Assay Technical Training Xpert® Xpress Flu/RSV For CE-IVD Use Only

Cepheid Training Center







Training Agenda

Xpert Xpress Flu/RSV Training

- Reagents
- Sample collection
- Kit storage and handling
- Preparing the cartridge
- Quality Controls
- Results analysis
- Discussion







Training Objectives

At the end of the training, users will be able to:

- Store and handle the Xpert® Xpress Flu/RSV kit
- Follow proper laboratory safety precautions
- Collect and transport appropriate specimen(s)
- Prepare a cartridge and run the assay
- Report and understand various software-generated results
- Understand the assay control strategy





The Cepheid Solution



- Detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV)
- On-board internal controls for each sample
 - Probe Check Control (PCC)
 - Sample Processing Control (SPC)
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access



Intended Use

- The Cepheid Xpert® Xpress Flu/RSV test, performed on the GeneXpert® Instrument Systems, is an automated, multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV). The Xpert Xpress Flu/RSV test uses nasopharyngeal (NP) swab or nasal swab (NS) specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Xpress Flu/RSV test is intended as an aid in the diagnosis of influenza and respiratory syncytial virus infections in conjunction with clinical and epidemiological risk factors.
- Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
- Performance characteristics for influenza A were established during the 2015-2016 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary.
- If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.



Xpert Xpress Flu/RSV Requirements

GeneXpert Systems

- •GeneXpert Dx Software v 4.7b or higher
- Xpertise Software v 6.4b or higher

Test Kits (CE-IVD)

XPRSFLU/RSV-CE-10

Sample Collection

- •Xpert Nasopharyngeal Sample Collection Kit (#SWAB/B-100)
- Xpert Swab Sample collection Kit (#SWAB/F-100)

Other materials

- Personal Protective Equipment (PPE)
- 1:10 bleach
- 70% ethanol or denatured ethanol

Optional

- Uninterruptible Power Supply /Surge Protector
- Printer
- Vortex





Good Laboratory Practice

Personnel Protective Equipment (PPE)

- Wear clean lab coats and gloves
- Change gloves between processing samples

Lab Bench Area

- Clean work surfaces routinely with:
 - √ 1:10 dilution of household bleach
 - ✓ 70% ethanol solution
- * Final Active Chlorine concentration should be 0.5% regardless of the household bleach
- After cleaning, ensure that the work surfaces are dry

Specimens, Samples, and Kits Storage

Store specimens and sample away from kit to prevent contamination

Equipment

- Use filtered tips when recommended
- Follow the manufacturer's requirements for calibration and maintenance of equipment



Kit Handling

Xpert Flu/RSV Kit Contents

Catalog Number	XPRSFLU/RSV-CE-10		
Cartridges Per Kit	10		
Transfer Pipettes	1 bag of 12 (300µl volume)		
Kit CD	Assay Definition File (ADF)		
	Assay Import Instructions		
	Package Insert (PDF)		
Storage	2-28 °C		







Xpert Xpress Flu/RSV Kit Storage and Handling

Store the Xpert Assay cartridges and reagents at 2–28°C



- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use sample collection devices that have not been validated by Cepheid
- Open the cartridge lid only when adding the sample, close the lid, and proceed with processing





Warnings and Precautions

- Do not shake the cartridge
- Do not use a cartridge that...:
 - appears wet, has leaked, or if the lid seal appears to have been broken
 - appears damaged
 - has been dropped after removing it from packaging
 - has been dropped or shaken after adding the sample to it
 - has a damaged reaction tube
 - has been used; each cartridge is single-use to process one test
 - is expired
- Do not reuse disposable pipettes



Warnings and Precautions

- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents and require use of standard precautions.
- Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures.
- If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.







