TRAINING UPDATE

Lab Location:	SGMC & WOMC	**Date Distributed:**	5/14/2020
Department:	Core Lab	**Due Date:**	5/31/2020
		Implementation:	**5/18/2020**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Influenza A & B and RSV PCR using Cepheid GeneXpert® SGMC.M1009 v5

Flu / RSV PCR Quality Control Log AG.F503.4

Description of change(s):

SOP

Section	Reason
6.3	Changed external QC frequency per IQCP (*deleted day of testing QC*)
16	Added IQCP info

FORM – removed requirement to perform external QC each day of testing

This revised SOP and FORM will be implemented on May 18, 2020

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Influenza A & B and RSV PCR using Cepheid GeneXpert®**
Prepared by	Ron Master Date: 3/02/2020
Owner	Ron Master Date: 3/02/2020

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Cepheid Xpert® Xpress Flu/RSV Assay	Real-time Polymerase Chain Reaction (PCR) Assay / GeneXpert System	IFRSV

Synonyms/Abbreviations
Flu/RSV PCR, Influenza/RSV PCR, Xpert Flu/RSV

Department
Core Lab

2. ANALYTICAL PRINCIPLE

The Xpert Xpress Flu/RSV Assay is an automated in vitro diagnostic test for qualitative detection of influenza A, influenza B, and RSV viral RNA. The assay is performed on Cepheid GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample extraction, nucleic acid purification and amplification, and detection of target sequences from clinical specimens by using reverse transcription (conversion of RNA templates into DNA) followed by real-time PCR. The primers and probes in the Xpert Xpress Flu/RSV Assay are designed to amplify and detect unique sequences in the genes that encode the following proteins: influenza A matrix (M), influenza A basic polymerase (PB2), influenza A acidic protein (PA), influenza B matrix (M), influenza B non-structural protein (NS), and the RSV A and RSV B nucleocapsid.

The GeneXpert 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 RT-PCR 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 or GeneXpert Infinity System Operator Manual.

The Xpert Xpress Flu/RSV Assay includes reagents for the detection and differentiation of influenza A, influenza B, and RSV viral RNA directly from NP swab specimens from patients with signs and symptoms of respiratory tract 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 PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Xpert Xpress Flu/RSV Assay can be run to detect Flu A, Flu B, and RSV by selecting Xpert Xpress Flu-RSV from the Select Assay menu. Xpert Xpress Flu and Xpert Xpress RSV

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 have been completed. When Flu A or Flu B viral titers are high enough to generate very early cycle thresholds (Cts) with the Xpert Xpress Flu Assay, SPC amplification curves will not be seen and their results will not be reported. When RSV titers are high enough to generate very early Cts with the Xpert Xpress RSV Assay, SPC amplification curves will not be seen and their results will not be reported.

The specimen for testing (NP swab) should be collected according to the institution's standard procedures and placed into a viral transport tube (containing 3 mL transport medium) using the Xpert Nasopharyngeal Sample Collection Kit for Viruses.

Following brief mixing by inverting the viral transport tube five times, the eluted material is transferred to the sample chamber of the disposable Xpert Xpress Flu/RSV Assay cartridge. The user initiates a test from the system user interface and places the cartridge into the GeneXpert instrument, which performs nucleic acid preparation and real-time, multiplex RT-PCR for detection of viral RNA. On this platform, sample preparation, reverse transcription, amplification, and real-time detection are all fully- automated and completely integrated. Test results are obtained in approximately 30 minutes.

The results are interpreted by the GeneXpert software from measured fluorescent signals and embedded calculation algorithms and are shown in the "View Results" window in tabular and graphic formats. The Xpert Xpress Flu/RSV Assay provides test results for influenza A, influenza B, and RSV. It also reports if the test is invalid, has encountered an error or produces no result.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	None
Specimen Collection and/or Timing	In order to obtain an adequate specimen, the procedure for specimen collection must be followed closely **Collect nasopharyngeal specimens according to the following procedure using the recommended swab:** For collection and transport of nasopharyngeal swab specimens, use only the Xpert Nasopharyngeal Sample Collection Kit for Viruses Cepheid Catalog number: SWAB/B-100 (Copan UTM P/N 330C and Copan nylon swab P/N 503CS01) or Quest 3-mL VCM/NP.
Special Collection Procedures	See above
Other	None

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Nasopharyngeal swab in viral transport medium None
Collection Container	Swab in transport medium
Volume - Optimum **- Minimum**	NP swab in viral transport medium N/A
Transport Container & Temperature	Xpert Nasopharyngeal Sample Collection Kit for Viruses Cepheid Catalog number: SWAB/B-100 (Copan UTM P/N 330C and Copan nylon swab P/N 503CS01) or Quest 3-mL VCM/NP 24 hours at 15-30°C
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated: 7 days
	Frozen: Not acceptable
Timing Considerations	Not applicable
Unacceptable Specimens & Actions to Take	• Any specimen, which does not meet the above criteria • Follow specimen rejection process
Compromising Physical Characteristics	Not applicable
Other Considerations	None

