

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) and nasal swabs (NS) 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	-	-	-	-

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 - ZeptoMetrix SARS-CoV-2 positive control 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). Refer to the Cepheid test's package insert for full details of supported sample types and specimen collection methods
- 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
 - 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
8	Protocol for "enhanced" simulated NP or NS samples	1	Same as section 5 or 6	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 or NS) 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 or NS), 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 “Enhanced” Simulated NP or NS Samples

Note: This step uses three mLVTM, Saline or two mL eNAT™ collection devices, that had been previously tested as per sections 5, 6, or 7, but test result was not as expected. The unexpected result is due to inconsistent amounts of control material being adsorbed onto the swab, prior to its inoculation into the VTM. Thus, Cepheid is recommending the following procedure when this occurs.

- 8.1. Vortex the NATtrol organism vial in question, and place in holder to keep the vial upright.
- 8.2. Remove 300 ul from the NATtrol vial using a sterile pipette or calibrated pipeter and deliver this volume into the previously inoculated collection device [3 mL Viral Transport Media (VTM), Saline or 2mL eNAT™].
- 8.3. Cap the tube and mix the specimen by rapid inversion five times.

8.4. 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.

8.5. Perform the test as per the package insert instructions.

▼ 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 and nasal 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. (CE-IVD) 302-7085.

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

© 2021 Cepheid 0848-03