**St. Elizabeth’s Medical Center**

**Department of Laboratory Services**

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| **POLICY NO: SE.MIC.00.0000.00**  **SUBJECT: Xpert Flu/RSV XC** |
| **Effective Date: 10/16** |
| **Supersedes: none** |
| **Medical Director: David Ricklan, MD, Ph.D** |
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| **Reviewed by:**  **Note Annual Review on Last Page** |

# Principle:

The Xpert Flu/RSV XC Assay is an automated in vitro diagnostic test for qualitative detection of influenza A, influenza B, and RSV. The assay is performed on Cepheid GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample extraction, purification, amplification, and detection of nucleic acid target sequences in clinical specimens by using reverse transcription (conversion of RNA templates into DNA) followed by real-time PCR. The primers and probes in the Xpert Flu/RSV XC Assay are designed to amplify and detect unique sequences in the genes which encode for the following proteins: Flu A Matrix (M), Flu A Basic Polymerase (PB2), Flu A Acidic protein (PA), Flu B Matrix (M), Flu B Non-Structural protein (NS), and the RSV A and RSV B Nucleocapsid gene. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. Each test requires the use of a single-use disposable GeneXpert cartridge that contains target-specific reagents and carries out the RTPCR and PCR processes. Because the cartridges are self-contained, the risk of cross- contamination between samples is minimized. For a full description of the systems, refer to the appropriate GeneXpert Dx System Operator Manual. The Xpert Flu/RSV XC Assay includes reagents for the detection and differentiation of influenza A, influenza B, and RSV viral RNA directly from nasal aspirate/wash (NA/W) specimens and nasopharyngeal (NP) swab specimens from patients with signs and symptoms of respiratory infection. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate extraction and processing of the target sequences and to monitor for the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The Xpert Flu/RSV XC Assay can be run to detect Flu A and Flu B only by selecting Xpert Flu XC US-IVD; RSV only by selecting Xpert RSV US-IVD; or Flu A, Flu B, and RSV by selecting Xpert Flu-RSV XC US-IVD from the Select Assay menu. Xpert Flu XC US-IVD and Xpert RSV US-IVD assays have an Early Assay Termination (EAT) function that enables early result reporting. EAT is activated when the pre-determined threshold for a positive test result is reached before the full 40 PCR cycles (Ct) have been completed. When Flu A or Flu B viral titers are high enough to generate very early Cts with the Xpert Flu XC US-IVD Assay, SPC amplification curves will not be seen and its results will not be reported. When RSV titers are high enough to generate very early Cts with the Xpert RSV US-IVD Assay, SPC amplification curves will not be seen and its results will not be reported.

# Purpose:

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily airborne (i.e., coughing or sneezing); the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, muscle aches, malaise, cough, and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting, or diarrhea) may also occur, primarily in children, but are less common in adults. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication of influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations. Influenza viruses are classified into types A, B, and C, the former two of which cause most human infections. Influenza A is the most common type of influenza virus in humans, and is generally responsible for seasonal flu epidemics and occasionally for pandemics. Influenza A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B virus are generally restricted to humans and are less frequent causes of epidemics. Influenza A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by subtypes H1, H2, H3, and N1 and N2. In addition to seasonal flu, a novel H1N1 strain was identified in humans in the United States in early 2009.Respiratory syncytial virus (RSV), a member of the Paramyxoviridae family consisting of two subgroups (subgroups A and B), is also the cause of a contagious disease that afflicts primarily infants and the elderly who are immunocompromised, e.g., patients with chronic lung or heart disease or undergoing treatment for conditions that reduce the strength of their immune system.3 The virus can live for hours on countertops and toys and causes both upper respiratory infections, such as bracheobronchitis and lower respiratory infections manifesting as bronchiolitis and pneumonia. By the age of two, most children have already been infected by RSV, but because only weak immunity develops, both children and adults can become reinfected.Symptoms usually appear four to six days after infection. The disease is typically self-limiting, lasting about one to two weeks in infants. In adults, the infection lasts about five days and presents with symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season overlaps with influenza season somewhat as infections begin to rise during the fall and continues through early spring. RSV infections, however, also occur at other times of the year, although rarely: Active surveillance programs in conjunction with infection control precautions are important components for preventing transmission of influenza and RSV. Identifying patients infected with these seasonal infections is also an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