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits or reagents must be reviewed for any changes before the kit is used.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
Xpert Xpress Flu/RSV Assay kit	Cepheid XPRSFLU/RSV-10 contains sufficient reagents to process 10 samples

4.2 Reagent Preparation and Storage

Assay Kit - Xpert® Xpress Flu/RSV - XPRSFLU/RSV-10	
Xpert Xpress Flu/RSV Assay Cartridges with integrated reaction tubes	Cartridge: • Bead 1 • Bead 2 • Bead 3

	• Lysis Reagent 1 (Guanidinium thiocyanate) – 1.5 mL per cartridge • Binding Reagent 2 – 1.5 mL per cartridge • Elution Reagent – 3.0 mL per cartridge
Storage/ Stability	Store the Xpert Xpress Flu/RSV 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 testing. Do not use a cartridge that has leaked
Preparation	None required

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Xpert Xpress Flu/RSV	Supplier and Catalog Number
Sample Processing Control (SPC)	Cartridge component
Probe Check Control (PCC)	Cartridge component
Negative External Control	ZeptoMetrix NATtrol Negative Control (NATCXVA9-6C)
Positive External Control	ZeptoMetrix NATtrol Positive Control (NATFLURSV-6C)

6.2 Control Preparation and Storage

Sample processing control (SPC) - Included in the Cartridge	
Storage	Refer to section 4
Stability	Refer to section 4
Preparation	Ready to use

Probe Check Control (PCC) - Included in the Cartridge	
Storage	Refer to section 4
Stability	Refer to section 4
Preparation	Ready to use

External Characterized Positive & Negative Controls	
Storage	Store at 2-8°C
Stability	Stable until manufacturer's expiration date.
Preparation	Ready for use

6.3 Number and Frequency

QC Frequency and Procedure	
1	A Sample Processing Control (SPC) and a Probe Check Control (PCC) (internal controls) are run within each test
2	External Controls are run with each new kit lot number or shipment or every 31 days, whichever is more frequent. ~~External Controls are run once per day of testing and should be included with the assay run.~~ External controls must be treated in the same manner as a patient samples.
3	Mix NATtrol™ sample by inverting the tube 5 times
4	Open the cartridge lid. Using a clean 300 uL transfer pipette, transfer 300 µL of each of the Negative and Positive NATtrol controls to the Sample Chamber (large opening) in the cartridge.
5	Close the cartridge lid and start the test following instructions in Section 8.2, GeneXpert Analysis

6.4 Tolerance Limits and Criteria for Acceptable QC

A. Tolerance Limits

Control Type	Instrument-Reported Assay Result	Interpretation of Result
External Positive Control	See Section 10.1	See Section 10.1
External Negative Control	See Section 10.1	See Section 10.1
SPC	Passes if Meets the Assigned Acceptance Criteria. See Section 10.1	
PCC		

B. Criteria for Acceptable QC

- All controls must yield acceptable result.
- Controls and patient data must be reviewed for acceptability and for atypical or unexpected results or trends prior to reporting patient results.
- DO NOT release results from runs with unacceptable controls or with unusual patterns, trends or distribution in patient values.

C. Corrective Action

- Report problem to supervisor or designee.
- All rejected runs must be effectively addressed and include the following documentation:
  - Control(s) that failed (e.g., positive control with negative result) and/or atypical or unexpected patient results
  - Actions taken
  - Statement of what was done with the patient samples from the affected run/batch,
  - Date and tech code of the person recording the information.

- Patient samples in failed analytical runs must be reanalyzed.

NOTE: The laboratory director or designee may override rejection of partial or complete runs. Justification for the override must be documented in detail.

6.5 Documentation

- Record all Quality Control results (failed and successful) manually or electronically.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.
- Refer to Quest Diagnostics Records Management Program for Quality Control record retention requirements.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

- Cepheid GeneXpert System

7.2 Equipment

- Computer, monitor, printer, and required application software
- Biological Safety Cabinet
- Timer
- Refrigerator, 2-8°C
- Vortex mixer

7.3 Supplies

- Sterile transfer pipette
- Plastic-backed absorbent pads (Blood Bloc or equivalent)
- Personal protective equipment (lab coat, powder-free gloves, face shields, and etc.)
- Disposable biohazard waste containers (sharps, etc.)
- 4x4 gauze
- 10% bleach
- 70% ethanol

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Preparation of Cartridge
Notes: • All work must be performed in an appropriate BSC. • Before testing, clean the work area with a solution of 1:10 dilution of household chlorine bleach and then repeat the cleaning of the work area with 70% ethanol. Wipe work surfaces dry completely before proceeding • Do not open a cartridge until you are ready to perform testing • Start the test within 15 minutes of adding the sample to the cartridge. • Do not touch the integrated reaction tube that is attached to the cartridge.	
1.	Remove the cartridge and Elution Reagent from the package.
2.	Mix specimen by inverting the Xpert Viral Transport Medium or the Copan UTM tube five times.
3.	Open the cartridge lid. Using a clean 300 µL transfer pipette, transfer 300 µL (one draw) of the specimen from the transport medium tube to the sample chamber by expressing the fluid into the large opening in the cartridge. 
4.	Close the cartridge lid and proceed to Section 8.2.