Specimen Collection Device

	Nasopharyngeal Swab and Transport Medium	Nasal Swab and Transport Medium			
Catalog #	SWAB/B-100	SWAB/F-100			
Intended Use	Designed to collect, preserve and transport respiratory virus specimens				
Kit	1 tube swab transport medium (3 mL, red cap)				
Contents	1 flocked swab with flexible shaft	1 flocked swab with rigid shaft			





Specimen Collection- Nasal Swab

Nasal Swab Specimen Collection

For use with Xpert® Swab Sample Collection Kit - Catalog # SWAB/F-100

Open the package that contains the swab and transport medium tube. Set the tube aside before collecting the specimen.



Repeat Step 4 on the other nostril with the same swab

To avoid specimen contamination, do not touch the swab tip to anything after collecting the specimen.



Open the swab wrapper and remove the swab, taking care not to touch the tip of the swab to any surface.



Remove the cap from the tube. Insert the swab into the transport



Hold the swab in your hand, pinching in the middle of the swab shaft on the scoreline

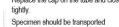


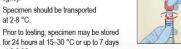
Break the swab shaft against the side of the tube at the scoreline.



Avoid splashing contents on the skin. Wash with soap and water if exposed.









Do not insert the swabs more than

Rotate swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of



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the nostril.

In Vitro Diagnostic Use (F IVD



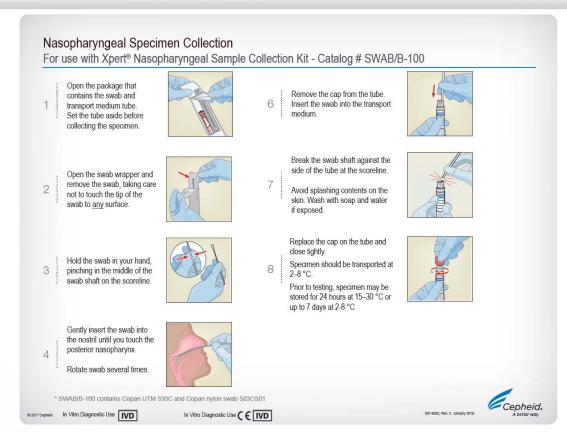




at 2-8 °C.

at 2-8 °C

Specimen Collection- Nasopharyngeal Swab





Specimen Collection, Transport and Storage

Sample type	Transportation	Storage Conditions	
Xpert Viral Transport Medium containing: Nasopharyngeal Swab	+2 P*8	+15 C Up to 24 hours +2 C Up to 7 days	
Or Nasal Swab			





Xpert Xpress Flu/RSV Cartridge Preparation

Xpert® Xpress Flu and Flu/RSV Cartridge Preparation

- Xpert® Xpress Flu/RSV Xpert® Xpress Flu*
- Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com Cepheid Technical Support US office (888) 838-3222, Option 2 techsupport@cepheid.com

European office +33 563 825 319 support@cepheideurope.com



Take one Xpert cartridge for each sample.



2 Invert the tube 5 times.





4 Using a clean 300 µL pipette (supplied), transfer 300 µL (one draw), of the sample to the opening of the cartridge.



5 Close the cartridge lid.



6 Start the test within the timeframe specified in the package insert.

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*FDA Cleared Assay Only

301-7307, Rev. B February 2017



Xpress Flu/RSV Cartridge Preparation



Take one Xpert cartridge for each sample.



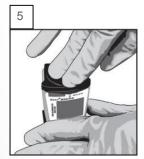
Invert the tube 5 times.



Open the cartridge lid.



Using a clean 300 µL pipette (supplied), transfer 300 µL (one draw), of the to the sample to the cartridge.



Close the cartridge lid.



Start the test within the timeframe specified in the packageinsert.



Run a Test

Create Test

GeneXpert



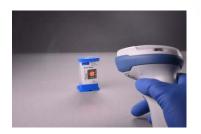
Start the test within 30 minutes after adding the sample to the cartridge

Scan barcode: Cartridge/ Patient and/or Sample ID



By default, do not click on Manual Entry or Cancel

Scan the cartridge

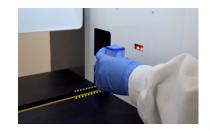


GeneXpert Infinity



Place the cartridge on the conveyor within 30 minutes of adding the sample.



