TITLE: Cepheid - XPERT® Flu

PRINCIPLE/PURPOSE: The Cepheid Xpert® Flu Assay, performed on the GeneXpert® Instrument Systems, is an automated, multiplex real-time RTPCR assay intended for the *in vitro* qualitative detection and differentiation of influenza A and influenza B viral RNA. The Xpert Flu Assay is intended as an aid in the diagnosis of influenza.

Influenza viruses are classified into types. Influenza A is the most common type of influenza virus in humans, and is generally responsible for seasonal flu epidemics and potentially can cause pandemics.

Active surveillance programs in conjunction with infection control precautions are important components for preventing transmission of influenza. The use of assays that provide rapid results to identify and differentiate the infecting agent in patients infected with seasonal flu is also an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks of influenza.

SCOPE: To provide instruction on the processing and testing of patient and quality control specimens for influenza PCR testing.

COMPLEXITY LEVEL: Moderate

SAFEY:

The required personal protective equipment for this procedure: Gloves, approved lab coats (worn closed), and biological safety cabinet.

SPECIMEN:

Patient Prep: N/A

Type: Nasopharyngeal swab placed in universal transport media (UTM).

Accepatble containers: Approved UTM

Volume required: 300 µL

Storage and stability: Specimens can be stored at room temperature for up to 24 hours and refrigerated up to 7 days.

Unacceptable specimens: Nasal washing/aspirate.

Handling Conditions: Specimens should be processed under the biological safety cabinet.

EQUIPMENT AND MATERIALS:

Equipment:

GeneXpert DX

Vortex

Safety Hood

Safety Shield

Materials:

Xpert Flu Assay Cartridges

UTM

Disposable 300 μL Transfer Pipettes

Gloves

Lab Coat

CALIBRATION: Calibration is to be performed on each GeneXpert module after every 2,000 PCR tests or 1 year of utilization, whichever comes first. Refer to Cepheid GeneXpert Calibraion Procedure for details.

QUALITY CONTROL:

Internal Quality Controls:

Each test includes a Sample Processing Control (SPC) and a Probe Check Control (PCC).

• Sample Processing Control (SPC): Ensures the sample was correctly processed. The SPC is an Armored RNA® in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample virus. The SPC verifies that

lysis of influenza virus has occurred if the organism is present and verifies that the specimen processing is adequate.

Additionally this control 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.

• Probe Check Control (PCC): Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the assigned acceptance criteria.

External Controls:

External Controls are performed with each new lot number and/or shipment.

FLU A/B POSITIVE CONTROL – NATrol Flu A/B Positive

FLU A/B NEGATIVE CONTROL – NATrol Flu A/B Negative

QC Procedure – Stepwise:

1. Clean work area with 70% ethanol.
2. Change gloves.
3. Open the cartridge lid. Vortex NATrol™ sample for 5-10 seconds. Using a clean transfer pipette, transfer 300 µL into the chamber with the LARGER opening.
4. Close cartridge lid.
5. Sign onto the GeneXpert using your username and password.
6. In the GeneXpert system window, click Create Test. The Create Test window will open.
7. Type the Control ID. The Control ID is associated with the test results and is shown in the View Results window.



1. Scan the barcode on the Xpert Flu Assay cartridge. Using the barcode information, the software automatically fills in the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
2. Click Start Test. In the dialog box that appears, type your password
3. Open the instrument module door with the blinking green light and load the cartridge.
4. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
5. Wait until the system releases the door lock before opening the module door and removing the cartridge.
6. The used cartridges should be disposed in the appropriate specimen waste containers.

Repeat steps 1 – 13 for ALL controls.

PROCEDURE:

Note: Start the test within 30 minutes of adding the sample and reagent to the cartridge.