8.2	GeneXpert Analysis
1.	Turn on the GeneXpert Instrument System, and then turn on the computer.
2.	On the desktop, double-click the GeneXpert software icon.
3.	Log on to the GeneXpert Instrument System software using user name and password.
4.	In the GeneXpert Dx Systems window, click **Create Test**.
5.	In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the View Results window and all the reports.
6.	Scan the barcode on the Xpert Xpress Flu/RSV Assay cartridge. Using the barcode information, the software automatically fills in the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.
7.	In the GeneXpert Dx Systems, click **Start Test**.
8.	Open the instrument module door with the blinking green light and load the cartridge.
9.	Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
10.	Wait until the system releases the door lock before opening the module door and removing the cartridge. Dispose of the used cartridges in biohazard waste container.
11.	A report is printed for each sample at the completion of testing.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

Not applicable

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

The results are interpolated by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window. Possible results are:

Assay Result Reported	Interpretation of Result
Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE	Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is not detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA (not applicable); SPC is ignored because the Flu A target amplification may compete with this control. • Probe Check – PASS; all probe check results pass
Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE**	Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is not detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA (not applicable); SPC is ignored because the Flu A and Flu B target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE**	Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA (not applicable); SPC is ignored because the Flu A and RSV target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.

Assay Result Reported	Interpretation of Result
Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE**	Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA (not applicable); SPC is ignored because the Flu A, Flu B, and RSV target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE	Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is not detected. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA (not applicable); SPC is ignored because the Flu B target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is detected. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA (not applicable); SPC is ignored because the RSV target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE**	Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is detected. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA (not applicable); SPC is ignored because the Flu B and RSV target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. • Flu A, Flu B and RSV target RNAs are not detected. • SPC – PASS; SPC has a Ct within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results pass.
INVALID	SPC does not meet acceptance criteria. Presence or absence of the target RNAs cannot be determined. Repeat test according to the instructions in Section 10.6, Retest Procedure

Assay Result Reported	Interpretation of Result
ERROR	Presence or absence of Flu A, Flu B, and/or RSV target RNA cannot be determined. Repeat test according to the instructions in Section 10.6, Retest Procedure. • Flu A – NO RESULT • Flu B – NO RESULT • RSV – 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, Flu B, and/or RSV target RNA cannot be determined. Repeat test according to the instructions in Section 10.6, Retest Procedure. 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 • RSV – NO RESULT • SPC – NO RESULT • Probe Check – NA (not applicable)

** Note: Because the incidence of co-infection with two or more viruses (Influenza A, Influenza B, or 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 Section 10.6, Retest Procedure.

10.2 Rounding

Not applicable

10.3 Units of Measure

Not applicable

10.4 Analytical Measurement Range (AMR)

Not applicable

10.5 Review Patient Data

- Review patient results for unusual patterns, trends or distribution.
- Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.

10.6 Repeat Criteria and Resulting

Repeat Criteria	
IF the PCR result is …	**THEN…**
Error/No Result/ Invalid result upon repeat testing	Report as INVLD; Add comment MPSP
Positive	Report as "Detected"
Negative	Report as "Not Detected"

Message	**Code**
Detected	DET
Not Detected	NTD
Non-amplification of the internal control suggests the presence of PCR inhibitors in the patient sample. An additional sample should be submitted for testing if clinically warranted.	MPSP

Retest Procedure	
For retest of an indeterminate result or a result indicating co-infection, use a new cartridge (do not re-use the cartridge). Use 300 µL of the left over specimen from the original transport medium tube.	
1.	Remove a new cartridge from the kit.
2.	Mix the specimen by inverting the Xpert Viral Transport Medium or the Copan UTM tube five times.
3.	Open the cartridge lid. Use a clean 300 µL transfer pipette (supplied) to transfer 300 µL of the sample to the chamber by expressing the fluid into the large opening in the cartridge.
4.	Close the cartridge lid.
5.	Follow the procedure in Section 8.2, Starting the Test.

If manually entering in results, use function **MEM** to enter results.

Enter Shift (1, 2, or 3), Press Enter to default in current shift
Worksheet: Use WIM2 for WOMC or SIM2 for SGMC.
Test: <Enter>
Enter "A" (Accept)
Enter Accession number
Press <Enter> until Result screen displayed
Key in result using appropriate code from above

If instrument is interfaced with Sunquest, use function **OEM** to view and release results.

Shift: Press Enter
Device: Type in **WOCE** (White Oak) **or SGCE** (Shady Grove)
Refer to addendum A for additional information on interfaced results.

