Xpert® Xpress SARS-CoV-2/Flu/RSV Verification Guide

*Disclaimer: This protocol was developed by Cepheid Medical/Scientific Affairs, to provide assistance to customers who are performing verification studies of the Xpert® Xpress SARS-CoV-2/Flu/RSV test, thereby referred to as Xpress SARS-CoV2-/Flu/RSV. 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 Xpress SARS-CoV-2/Flu/RSV test by describing how to use inactivated organisms from ZeptoMetrix (ZeptoMetrix, Buffalo, NY) to prepare mock or simulated nasopharyngeal (NP), Nasal (NS) swabs, 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 Xpress SARS-CoV-2/Flu/RSV test. 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)	Xpert Expected Results			
		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 *in-vitro* Diagnostic Use. This guide is applicable to the EUA version of Xpert Xpress SARS-CoV-2/Flu/RSV

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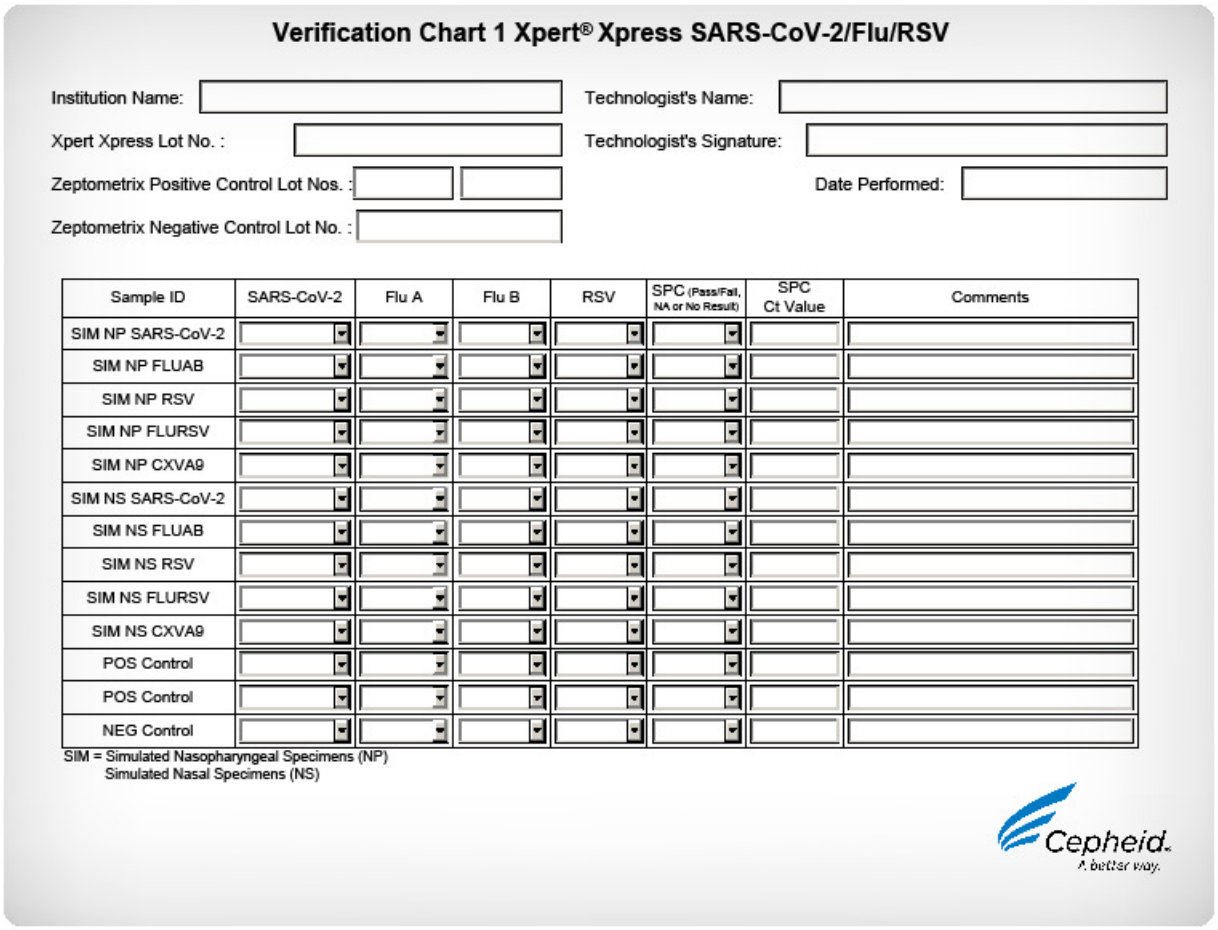
Note: Before you start, please read through the procedure in its entirety and print out **Verification Charts (1 and 2) Xpert Xpress SARS-CoV2/Flu/RSV** by clicking on the paperclip icon and selecting the PDF files (Figure 1). The charts contain specimen numbering/labeling information that is used throughout the procedure. Also, remember to change gloves between processing specimens.