1. Clean work area with 70% Ethanol.
2. Change gloves.
3. Remove the cartridge and reagents from the package.
4. Mix specimen by inverting the UTM tube five times.
5. Open the cartridge lid. Using a clean transfer pipette (supplied), transfer 300 µL of the UTM to the chamber with the large opening in the cartridge.
6. Clean work area with 70% Ethanol.
7. Change gloves.
8. Remove the cartridge and reagents from the package.
9. Close the cartridge lid.
10. Log on to the GeneXpert using your user name and password.



1. In the GeneXpert System window, click Create Test. The Create Test window will open.
2. Scan the patient barcode (accession number). The patient accession number is associated with the test results and is shown in the View Results window.
3. Scan the barcode on the Xpert Flu Assay cartridge. Using the barcode information, the software automatically fills in the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
4. Type the patients name in the notes section.
5. Click Start Test. In the dialog box that appears, type your password
6. Open the instrument module door with the blinking green light and load the cartridge.
7. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
8. Wait until the system releases the door lock before opening the module door and removing the cartridge.
9. The used cartridges should be disposed in the appropriate specimen waste containers.

INTERPRETATION & REPORTING RESULTS:

Reference Ranges: Not Detected – Influenza A, Influenza B

Reporting Format: Results are interfaced.

Record results in LIS AND on patient log.

INTERFACE RESULT ENTRY:

1. SELECT RESULT TAB.
2. SELECT INTERFACE AS THE RESULTING MODE.
3. ENTER THE CORRECT METHOD CODE IN THE SELECT METHODS FIELD AND CLICK ADD.
4. ONCE THE METHOD IS ACTIVATED, SELECT TRESULTS AT THE BOTTOM RIGHT OF THE SCREEN.
5. SELECT THE BINOCULAR ICON FROM THE BOTTOM FO THE PAGE TO ACCESS THE SEARCH WINDOW.
6. USE THE BARCODE SCANNER TO SCAN THE CID AND ACCESS PATIENT RESULTS.
7. REVIEW THE RESULTS AND CHECK FOR QA FAILURES. IF THERE IS A CRITICAL VALUE OR RESULT THAT REQUIRES A CALL, ENTER THE APPROPRIATE COMMENTS.
8. SELECT SAVE.

MANUAL RESULT ENTRY:

1. SIGN INTO SUNQUEST
2. SELECT “RESULT ENTRY”
3. SELECT “MANUAL”
4. ENTER WORKSHEET “ARURIN”
5. “TAB”
6. CLICK “RESULT”
7. ENTER ACCESSION
8. “TAB”
9. ENTER RESULT (NEG OR POS)
10. “TAB”
11. ENTER RESULT (NEG OR POS)
12. “TAB”
13. “SAVE”
14. REVIEW RESULTS
15. “ACCEPT”

Procedures for abnormal results:

 An INVALID result indicates one or more of the following:

* + The control SPC failed.
	+ The sample was not properly processed or PCR was inhibited.
	+ An ERROR result indicates that the assay was aborted. Possible causes include: the reaction tube was filled improperly, a reagent probe integrity problem was detected, or the maximum pressure limit was exceeded.
	+ 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.

Reporting Format:

POS – FLU A POSITIVE

NEG – FLU A NEGATIVE

POS – FLU B POSITIVE

NEG – FLU B NEGATIVE

Result Interpretations:

 Flu A POSITIVE

 Flu B NEGATIVE

Flu A target RNA detected, Flu B target RNA not detected.

 Flu A POSITIVE

Flu B NEGATIVE

Flu A target RNA detected, Flu B target RNA not detected.

 Flu A NEGATIVE

Flu B POSITIVE

 Flu B target RNA detected, Flu A target RNA not detected.

 Flu A NEGATIVE

 Flu B NEGATIVE

Flu A target RNA not detected Flu B target RNA not detected.

Flu A POSITIVE

Flu B POSITIVE

Flu A target RNA detected, Flu B target RNA detected.

Rebooting:

Shutdown:

1. Click on the RED “X” in the top right corner.
2. Click on the windows icon in the bottom left corner.
3. Click on “shutdown”.