11. EXPECTED VALUES

11.1 Reference Ranges

Not detected

11.2 Critical Values

None

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily airborne (i.e., coughing or sneezing) and the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, malaise, cough and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting or diarrhea) may also occur, primarily in children, but are less common. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication due to influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.[1,2]

Influenza viruses are classified into types A, B, and C, the former two of which cause the most human infections. Influenza A is the most common type of influenza virus in humans, and is generally responsible for seasonal flu epidemics and potentially 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 less frequently cause 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, N1 and N2. In addition to seasonal flu, a novel H1N1 strain was identified in humans in the United States in early 2009.[3]

Respiratory Syncytial Virus (RSV), a member of the Paramyxoviridae family, consisting of two strains (subgroups A and B) is also the cause of a contagious disease that affects primarily infants, and the elderly who are immunocompromised (e.g. patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system).[3] The virus can remain infectious for hours on countertops and toys and can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.[4] By the age of two years, most children have already been infected by RSV and because only weak immunity develops, both children and adults can be reinfected.[3] Symptoms appear four to six days after infection and are usually self-limiting,

lasting approximately one to two weeks in infants. In adults, infection lasts about 5 days and presents as symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season mirrors influenza somewhat as infections begin to rise during the fall through early spring.[3,4]

Active surveillance programs in conjunction with infection prevention precautions are important components for preventing transmission of influenza and RSV. The use of assays providing rapid results to identify patients infected with these seasonal viruses can be an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

13. PROCEDURE NOTES

- **FDA Status: FDA Exempt/Cleared or Approved**
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a solution of 1:10 dilution of household chlorine bleach and then repeat the cleaning of the work area with 70% ethanol. Wipe work surfaces dry completely before proceeding.
- Do not substitute Xpert® Xpress Flu/RSV reagents with other reagents.
- Do not open the Xpert® Xpress Flu/RSV cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped or shaken after you have added the sample.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert® Xpress Flu/RSV cartridge is used to process one test. Do not reuse spent cartridges.

14. LIMITATIONS OF METHOD

14.1 Precision

Not applicable

14.2 Interfering Substances

As indicated in the package insert, potentially interfering substances in the nasal passage and nasopharynx included: 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 antivirals. None of the substances caused interference of the assay at the concentrations tested in this study. All positive and negative replicates were identified correctly using the Xpert Xpress Flu/RSV Assay.

14.3 Clinical Sensitivity/Specificity/Predictive Values

A total of 2051 NP swab specimens were tested for influenza A, influenza B and RSV by the Xpert Xpress Flu/RSV Assay and the comparator assay. Of the 2051 NP swab specimens, 1139 were fresh, prospectively collected and 912 were consecutively collected, frozen specimens. For the fresh, prospectively collected NP swab specimens, the Xpert Xpress Flu/RSV Assay demonstrated a PPA and NPA of 94.6% and 99.4%, detection of influenza A; 100% and 99.2% for influenza B, respectively; and 100% and 99.8%, for RSV, respectively, relative to the comparator assay. For the consecutively collected, frozen NP swab specimens, the Xpert Xpress Flu/RSV Assay demonstrated a PPA and NPA of 100% and 98.0% for the detection of influenza A, respectively; 100% and 99.0% for influenza B, respectively; and 97.9% and 98.7% for RSV, respectively, relative to the comparator assay (Table 9). For the combined dataset, the Xpert Xpress Flu/RSV Assay demonstrated a PPA and NPA of 98.1% and 98.8% for the detection of influenza A, respectively; 100% and 99.1% for influenza B respectively; and 98.4% and 99.3% for RSV, respectively, relative to the comparator assay.

The analytical specificity of the Xpert Xpress Flu/RSV Assay was evaluated by testing a panel of 44 cultures consisting of 16 viral, 26 bacterial, and two yeast strains representing common respiratory pathogens or those potentially encountered in the nasopharynx. Three replicates of each bacterial and yeast strain were tested at concentrations of ≥ 1 x 106 CFU/mL with the exception of one strain that was tested at 1 x 105 CFU/mL (Chlamydia pneumoniae). Three replicates of each virus were tested at concentrations of ≥ 1 x 105 TCID50/mL. The analytical specificity was 100%.

- The performance of the Xpert Xpress Flu/RSV 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/RSV 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 analytical studies show potential for competitive inhibition in specimens with two different viruses in which one virus is present at the LoD and the other virus is present at >2 logs higher $TCID_{50}$/mL. However, numerous studies have shown that infections with combinations of only these specific viruses (Flu A, Flu B, and RSV) occur in <1.6% of patients.[8, 9,10]

- When using the Xpert Xpress Flu/RSV Assay in the Flu Only mode, in the event of a mixed Flu A and Flu B infection where one target crosses the cycle threshold >5 cycles prior to the other target, the target with the higher titer of the two infections will be reported as **POSITIVE** and the lower titer target will be reported as **NEGATIVE**.
- Results from the Xpert Xpress Flu/RSV 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 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 2015-2016 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), 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.