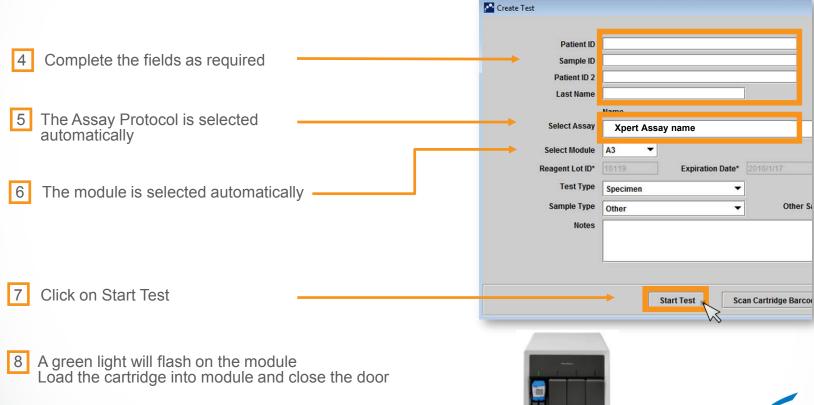


"For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx or Xpertise Operator Manuals.





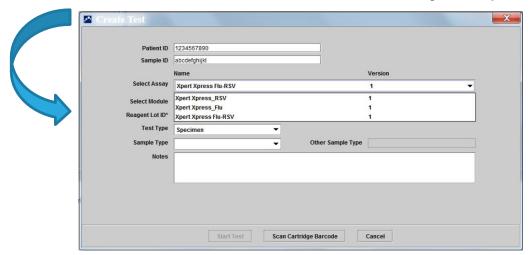
Create a Test on GeneXpert Dx Software





Combinatorial reporting: Xpert Xpress Flu/RSV

Choose the desired test from the "Select Assay" drop-down menu

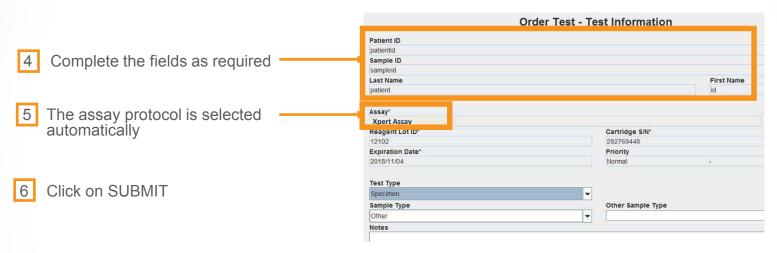


- Only the test result for the assay selected at this step will be collected once the test is started.
 - Example: If the operator selects Xpert Xpress_RSV, once the assay starts, the option cannot be changed to collect Flu data



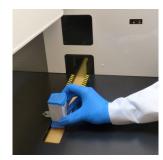


Create a Test on Xpertise Software





7 Place the cartridge into the conveyor belt





Automated Xpert Xpress Flu/RSV Test Steps

Nucleic acids are purified.

Purified nucleic acids mix with PCR reagents.

Place the cartridge into the instrument.

Add the sample to the cartridge



Simultaneous amplification and detection occurs.



Results are ready to view.







Assay Control Strategy



Xpert Assay Quality Controls

- Each Xpert cartridge is a self-contained test device
- Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge
 - Probe Check Controls (PCC)
 - Sample Processing Control (SPC)

Refer to 301-4868 GeneXpert Quality Control Features for All Cepheid Xpert Assays





Internal Quality Controls

Probe Check Controls (PCC)

- Before the PCR step, fluorescence signal is measured on all probes and compared with default factory settings to monitor
 - bead rehydration
- probe integrity
- reaction tube filling
- dye stability

Sample Processing Control (SPC)

