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Valley Laboratories	**Next Review:**	*2 years after approval*
	Owner:	*Lindsey Westerbeck: Dir, Lab*
	Policy Area:	*Lab - Serology*
	References:	
	Applicability:	*Valley Laboratories*

Performing an Xpress Flu Assay on the Cepheid GeneXpert Dx System

PURPOSE

The Cepheid Xpress Flu Assay, performed on the GeneXpert Dx 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 and influenza B viral RNA. The Xpress Flu Assay uses nasopharyngeal (NP) swab specimens collected from patients with signs and symptoms of respiratory infection. This test is performed on the Cepheid GeneXpert System using a self-contained, single-use disposable cartridge. This Xpress Flu Assay is intended as an aid in the diagnosis of influenza infections in conjunction with clinical and epidemiological risk factors.

POLICY

- The Xpress Flu Assay will be performed on upper respiratory specimens when a testing request for influenza A & B is ordered by the clinician.
- This procedure is to be followed when testing for influenza A & B using the Xpress Flu Assay is performed.
  - Test code: **FLUAB** – *"Flu A/B by PCR"*
- Flu A/B PCR testing is performed on all shifts upon receipt of the sample.

EQUIPMENT, REAGENTS AND SUPPLIES

Equipment	Reagents	Supplies
Biosafety Cabinet	Xpress Flu Cartridge	300 µL disposable transfer pipettes *(included in kit)*
GeneXpert System	External Positive and Negative Controls	3 mL BD™ Universal Viral Transport Media (UVT)
Vortex	10% Bleach (*made fresh weekly*)	Disposable Gloves
Cartridge Carrying Tray *(optional)*	70% Reagent Alcohol (*made fresh weekly*)	50 µl MLA Pipette and Tips (for external QC)

STORAGE & HANDLING OF REAGENTS

- Store the Xpress Flu Assay cartridges at 2-28°C until the expiration date provided on the label.
- Do not open a cartridge lid until you are ready to perform the testing.
- Do not use cartridges that have passed the expiration date.
- Do not use a cartridge that has leaked.

SPECIMEN REQUIREMENTS

- Nasopharyngeal swabs (NP) placed in 3 mL UVT collection kit
  - **NOTE:** Universal Transport Media (UTM) is equivalent to BD™ Universal Viral Transport Media (UVT) and is acceptable.
- Specimens are to be stored and transported at 2-8°C
  - Specimens can be stored at room temperature (15-30°C) for up to 24 hours and refrigerated (2-8°C) for up to seven days until testing is performed.

SPECIMEN REJECTION CRITERIA

- Specimens received unlabeled or leaking
- Dry swabs
- Specimens received outside of acceptable transport and storage conditions
- NP swabs received in a viral transport other than UVT or UTM
  - **NOTE** : No other viral transport media has been validated for this test
- Respiratory specimens from sources other than NP swabs.

INTERNAL QUALITY CONTROL

Each cartridge includes a built-in Sample Processing Control (SPC) and a Probe Check Control (PCC) and therefore, internal quality control id performed with each test.

- **Sample Processing Control (SPC)** — Ensures the sample was processed correctly. The SPC is an Armored RNA® that is

included in each cartridge to verify adequate processing of the sample. The SPC verifies that release of RNA from the
This control also detects specimen-associated inhibition of the RT-PCR and PCR reactions.
    - The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC **PASSES** if it meets the validated acceptance criteria.
    - The assay result is **INVALID** if all targets are reported negative and the SPC does not meet the validated acceptance criteria.
- **Probe Check Control (PCC, QC1, QC2)** — Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the first PCC (QC1 and QC2) performed before the reverse transcription step. QC1 checks for the presence of the EZR bead and QC2 checks for the presence of the TSR bead. The second PCC (Flu A 1, Flu A 2, Flu B and SPC) is performed after the reverse transcription step and before PCR begins. The PCC monitors bead rehydration, reaction tube filling, probe integrity, and dye stability.
    - The PCC **PASSES** if it meets the validated acceptance criteria.
    - If any of the PCC criteria fail, the test results in an **ERROR**.

EXTERNAL QUALITY CONTROL

- External controls are run with each new lot and and every 30 days.
    - **NOTE**: External controls are also to be run after a major service event.
- The external controls are not included in the Xpress Flu assay, but are commercial controls from Zeptometrix and purchased separately.
    - See **Procedure** *External QC* for instructions on preparing cartridge for testing.

NATtrol™ Influenza External Controls	External Control Designation	Expected Results
Influenza A/Brisbane/59/07 Influenza B/Florida/02/06	**Flu AB Positive**	• Flu A Positive • Flu B Positive
Coxsackie virus A9	**Flu AB Negative**	• Flu A Negative • Flu B Negative

- QC results and any actions taken are documented on *Form A: Xpress Flu External QC.*
- Patient testing is not to be performed unless QC is acceptable.