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

- Biological Safety Cabinet, Micro procedure
- Laboratory Quality Control Program
- Laboratory Safety Manual
- Safety Data Sheets (SDS)
- Quest Diagnostics Incorporated Records Management Program for Record Retention Requirements SOP.
- GeneXpert Dx System Operator Manual
- Cepheid GeneXpert® Dx System Maintenance, Micro procedure
- Flu / RSV PCR Quality Control Log (AG.F503)
- Cepheid Xpert® Xpress Flu/RSV package insert 301-7239, Rev. D.
- Cepheid GeneXpert® Xpert Xpress Flu/RSV PCR Assay Individual Quality Control Plans (VC 649, VC 650)

17. 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.
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18. DOCUMENT HISTORY

Version	Date	Section	Revision	Revised By	Approved By
1	3/16/20	6.3	Require external QC each day of testing	R Master	R Master
2	3/24/20	7	Clarify supply list	R Master	R Master
		8.2	Removed 'Select test' step		
		11.2	Deleted critical value		
		19	Added addendum A	L Barrett	
3	4/23/20	3	Added Quest 3-mL VCM/swab set as acceptable collection and transport medium	L Barrett	R Master
		Add A	Added SARS-CoV-2		
4	5/13/20	6.3	Changed external QC frequency per IQCP	R Master	R Master
		16	Added IQCP info		

19. ADDENDA

A. Cepheid Testing and Running via Sunquest Interface

Addendum A

Cepheid Testing and Running via Sunquest Interface

A. General Information:

1. This interface does NOT go through DI-Instrument Manager. Cepheid is interfaced directly to Sunquest. The Sunquest interface is set up for Autoverification.
2. All tests will auto-file with the following exceptions:
   - Positive *C. difficile* results
   - Positive MRSA results
   - Positive SARS-CoV-2 results (on inpatients only)
3. If the test is one of the above exceptions, then the results will be held in Sunquest. These results must be called and documented per routine process.
4. Use function OEM on Sunquest SmarTerm to review results.
   a. Access OEM
      - At DEVICE: prompt, type in Method code **WOCE** (WOMC) or **SGCE** (SGMC).
      - Results will display cup by cup.
        - Those that were auto-filed require no action, proceed to next cup.
        - For positive results that were held, continue with steps b and c below.
      - Refer to *OEM - On Line Result Entry Method* procedure (LIS SOP) for additional information about review and release of results.

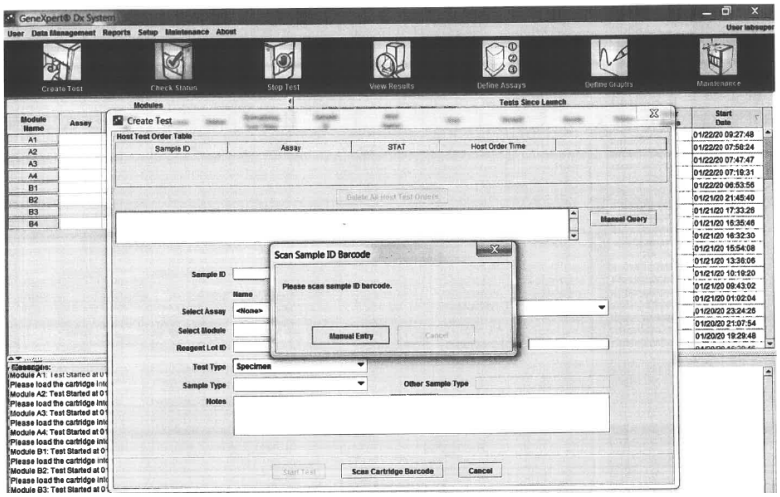
   b. Call results. Append CBACK documentation to results including who you called, date, time and tech code. Required format is:

      -CBACK-;full name of person called DATE TIME Tech code
      Example -CBACK-;Sue Smith 032420 1420 4568