- Verifies that conditions for adequate amplification process were met
- Detects PCR inhibition
- Should be positive in a negative sample
- Can be positive or negative in a positive sample



Commercially Available External Controls

Zeptometrix Part Number	Description	Configuration		
NATFLUA/B-6C	FLU A/B Positive	6 X 0.5 mL/box		
NATCXVA9-6C	Negative Control	6 X 0.5 mL/box		
NATRSV-6C	RSV Positive	6 X 0.5 mL/box		
http://www.zeptometrix.com				

- Storage conditions for external controls: 2-8°C
- Other options: known positive and negative patient samples





Results Summary

Result displayed	Flu A 1	Flu A 2	Flu B	RSV	SPC
Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE	+	+/-	-	-	+/-
	+/-	+			
Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE	+	+/-	+	-	+/-
	+/-	+			
Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE	+	+/-			
	+/-	+	-	+	+/-
Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE	+	+/-			
	+/-	+	+	+	+/-
Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE	-	-	+	-	+/-



Results Summary continued

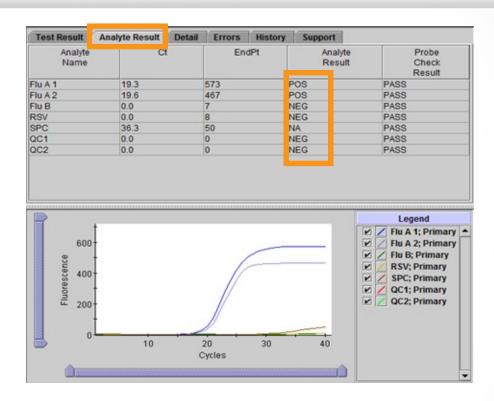
Result displayed	Flu A 1	Flu A 2	Flu B	RSV	SPC
Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE	-	-	-	+	+/-
Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE	-	-	+	+	+/-
Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	-	-	-	-	+
INVALID	-	-	-	-	-
ERROR	NO RESULT				
NO RESULT	NO RESULT	NO RESULT	NO RESULT	NO RESULT	NO RESULT



Flu A POSITIVE



- Flu A target RNA detected;
 Flu B target RNA not detected;
 RSV target RNA not detected
- Flu A POSITIVE; Flu A target has a valid Ct.
- SPC Not Applicable; The SPC is ignored because the Flu A target amplification may compete with this control.
- PCC PASS; All probe check results pass

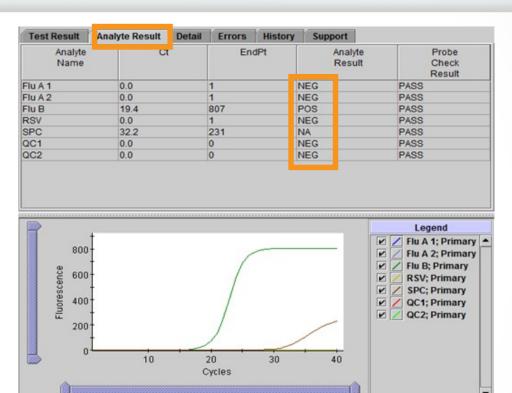




Flu B POSITIVE



- Flu B target RNAdetected; Flu A target RNA not detected; RSV target RNA not detected
- Flu B POSITIVE; Flu B target has a valid Ct.
- SPC Not Applicable ;The SPC is ignored because the Flu B target amplification may compete with this control.
- PCC PASS; All probe check results pass

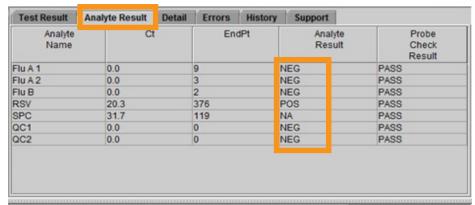


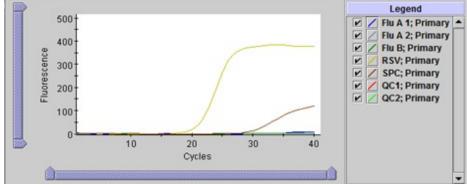


RSV POSITIVE



- RSV target RNA detected; Flu A and Flu B target RNA not detected
- RSV POSITIVE; RSV target has a valid Ct.
- SPC Not Applicable; The SPC is ignored because the RSV target amplification may compete with this control.
- PCC PASS; All probe check results pass