# Safety:

* Safety Data Sheets (SDS) is available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.
* Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
* Wear clean lab coats and gloves. In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a 1:10 dilution of household chlorine bleach and then 70% denatured ethanol.
* Wipe work surfaces dry completely before proceeding.

# Specimen Requirements:

* Specimen collection and handling procedures require specific training and guidance.
* For collection and transport of nasopharyngeal swab specimens, use only Viral Transport Media, before the expiration date of the tube.
* Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen.
* NA/W specimens and NP swab specimens can be collected following the standard FLU collection procedure and placed into VTM (3 mL tube with transport medium). Specimens should be transported at 2–8 °C.
* Specimens placed in transport medium following collection can be stored for up to 24 hours at 2–30 °C or up to seven days at 2–8 °C prior to testing with the Xpert Flu/RSV XC Assay.

# Reagents, Equipment and Materials:

* The Xpert Flu/RSV XC Assay kit contains sufficient reagents to process 10 specimens or quality control samples.

*The kit contains the following:*

* Xpert Flu/RSV XC Assay Cartridges with 10 Integrated Reaction Tubes
* Bead 1, Bead 2, and Bead 3 (freeze-dried) 1 of each per cartridge
* Lysis Reagent (Guanidinium Thiocyanate) 1.5 mL per cartridge
* Binding Reagent 1.5 mL per cartridge
* Elution Reagent 3.0 mL per cartridge
* Disposable 300 μL Transfer Pipettes 2 bags of 12 per kit
* CD 1 per kit
* Assay Definition Files (ADF)
* Instructions to import ADF into GeneXpert software
* Package Insert
* The assay has been validated using Cepheid GeneXpert software version 4.3 or higher. Cepheid will validate future software versions for use with the Xpert Flu/RSV XC Assay.
* Performance may be impacted when using frozen specimens.
* Do not open the Xpert Flu/RSV XC Assay cartridge lid except when adding sample.
* Do not use a cartridge that has been dropped after removing it from the packaging.
* Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
* Do not place the sample ID label on the cartridge lid or on the barcode label.
* Do not use a cartridge that has a damaged reaction tube.
* Each single-use Xpert Flu/RSV XC Assay cartridge is used to process one test. Do not reuse spent cartridges.
* Each single-use disposable pipette is used to transfer one specimen. Do not reuse spent disposable pipettes, except when diluting N/A specimens.
* Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
* Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
* Wear clean lab coats and gloves. In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a 1:10 dilution of household chlorine bleach and then 70% denatured ethanol.
* Wipe work surfaces dry completely before proceeding.

# Quality Control:

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

1. **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 influenza and RSV viruses 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.

***There are two exceptions in which SPC is ignored and the result is valid:***

* The SPC may be negative in a sample with a high titer of Flu A or Flu B when tested with the Xpert RSV US-IVD ADF.
* The SPC may be negative in a sample with a high titer of RSV when tested with the Xpert Flu XC US-IVD ADF.
* **Probe Check Control (PCC, QC1, and 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, RSV, and SPC) is performed after the reverse transcription step and before PCR begins. The Probe Check Control (PCC, QC1 and QC2) monitors bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

# Procedure:

*Preparing the Cartridge*

*For NP Swab Specimens:*

* Remove a cartridge from the package.
* Mix specimen by inverting the Viral Transport Medium tube five times.
* Open the cartridge lid. Using a clean 300 μL transfer pipette (supplied), transfer 300 μL (one draw) of the specimen from the transport medium tube to the sample chamber with large opening in the cartridge.
* Close the cartridge lid.