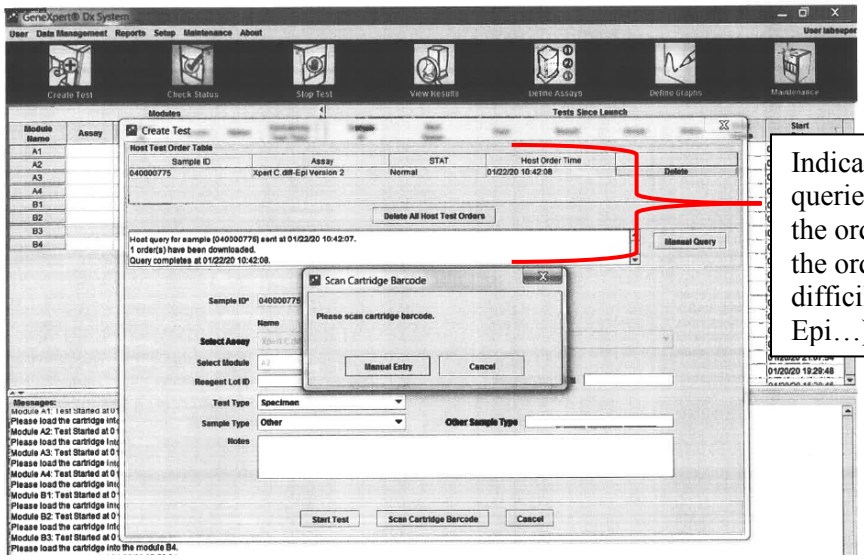
   c. Click on Accept to release results.
5. Perform an OFC (Online File Cleanup) at least once per shift. This process cleans up the online data that was sent to Sunquest.
   a. In Sunquest (SmarTerm) access function OFC
   b. Type in the method code (WOCE or SGCE).
   c. At the Start at Cup Number prompt, type in 1 and then press ENTER.
   d. At the Stop at Cup Number prompt, press ENTER.

B. Running Tests on Cepheid:

1. Create Test
   a. In the GeneXpert Dx System window, click **Create Test** on the menu bar. The Scan Sample ID Barcode dialog box appears.



   b. Scan the Sunquest barcode label.



Indicates the Cepheid queried Sunquest and found the order. In this example the order was for a C difficile PCR (Xpert C diff-Epi…)

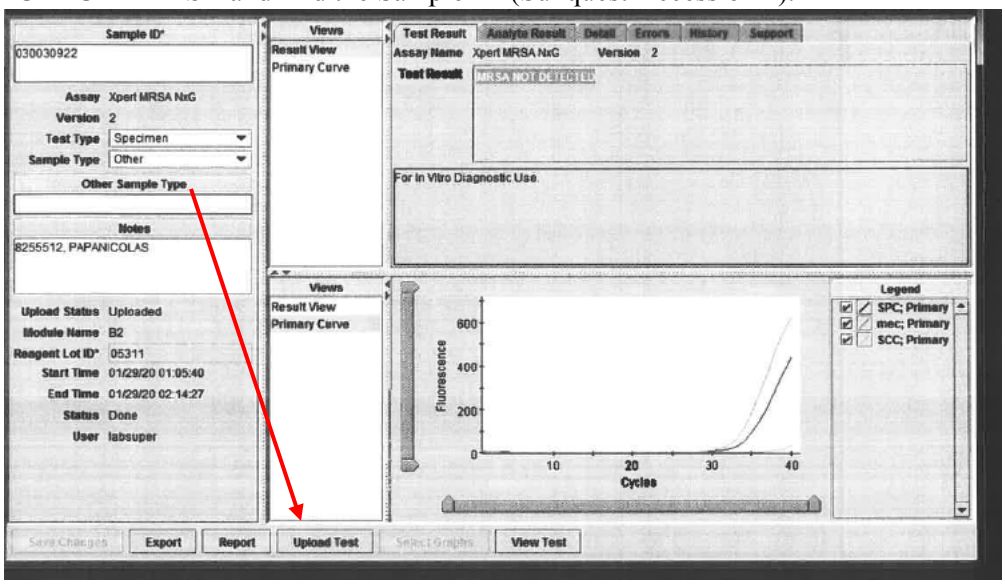
   c. Scan the cartridge barcode.

2. Click **OK**
3. Click **Create Test**
4. Load cartridge
5. Verify that the test has started before walking away
6. When testing is completed results will print to Cepheid printer.

C. Manually uploading results to Sunquest (Example Sunquest downtime)

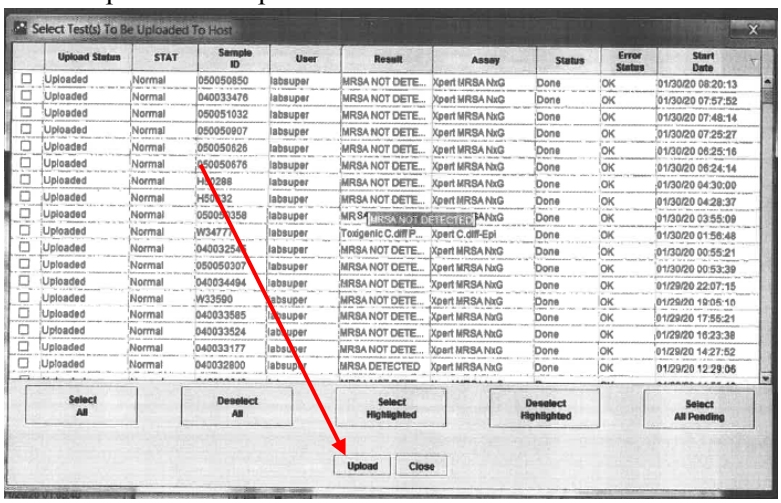
1. From the Cepheid, go to VIEW RESULTS

   a. Click on **UPLOAD TEST** and find the Sample ID (Sunquest Accession #).



   b. Check off the one that you want to upload (located to the left of the Update Status column).
   Note: You can check off one or more accession numbers at the same time.