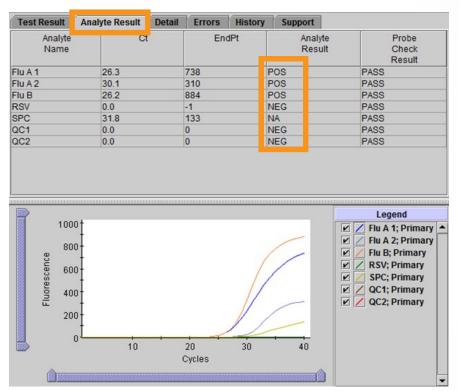




Flu A and Flu B POSITIVE



- Flu A and Flu B target RNA detected; RSV target RNAnot detected
- Flu A and Flu B targets have a valid Ct.
- SPC Not Applicable; The SPC is ignored because the Flu A and Flu B target amplification may compete with this control.
- PCC PASS; All probe check results pass

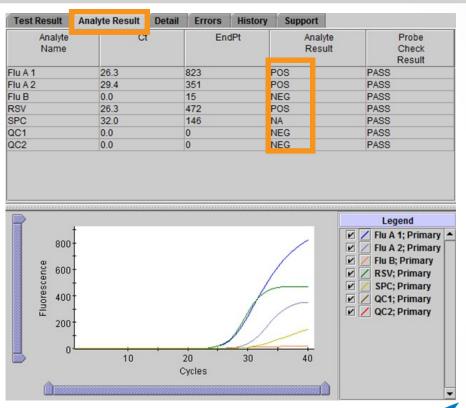




Flu A and RSV POSITIVE



- Flu A and RSV target RNA detected: Flu B target RNAnot detected
- Flu Aand RSV targets have a valid Ct.
- SPC Not Applicable; The SPC is ignored because the Flu A and RSV target amplification may compete with this control
- PCC PASS; All probe check results pass

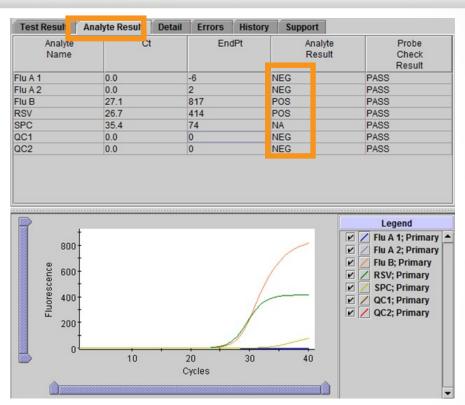




Flu B and RSV POSITIVE



- Flu B and RSV target RNA detected: Flu A target RNA not detected
- Flu B and RSV targets have a valid Ct.
- SPC Not Applicable; The SPC is ignored because the Flu B and RSV target amplification may compete with this control
- PCC PASS; All probe check results pass

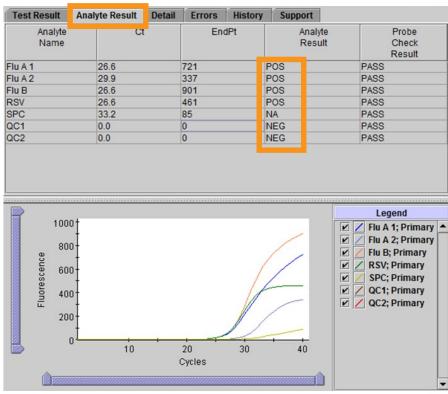




Flu A, Flu B, and RSV POSITIVE



- Flu A, Flu B, and RSV target **RNA** detected
- Flu A, Flu B, and RSV targets have a valid Ct.
- SPC Not Applicable; The SPC is ignored because the Flu A, Flu B, and RSV target amplification may compete with this control.
- PCC PASS; All probe check results pass