Startup:

1. Turn on computer tower.
2. Double click on the FilmArray icon.

PROCEDURE NOTES:

In a non-clinical study, potentially interfering substances that may be present in the nasopharynx were evaluated directly relative to the performance of the Xpert Flu Assay. Potentially interfering substances in the nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and anti-virals. These substances are listed below. Highly viscous samples resulting from the addition of 1.5% (w/v) and 2.5% (w/v) mucin yielded false-negative test results from the Xpert Flu Assay. Inhibition of the Xpert Flu Assay was also observed from the addition of 1% (w/v) mucin, resulting in delayed detection of influenza A and influenza B.

Potentially Interfering Substances in Xpert Flu Assay Substance Description/Active Ingredient Concentration Tested

Blood (human)

Mucin Purified mucin protein

Neo-Synephrine® Nasal

Drops Phenylephrine HCl 15%

Anefrin Nasal Spray Oxymetazoline Hydrochloride

Zicam® Nasal Gel Luffa opperculata, Galphimia glauca,

Histaminum hydrochloricum Sulfur

Saline Nasal Spray Sodium Chloride with preservatives

Antibiotic, nasal ointment Mupirocin

Antibacterial, systemic Tobramycin

Antiviral Oseltamivir (TamiFlu)

Throat lozenges, oral anesthetic and analgesic Menthol

RELATED PROCEDURES:

Specimen Collection and Labeling

Microbiology Specimen Collection

Specimen Labeling

GeneXpert Calibration

LIMITATIONS OF THE PROCEDURE:

False negative test results have been observed in the presence of mucin, likely due to the inhibition by high sample viscosity.

• In the presence of blood, delayed Ct values of less than 1 have been observed.

• The performance of the Xpert 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 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.

• 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 the Xpert 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 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 2009–2010, 2010–2011 and 2011–2012 influenza seasons. The performance may vary depending on the prevalence 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 product for the presence of influenza

SUPPLEMENTAL MATERIALS/ADDENDUM:

Package inserts:

* NATrol Influenza External Run Controls
* Xpert Flu
* Copan Universal Transport Medium (UTM) System

REFERENCES:

1. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential

pandemics. *J Infect Dis*. 2006;194:S98–110.

2. Schweiger B, Zadow I, Heckler R, et al. Application of a fluorogenic PCR assay for typing and subtyping of influenza

viruses in respiratory samples. *J Clin Micro*. 2000;38:1552–1558.

3. Centers for Disease Control and Prevention. Seasonal Influenza. http://www.cdc.gov. Accessed on September 19, 2012.

4. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical laboratories*. Richmond JY and

McKinney RW (eds) (1993). HHS Publication number (CDC) 93-8395.

5. Centers for Disease Control and Prevention. Interim Biosafety Guidance for All Individuals Handling Clinical Specimens or

Isolates Containing 2009-H1N1 influenza A Virus (Novel H1N1), including Vaccine Strains, August 15, 2009; (http://

www.cdc.gov/h1n1flu/guidelines\_labworkers.htm).

6. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). *Protection of*

*Laboratory Workers from Occupationally Acquired Infections; Approved Guideline*. Document M29 (refer to latest edition).

7. Xpert Flu package insert.

8. NATrol Influenza External Run Controls package insert.

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|  |  Signature Date |
| Medical Director Approval – ARMC Cancer CenterARMC Main Lab | Original hard copy signed |  |
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HISTORY PAGE

SOP Number: MICRO-723-CH

SOP Title: Cepheid Xpert Flu

Written By: Jacee Farmer

Manual in which Hard Copy of this SOP is located: Microbioloby Manual

Distribution: Sharepoint

Supersedes Procedure:

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|   | Approvals |   | Action | In |
| Mgmt. | Date |  Director | Date |   | Effect |
| Jacee Farmer | 10/31/17 |  |  | Updated Xpert Flu information (H1N1 no longer dected) | 10/31/17 |
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SOP CHANGE CONTROL