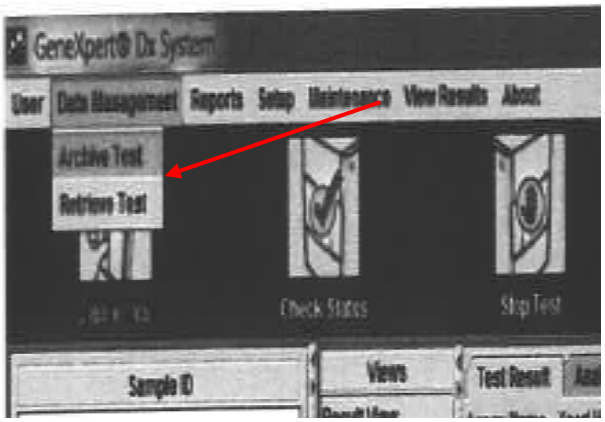
   c. Click on **UPLOAD** to resend to Sunquest. Results will now upload into Sunquest. It make take a little time for upload to complete.



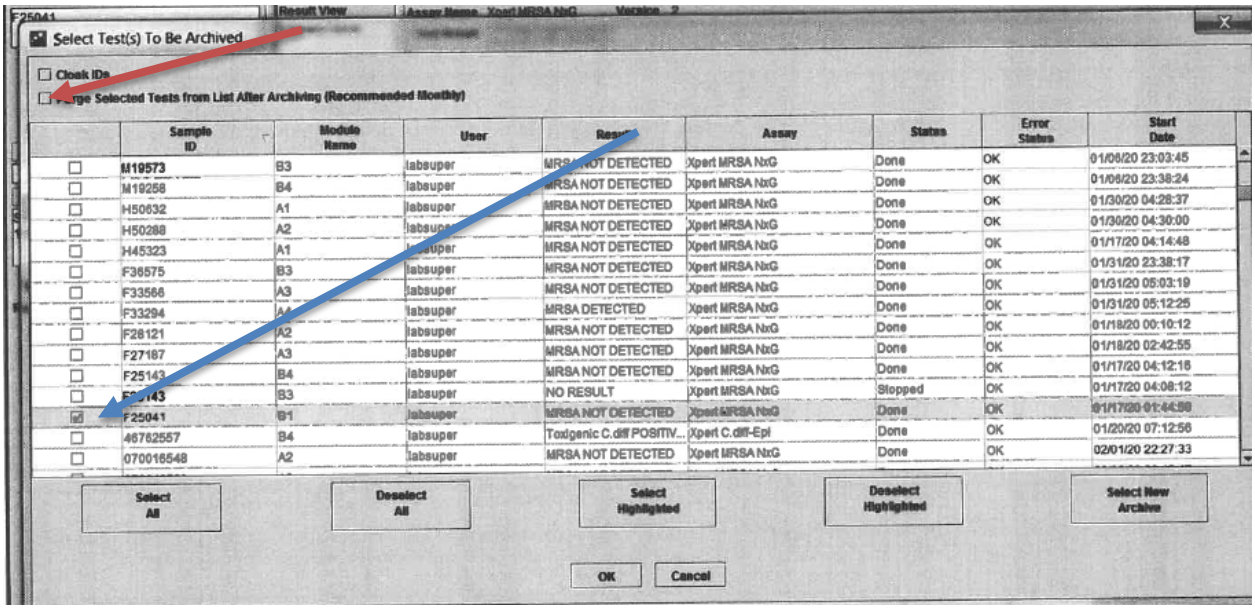
   d. Review in Sunquest OEM to document any positive result call notification.

D. Editing Sample ID (SQ Accession #)

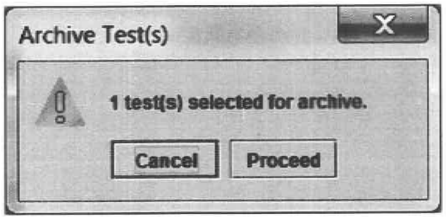
1. From the main screen -→Data Management-→ Click on Archive Test



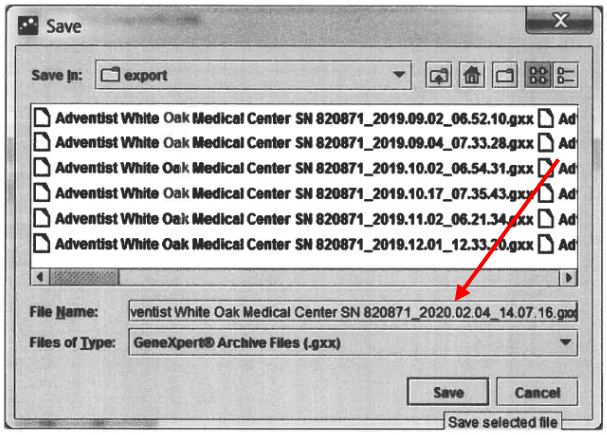
2. In the upper left corner click on **Purge Selected Tests from the LIS after Archiving** (red arrow). Then locate the Sample ID (SQ Accession#) that you want and select it by clicking on box to the left of the Sample ID (blue arrow). Then click on OK.