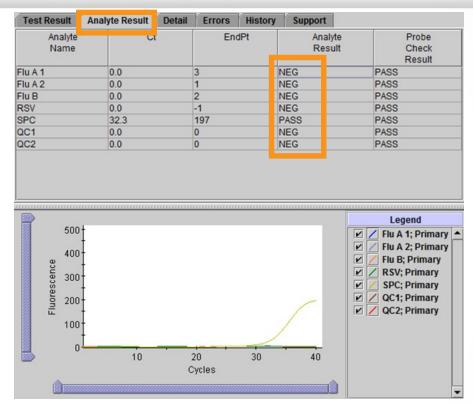




Flu A, Flu B, and RSV Negative



- Flu A, Flu B, and RSV target RNA not detected
- SPC PASS; SPC has a Ct within the valid range and endpoint above the threshold setting.
- PCC PASS; All probe check results pass





Xpert® Xpress Flu/RSV Early Assay Termination (EAT)

- EAT for Flu-only and RSV-only ADFs
 → positive results available as early as 20 mins depending on viral titer
- EAT activated when pre-determined threshold for a positive test result is reached before the full 40 PCR cycles completed

Please note:

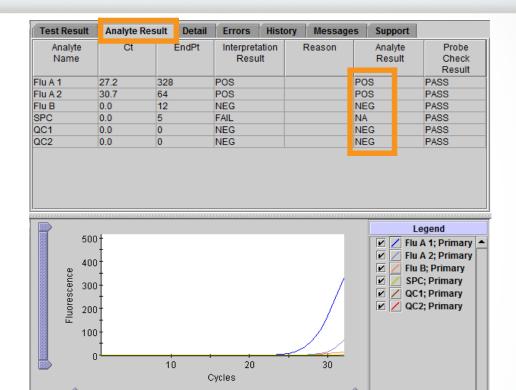
- When Flu A or Flu B titers are very high, generating very early Cts with Xpert Xpress Flu, SPC amplification curves may not be seen and not reported.
- When RSV titers are very high, generating very early Cts with Xpert Xpress RSV, SPC amplification curves may not be seen and not reported.



Flu A POSITIVE and Flu B NEGATIVE



- Flu A target RNA detected.
- Flu A targets have a valid Ct.
- SPC Not Applicable; The SPC is ignored because the Flu A and Flu B target amplification may compete with this control.
- PCC PASS; All probe check results pass

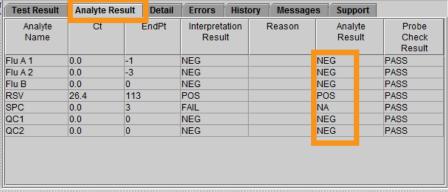


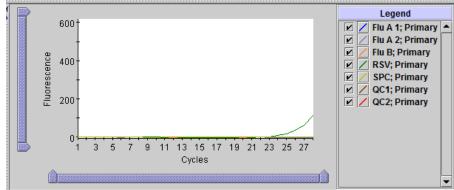


RSV POSITIVE

Test Result RSV POSITIVE

- RSV target RNA detected
- RSV POSITIVE; RSV target has a valid Ct.
- SPC Not Applicable; The SPC is ignored because the RSV target amplification may compete with this control.
- PCC PASS; All probe check results pass