*For NA/W Specimens:*

* Using a clean 300 μL transfer pipette (supplied), transfer 600 μL of the sample (two draws, using the same transfer pipette) into the 3 mL Xpert Viral Transport Medium tube and then cap the tube.
* Mix specimen by inverting the transport medium tube five times.
* Remove the cartridge from the package.
* Open the cartridge lid. Using a clean 300 μL transfer pipette (supplied), transfer 300 μL (one draw) of the diluted specimen to the sample chamber with the large opening in the cartridge.
* Close the cartridge lid.

*Starting the Test:*

* Turn on the GeneXpert instrument:
* Log on to the GeneXpert Instrument System software using your user name and password.
* In the GeneXpert System window, click Create Test.
* Scan (or type in) the Sample ID (Meditech barcode). Using the barcode information, the software automatically fills in the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.
* Open the instrument module door with the blinking green light and load the cartridge.
* Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
* Wait until the system releases the door lock before opening the module door and removing the cartridge.
* The used cartridges should be disposed in the appropriate biohazard waste container.

# Limitations/ Interferences:

* The performance of the Xpert Flu/RSV XC 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/RSV XC 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 or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
* Results from the Xpert Flu/RSV XC 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 viruses (es) are infectious nor 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 and/or RSV 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 2013-2014 influenza season. 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 or RSV infection.
* This test has not been evaluated for monitoring treatment of influenza or RSV infection.
* This test has not been evaluated for screening of blood or blood products for the presence of influenza or RSV.
* 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), /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) /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.
* The test is a qualitative test and does not provide the quantitative value of detected organism present.

# Expected Results and Reporting:

* The Xpert Flu/RSV XC Assay has two channels (Flu A 1 and Flu A 2) to detect most influenza A strains. The primers and probes in the Flu A 1 channel have 100% homology to human influenza A strains.
* The primers and probes in the Flu A 2 channel have approximately 80% homology to human influenza A strains.
* All influenza A strains detected by the Xpert Flu/RSV XC Assay is reported as **Flu A POSITIVE**.
* The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window.
* A Flu A result in the Xpert Flu/RSV XC 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. •
* Probe Check: PASS; all probe check results pass.



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*Reasons to Repeat the Assay*

* If any of the test results mentioned below occur, repeat the test according to the instructions in Retest Procedure.
* An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited or the
* sample was not properly collected.
* An **ERROR** result could be due to, but not limited to, Probe Check Control failed or the 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, or a power failure occurred.
* 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. Repeat test according to the instructions in Retest Procedure below.

***Retest Procedure***

* For retest of an indeterminate result, use a new cartridge (do not re-use the cartridge).
* For NP swab specimens, use 300 μL of the leftover specimen from the original transport medium tube.
* For NA/W specimens, use 300 μL of the leftover diluted specimen from the 3 mL transport medium tube.
* Remove a new cartridge from the kit.
* Mix the specimen by inverting the Viral Transport Medium tube five times.
* Open the cartridge lid. Use a clean 300 μL transfer pipette (supplied) to transfer 300 μL (one draw) of the specimen from the transport medium tube to the sample chamber with large opening in the cartridge.
* Close the cartridge lid.
* Follow the procedure in Starting the Test.

# Staff Education and Competency:

Training activities will be documented on the Microbiology Training Checklist form and maintained on file in the laboratory. Competency will be maintained by continuing education as necessary, participation in CAP surveys documented annually on the Training and Competency Checklist.

# References:

1. Xpert Flu/RSV XC , Package insert 2016, Cepheid.

**St. Elizabeth’s Medical Center**

**Clinical Laboratory**

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| Standard Operating Procedure File #: | File Name: .doc Xpert Flu/RSV XC |
| Title of Procedure: **Xpert Flu/RSV XC** | |

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| Standard Operating Procedure Historical Record |

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| Review Date | Annual Review/  Revision Summary | Supervisor Review | Laboratory Director/Approval | |
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