSV, RSV, SARS-CoV-2, and Coxsackievirus from the ZeptoMetrix Kit

Note: This step uses new 3 mL Viral Transport Media (VTM), saline or 2 mL eNAT collection devices, not previously inoculated with a patient specimen. These specimens will be labeled as Sim NP xxx.

One vial of each of the following ZeptoMetrix materials will be used in this step: NATSARS(COV2)-ERC, NATFLUAB-6C, NATRSV-6C, NATFLURSV-6C, and NATCV9-6C. Each NATtrol vial contains 0.5 mL of a viral suspension. You will be preparing one simulated NP specimen for each NATtrol control for a total number of five specimens.

- 5.1. Vortex or shake each NATtrol vial vigorously for 10 seconds and place in a small rack.
- 5.2. Obtain five VTM, saline or eNAT collection devices, place the five collection device tubes in a test tube holder, and label the tubes with respective NATtrol viral strain to be spiked.
- 5.3. Label five cartridges with the virus and strain name and Sim NP xxx as outlined in the verification chart.

- 5.4. Remove the flocked or similar swab from the collection device packaging and drop swab into the NATtrol organism vial, being careful to keep the vial upright.
- 5.5. Leave the swab in the vial for 10 – 20 seconds.
- 5.6. Remove the swab from the vial and break off the swab into the pre-labeled VTM, saline or eNAT tube, being careful to place the correct virus to its corresponding collection device tube.
- 5.7. Cap the tube and mix by rapid inversion five times. Add 300 µL of the simulated NP specimen using the transfer pipette supplied in the kit to the appropriate labeled cartridge.
- 5.8. Perform the test as per the package insert.

6 Preparing Simulated Nasal Samples (NS) with NATtrol-Inactivated Influenza A/B, Influenza A/B/RSV, RSV, SARS-CoV-2, and Coxsackievirus from the ZeptoMetrix kit

Note: This step uses new 3 mL Viral Transport Media (VTM), saline or 2 mL eNAT collection devices, not previously inoculated with a patient specimen. These specimens will be labeled as SIM NS xxx.

One vial of each of the following ZeptoMetrix materials will be used in this step: NATSARS(COV2)-ERC, NATFLUAB-6C, NATRSV-6C, NATFLURSV-6C, and NATCXVA9-6C. Each NATtrol vial contains 0.5 mL of a viral suspension. You will be preparing one simulated NS specimen for each NATtrol control for a total number of five specimens.

- 6.1. Vortex or shake each NATtrol vial vigorously for 10 seconds and place in a small rack.
- 6.2. Obtain five VTM, saline or eNAT collection devices, place the five collection device tubes in a test tube holder, and label the tubes with respective NATtrol viral strain to be spiked.
- 6.3. Label five cartridges with the virus, strain name, and NS xxx as outlined in the verification chart.
- 6.4. Remove the flocked or similar swab from the collection device packaging and drop swab into the NATtrol organism vial, being careful to keep the vial upright.
- 6.5. Leave the swab in the vial for 10 – 20 seconds.
- 6.6. Remove swab from the vial and break off the swab into the pre-labeled VTM or saline tube, being careful to place the correct virus into its corresponding collection device tube.
- 6.7. Cap the tube and mix by rapid inversion five times. Add 300 µL of the simulated NS specimen using the transfer pipette supplied in the kit to the appropriate labeled cartridge.
- 6.8. Perform the test as per the package insert.

7 Procedure for Spiking Negative Clinical Specimens with NATtrol-Inactivated Influenza A/B, Influenza A/B/RSV, RSV, and SARS-CoV-2 from the ZeptoMetrix Kit

Note: This step uses four negative patient specimens (either NP,NS, or NA/W) collected in 3 mL Viral Transport Media (VTM), saline or 2 mL eNAT. These specimens will be labeled CL xxx for clinical specimens.