If	Then
All three controls give the expected results	• QC is acceptable.
One or more of the controls did not give the expected results	• QC is not acceptable. • Repeat the control that was "out" using a new aliquot of control material.
Unable to resolve upon repeat testing	• QC is not acceptable. • **Do not perform patient testing** and notify supervisor. • Contact Cepheid technical support for additional troubleshooting.

PROCEDURE - EXTERNAL QC TESTING

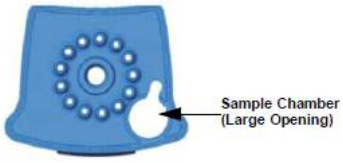
Step	Action
1.	Obtain commercial external controls from refrigerator.
2.	Obtain one cartridge and one Binding Reagent vial per control to be tested and take care to handle the cartridge without touching the reaction tube. • Do not open the cartridge lid except when adding sample and reagent.
3.	Visually inspect the cartridge, reaction tube, and vial. • Do not use a cartridge that has been dropped. • Do not use a cartridge that has a damaged reaction tube.
4.	Label one side of the cartridge with the external QC to be tested.
5.	Processing ONE control sample at a time, pipette 50µl of the control sample and expel into a new UVT vial. • Ensure the UVT vial is clearly labeled with control name, lot #, date made and expiration date (7 days post date made). • Store UVT vials of controls at 2-8°C.
6.	Follow Steps 7-12 in **Procedure** *Patient Testing*
7.	Change gloves and repeat steps for the next control sample.
8.	When programming External QC on Cepheid, scan appropriate QC barcode at **Sample ID** prompt for the cartridge being loaded: • **FLUAB POS CTRL:**  • **FLUAB NEG CTRL**: 
9.	Upon completion of the assay for external QC, retrieve instrument printouts.

Step	Action
10.	Check printouts to make sure the external QC gave anticipated results and document as needed.
11.	Date and initial QC printouts and file behind *Form A: Xpress Flu External QC.*

PROCEDURE - PATIENT TESTING

IMPORTANT: Work surfaces should be cleaned and decontaminated before and after processing samples to prevent cross-contamination.

- Perform **all** cartridge and sample preparation under the biosafety cabinet.
- Change gloves between samples.
- Change or remove gloves before using computer.
- Use Standard Precautions when handling samples and used cartridges, as they are capable of transmitting infectious agents.

Step	Action
1.	Clean work surface inside hood with 10% bleach, followed by a 70% reagent alcohol rinse before beginning any PCR testing.
2.	Obtain one cartridge per specimen to be tested and take care to handle the cartridge without touching the reaction tube. • Do not open the cartridge lid except when adding sample and reagent.
3.	Visually inspect the cartridge and reaction tube. • Do not use a cartridge that has been dropped. • Do not use a cartridge that has a damaged reaction tube.
4.	Label one side of the cartridge with a small Sunquest barcode label. • Do not cover the barcode label on the cartridge itself.
5.	Assess sample being tested for acceptability. Verify NP swab is **not** a green-top wire shaft swab and that the NP swab is in the appropriate transport media.
6.	Processing ONE sample at a time, mix specimen by inverting the UVT tube five times.
7.	Open the cartridge lid and uncap the corresponding patient specimen. Using the 300 µL pipette supplied in the kit, transfer 300 µL (one draw) of the specimen from the transport medium tube to the sample chamber by expressing fluid into the large opening of the Flu cartridge. 
8.	Close the cartridge lid and place cartridge on carrying tray or worksurface outside of the biosafety cabinet. • **NOTE:** Test must be started within **30 minutes** of adding the sample and reagent to the cartridge.
9.	Change gloves and repeat steps for each additional specimen to be setup.
10.	Once all samples are processed, carry cartridge(s) to the instrument to load for testing.
11.	Upon completion of patient testing, samples are to be stored refrigerated for 7 days post collection.

INTERPRETING RESULTS

The Xpress Flu Assay has two channels (Flu A 1 and Flu A 2) to detect most influenza A strains. All influenza A strains detected by the Xpress Flu Assay are reported as **Flu A POSITIVE**. The Xpress Flu Assay requires either the Flu A 1 or Flu A 2 channel to be positive in order for a **Flu A POSITIVE** test result to be reported. Table below lists all the possible test results for Flu A.

Flu A Test Result	Flu A 1 Channel	Flu A 2 Channel
Flu A POSITIVE	POS	POS/NEG
	POS/NEG	POS
Flu A NEGATIVE	NEG	NEG

The results reported from testing with the Xpress Flu Assay are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window.