(Insert snapshot here of embedded verification charts 1 and 2 and label it as Figure 1.)



Verification Chart 1 Xpert® Xpress SARS-CoV-2/Flu/RSV

Institution Name: | Technologist's Name:

Xpert Xpress Lot No. : | Technologist's Signature:

Zeptometrix Positive Control Lot Nos. : | Date Performed:

Zeptometrix Negative Control Lot No. :

Sample ID	SARS-CoV-2	Flu A	Flu B	RSV	SPC (Pass/Fail, NA or No Result)	SPC Ct Value	Comments
SIM NP SARS-CoV-2							
SIM NP FLUAB							
SIM NP RSV							
SIM NP FLURSV							
SIM NP CXVA9							
SIM NS SARS-CoV-2							
SIM NS FLUAB							
SIM NS RSV							
SIM NS FLURSV							
SIM NS CXVA9							
POS Control							
POS Control							
NEG Control							

SIM = Simulated Nasopharyngeal Specimens (NP)
Simulated Nasal Specimens (NS)

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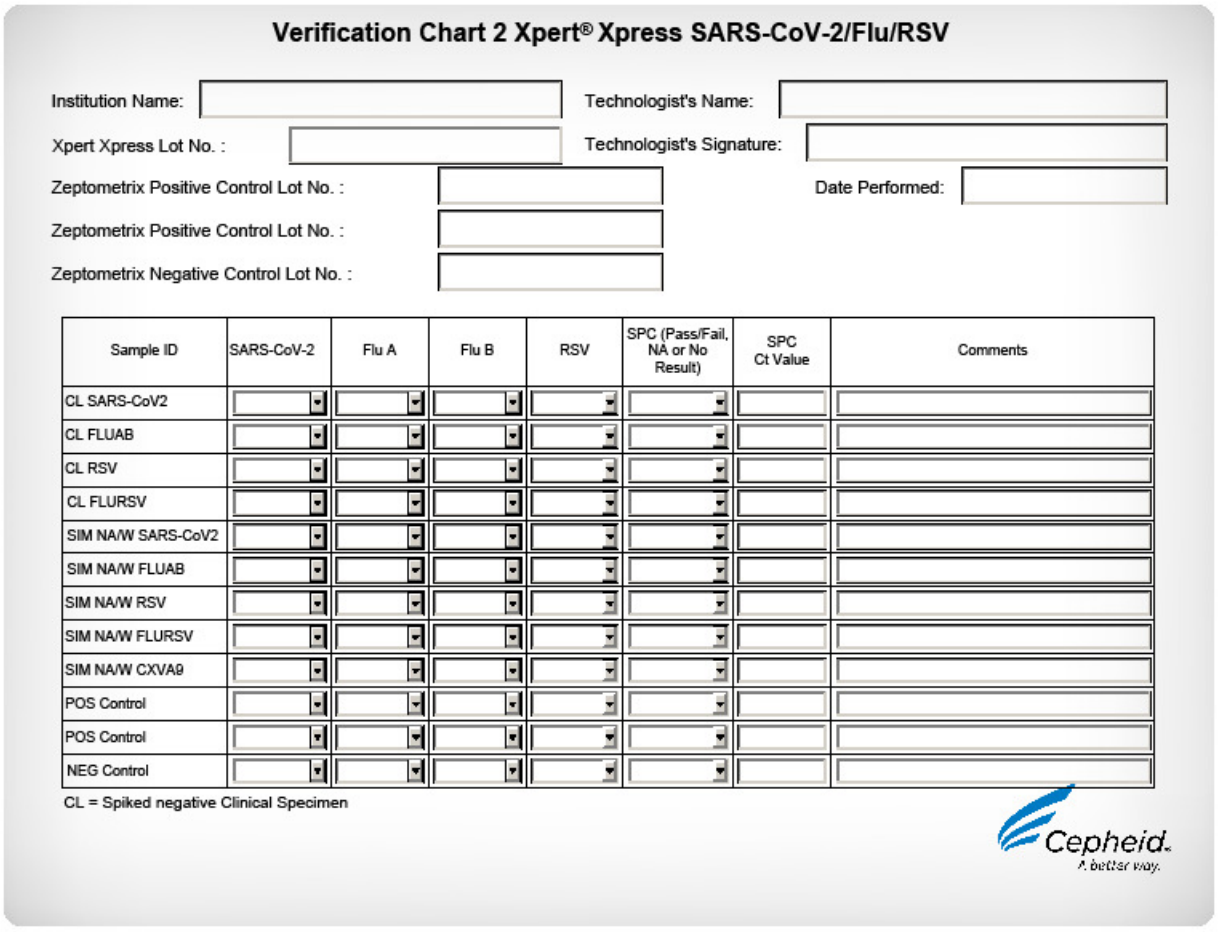