One vial of each of the following ZeptoMetrix materials will be used in this step: NATSARS(COV2)-ERC, NATFLUAB-6C, NATRSV-6C, and NATFLURSV-6C. Each NATtrol vial contains 0.5 mL of a viral suspension. You will be preparing one spiked clinical specimen for each NATtrol positive controls for a total number of four specimens, as you will not be using the negative control to spike.

- 7.1. Vortex or shake each NATtrol vial vigorously for 10 seconds and place in a small rack.
- 7.2. Obtain four negative SARS-CoV-2/Flu/RSV clinical (patient) specimens (either NP, NS or NA/W specimens), collected in 3 mL VTM, Saline or 2 mL eNAT. If there is a patient swab already in the transport tube, it can remain in the device during this procedure.
- 7.3. Place the four clinical specimens in a test tube holder and label them with the respective virus to be spiked into each.
- 7.4. Label four cartridges with the organism and strain name and CL xxx as outlined in the verification chart.
- 7.5. Obtain a brand new flocked or similar swab and drop swab into the NATtrol organism vial, being careful to keep vial upright.
- 7.6. Leave the swab in the vial for 10 – 20 seconds.
- 7.7. Remove the swab from the vial and break off swab into the pre-labeled clinical tube, ensuring that the organism on the swab matches pre-labeled spiked organism name on the collection device.
- 7.8. Cap the tube and mix by rapid inversion five times. Add 300 µL of the spiked clinical specimen to the Xpert cartridge using the transfer pipette supplied with the kit.
- 7.9. Perform the test as per the package insert.

8 Preparing Simulated Nasal Aspirate/Wash Specimens with NATtrol-Inactivated Influenza A/B, Influenza A/B/RSV, RSV, SARS-CoV-2, and Coxsackievirus from the ZeptoMetrix Kit

Note: This step uses 3 mL VTM, saline or 2 mL eNAT collection devices, not previously inoculated with a patient specimen. These specimens will be labeled SIM NA/W xxx.

One vial of each of the following ZeptoMetrix materials will be used in this step: NATSARS(COV2)-ERC, NATFLUAB-6C, NATRSV-6C, NATFLURSV-6C, and NATCV9-6C. Each NATtrol vial contains 0.5 mL of a viral suspension. You will be preparing one simulated NA/W specimen for each NATtrol control for a total number of five specimens.

- 8.1. Label five small test tubes (Eppendorf or similar) with the appropriate ZeptoMetrix identification (NATtrol #) according to [Table 1](#). Add 300 µL of sterile saline to the five test tubes and place each in a rack.
- 8.2. Vortex or shake vigorously each of the NATtrol vials for 10 seconds and place in the rack, adjacent to their matching pre-labeled test tube.

- 8.3. Add 300 µL of the NATtrol viral material to the pre-labeled saline-filled tubes. These are the simulated NA/W specimens. There should be a total volume of 600 uL in each tube (300 uL saline + 300 uL control).
- 8.4. Obtain five VTM, saline or eNAT collection devices and place them in a test tube holder, then label the device with the NATtrol viral strain to be tested.
- 8.5. Mix each small tube and then add the total volume (~600 µL) of the simulated NA/W specimens (i.e., saline plus virus) to the pre-labeled collection devices. Ensure that the total volume is added to the collection device.
- 8.6. Label five cartridges with the organism and strain name and NA/W xxx as outlined in the verification chart. Cap the tube and mix by rapid inversion five times. Add 300 µL of the simulated NA/W to the Xpert cartridge using the transport pipette supplied in the kit.
- 8.7. Perform the test as per the package insert.

9 Retest Procedure for all Specimens

In case of INVALID, ERROR, or NO RESULT, obtain the leftover sample from the appropriate collection device and repeat the test with a new cartridge. Note that leftover nasopharyngeal, nasal, and nasal wash/aspirate 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 Systems.

10 References

- 10.1. Cepheid Xpert **Xpress** CoV-2/Flu/RSV *plus* Package Insert. 302-6991.

This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by authorized laboratories; this test has been authorized only for the simultaneous qualitative detection and differentiation of nucleic acids from SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV), and not for any other viruses or pathogens; and this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

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