All Possible Final Test Results for the Xpert Xpress Flu/RSV Assay

Result Interpretation on Printout	Flu A 1	Flu A 2	Flu B	SPC
Flu A POSITIVE; Flu B NEGATIVE	POS	POS/NEG	NEG	POS/NEG
	POS/NEG	POS		
Flu A POSITIVE; Flu B POSITIVE**	POS	POS/NEG	POS	POS/NEG
	POS/NEG	POS		

Flu A NEGATIVE; Flu B POSITIVE	NEG	NEG	POS	POS/NEG
Flu A NEGATIVE; Flu B NEGATIVE	NEG	NEG	NEG	POS

****NOTE:** Because the incidence of co-infection with Influenza A and Influenza B viruses is low, it is recommended that testing of the original specimen is repeated if both Flu A and B are detected in a single specimen.

The following scenarios may cause a result ***other than*** Positive or Negative:

Result	Interpretation
INVALID	SPC does not meet acceptance criteria. Presence or absence of the target RNAs cannot be determined. Repeat test using new cartridge and new sample aliquot.
ERROR	Presence or absence of Flu A and/or Flu B target RNA cannot be determined. Repeat test using new cartridge and new sample aliquot. • Flu A: NO RESULT • Flu B: NO RESULT • SPC: NO RESULT • Probe Check: 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.*
NO RESULT	Presence or absence of Flu A and/or Flu B target RNA cannot be determined. Repeat test using new cartridge and new sample aliquot. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred. • Flu A: NO RESULT • Flu B: NO RESULT • SPC: NO RESULT • Probe Check: NA *(not applicable)*

- If the Xpress Flu assay gives an INVALID, ERROR, or NO RESULT; repeat the assay using a new cartridge.
- You **cannot** aspirate and re-use sample from original cartridge.
- If the assay fails again upon repeat, request a new sample be collected.

REPORTING RESULTS

Test Code	FLUAB
Xpress Flu Result	**If Xpert Flu/RSV Result** / **Sunquest Result Field** / **Then Report As** Flu A **POSITIVE** / FLUA / DET1 Flu B **POSITIVE** / FLUB / DET1 Flu A **NEGATIVE** / FLUA / NOTDET Flu B **NEGATIVE** / FLUB / NOTDET
Reference Range	Not Detected (NOTDET)
Critical Value	None
Technical Range	None

- File patient Xpress Flu printouts and worksheet in designated area.

TECHNICAL NOTES

- The performance of the Xpert Xpress Flu Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Results from the Xpert Xpress Flu Assay should be interpreted with other laboratory and clinical data available to the clinician.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; sample mix-up; or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- False negative results may occur if virus is present at levels below the analytical limit of detection.
- Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from analytical studies show potential for competitive inhibition in specimens with both influenza A and influenza B viruses present. However, numerous studies have shown that infections with combinations of only these specific viruses (Flu A, and Flu B) occur in <1.6% of patients.9.10,11
- The Xpert Xpress Flu Assay uses EAT. In the event of a mixed Flu A and Flu B infection, the target with the higher titer of the two infections may be reported as POSITIVE and the lower titer target may be reported as NEGATIVE.
- Results from the Xpert Xpress Flu Assay should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral nucleic acid may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- This test has been evaluated for use with human specimen material only.

- If the virus mutates or there are other sequence changes in the target region, influenza virus may not be detected, or may be detected less predictably.
- Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2015-2016 influenza season for NP swab specimens and during the 2016-2017 influenza season for NS specimens. The performance may vary depending on the prevalence of the different viruses and population tested.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for patients without signs and symptoms of influenza infection.
- This test has not been evaluated for monitoring treatment of influenza infection.
- This test has not been evaluated for screening of blood or blood products for the presence of influenza.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- This assay has not been evaluated for immunocompromised individuals.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
- Although this test has been shown to detect A/H1N1 (pre-2009 pandemic), A/H7N9 (detected in China in 2013) and A/ H3N2v viruses cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for the A/H1N1 (pre-2009 pandemic), A/H7N9 (detected in China in 2013) and A/ H3N2v viruses have not been established.
- This test is not intended to differentiate Influenza A subtypes or Influenza B lineages. If differentiation of specific influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.

REFERENCES

- Xpert® Xpress Flu Assay Product Insert 301-7268, Rev E April 2019.

All revision dates:

Attachments:

Form A: Xpress Flu External QC

Approval Signatures

Step Description	Approver	Date
Lab Medical Directors	Kristen Vandewalker: MD	pending
Lab Medical Directors	Andrea Ong: MD	pending
Lab Medical Directors	Hannah Wong: MD	pending
Lab Medical Directors	Rowberry Ron: MD	pending
Lab Medical Directors	Jamie Cassity: MD	pending
Lab Medical Directors	Mary Keohane: MD	pending
Lab Medical Directors	Marian Butcher: MD	pending
	Lindsey Westerbeck: Dir, Lab	10/16/2019