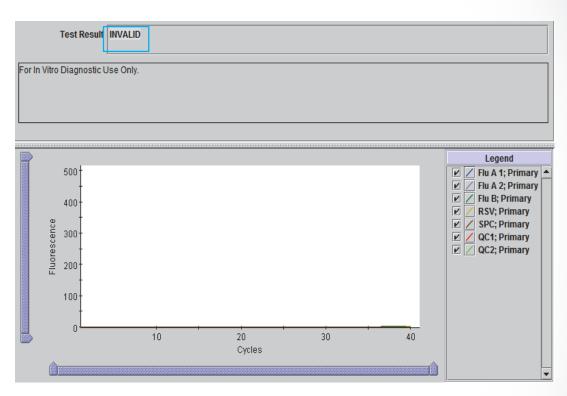


Reasons to Repeat the Assay

- An INVALID result indicates that the sample was not processed properly, PCR was inhibited, or the sample was inadequate.
- An ERROR result indicates that the Probe Check Control failed or maximum pressure limits were exceeded.
- A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, a load error occurred, or the software was closed prematurely.
- Because the incidence of co-infection with two or more viruses (Influenza A, Influenza B, and RSV) is low, it is recommended that specimens undergo repeat testing if nucleic acids from two or more analytes are detected in a single specimen.



- SPC does not meet acceptance criteria. Presence or absence of the target RNAs cannot be determined. Repeat the test according to the instructions in the Retest Procedure section of the package insert.
- SPC FAIL; SPC target result is negative. The SPC Ct is not within valid range and fluorescence endpoint is below the minimum setting.
- PCC PASS; all probe check results pass.

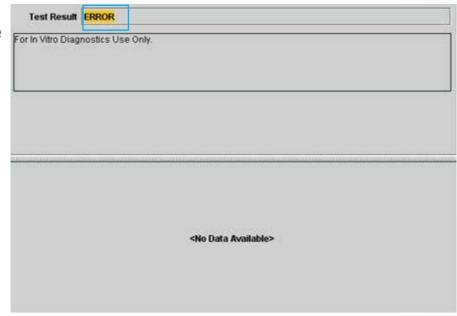




Presence or absence of Flu A, Flu B, and/or RSV target RNAs cannot be determined.

Repeat the test according to the instructions in the Retest Procedure section of the package insert.

- Flu A NO RESULT
- Flu B NO RESULT
- RSV NO RESULT
- SPC NO RESULT
- PCC FAIL.* All or one of the probe check results fail.
- * If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure





The presence or absence of Flu A, Flu B, and RSV target RNAs cannot be determined.

- Repeat the test according to the instructions in the Retest Procedure section of the package insert.
- Flu A NO RESULT
- Flu B NO RESULT
- RSV NO RESULT
- SPC NO RESULT
- PCC Not Applicable







Re-test Procedure

1

Discard used cartridge

Follow your institution's safety guidelines for disposal of cartridges

2



Obtain the residual sample, mix according to Package Insert

If the leftover sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample

3



Obtain a new cartridge

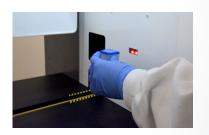
Label appropriately as retest on the new cartridge

Process the sample per the Package Insert

4



Run the test on the System







Factors That Negatively Affect Results

- Improper specimen collection
 - The viral load in the specimen is below the detection limit of the test
 - Performance with other specimen types has not been assessed
- Improper transport or storage of collected specimen
 - Storage and transport conditions are specimen specific
 - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
 - Modification to the testing procedures may alter the performance of the test
 - Careful compliance with the Package Insert is necessary to avoid erroneous results





Technical Assistance

- Before contacting Cepheid Technical Support, collect the following information:
 - Product name
 - Lot number
 - Serial number of the System
 - Error messages (if any)
 - Software version and, if applicable, Computer Service Tag number

• Log your complaint online using the following link http://www.cepheid.com/us/support: Create a Support

Region	Telephone	Technical Support Email
US	+ 1 888 838 3222	techsupport@cepheid.com
Australia and New Zealand	+ 1800 130 821 (AU) + 0800 001 028 (NZ)	techsupportANZ@cepheid.com
Brazil and Latin America	+ 55 11 3524 8373	latamsupport@cepheid.com
China	+ 86 400 821 0728	techsupportchina@cepheid.com
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