Verification Chart 2 Xpert® Xpress SARS-CoV-2/Flu/RSV

Institution Name: | Technologist's Name:

Xpert Xpress Lot No. : | Technologist's Signature:

Zeptometrix Positive Control Lot No. : | Date Performed:

Zeptometrix Positive Control Lot No. :

Zeptometrix Negative Control Lot No. :

Sample ID	SARS-CoV-2	Flu A	Flu B	RSV	SPC (Pass/Fail, NA or No Result)	SPC Ct Value	Comments
CL SARS-CoV2							
CL FLUAB							
CL RSV							
CL FLURSV							
SIM NA/W SARS-CoV2							
SIM NA/W FLUAB							
SIM NA/W RSV							
SIM NA/W FLURSV							
SIM NA/W CXVA9							
POS Control							
POS Control							
NEG Control							

CL = Spiked negative Clinical Specimen

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3 Materials Required

3.1. (1) ZeptoMetrix 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) XPCOV2/FLU/RSV-10 cartridges (Note: Less may be required depending on specimen types to be verified, see procedure reference table)

3.4. (10-15) Collection devices [3 mL Viral Transport Media (VTM)}

3.5. (4) Clinical Specimens

Note: For previously assayed clinical specimens, the following transport media are acceptable: Any viral transport media that does not contain guanidinium thiocyanate or any guanidium-containing compounds.
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 ℃, or up to seven days at 2–8 ℃. Additionally, these specimens should have ≥300 µL residual volume.

3.6. Other laboratory supplies

- 10 mL sterile saline
- Thirty 300 µL transfer pipettes (supplied in the Xpert 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	Four 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 XPCOV2/FLU/RSV cartridge.

4.2 Test the cartridges on a GeneXpert or Infinity 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 Viral Transport Media (VTM) collection devices, not previously inoculated with a patient specimen. These specimens will be labeled as Sim NPxxx.

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 1 simulated NP specimen for each NATtrol control for a total number of 5 specimens.

5.1. Vortex or shake vigorously each NATtrol vial for 10 seconds and place in a small rack.

5.2. Obtain 5 VTM collection devices, place the 5 collection device tubes in a test tube holder, and label the tubes with respective NATtrol viral strain to be spiked.

5.3. Label 5 cartridges with the virus and strain name and Sim NPxxx 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 swab from the vial and break off the swab into the pre-labeled VTM tube, being careful to place the correct virus to its corresponding collection device tube.

5.7. Cap the tube and mix by rapid inversion 5 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 Viral Transport Media (VTM) 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 NATCV9-6C. Each NATtrol vial contains 0.5 mL of a viral suspension. You will be preparing 1 simulated NS specimen for each NATtrol control for a total number of 5 specimens.

6.1. Vortex or shake vigorously each NATtrol vial for 10 seconds and place in a small rack.

6.2. Obtain 5 VTM collection devices, place the 5 collection device tubes in a test tube holder, and label the tubes with respective NATtrol viral strain to be spiked.

6.3. Label 5 cartridges with the virus and 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 tube, being careful to place the correct virus to its corresponding collection device tube.

6.7. Cap the tube and mix by rapid inversion 5 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 4 negative patient specimens (either NP,NS, or NA/W) collected in Viral Transport Media (VTM). These specimens will be labeled **CL** xxx for **Cl**inical 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 1 spiked clinical specimen for each NATtrol positive controls for a total number of 4 specimens, as you will not be using the negative control to spike.

7.1. Vortex or shake vigorously each NATtrol vial for 10 seconds and place in a small rack.

7.2. Obtain 4 negative SARS-CoV-2/Flu/RSV clinical (patient) specimens (either NP, NS or NA/W specimens), collected in the VTM. If there is a patient swab already in the VTM, it can remain in the device during this procedure.

7.3. Place the 4 clinical specimens in a test tube holder and label them with the respective virus to be spiked into each.

7.4. Label 4 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 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 5 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 VTM 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 1 simulated NA/W specimen for each NATtrol control for a total number of 5 specimens.

8.1. Label 5 small test tubes (Eppendorf or similar) with the appropriate ZeptoMetrix identification (NATtrol #) according to Table 1. Add 300 µL of sterile saline to the 5 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 5 VTM 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 5 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 5 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 ℃) for up to 8 hours and refrigerated (2–8 ℃) up to seven days until testing is performed on the GeneXpert Instrument Systems.