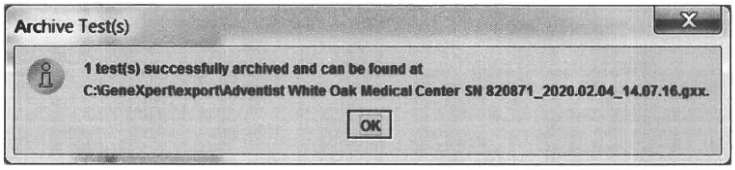
3. At the Archive Test prompt, click on **Proceed**.



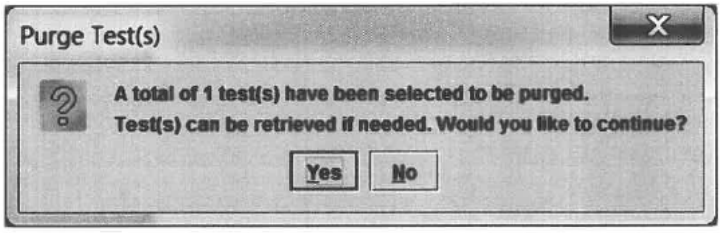
4. Archive file is generated (File name is system generated) and click on **SAVE**. Note that the File Name has the date and time as part of the file name. In the example below "2020.02.04_1407" is the date of 2/4/20 and time of 1407.



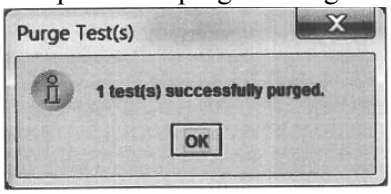
5. Archive message displays, click on **OK**



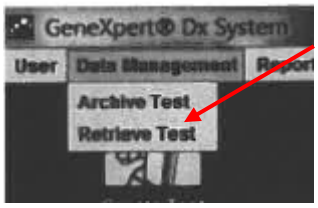
6. Purge message displays, click on **OK**



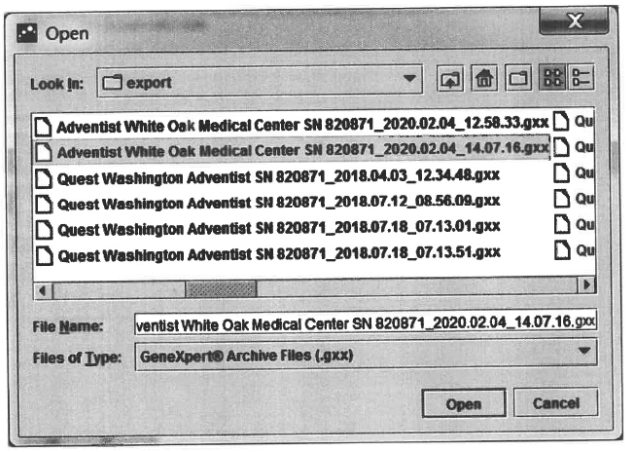
7. Completion of purge message displays



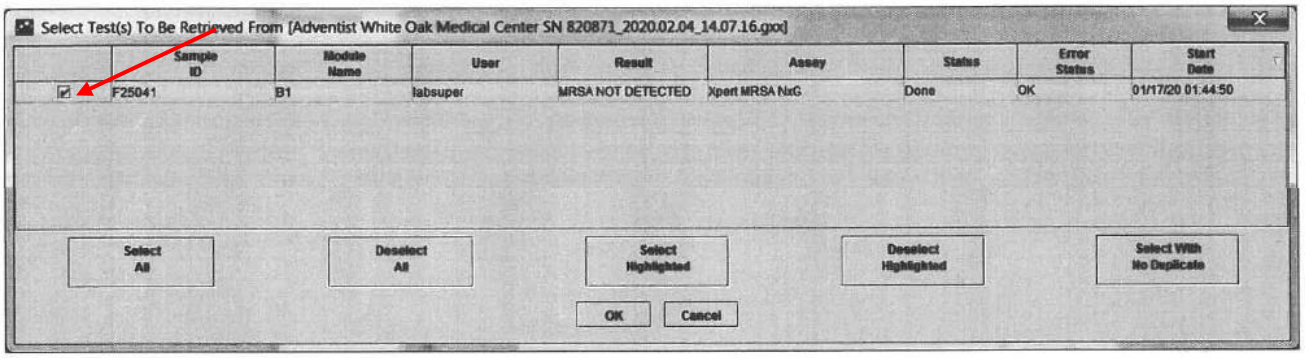
8. Retrieve test by going to Main screen -→ Data Management-→ Retrieve Test



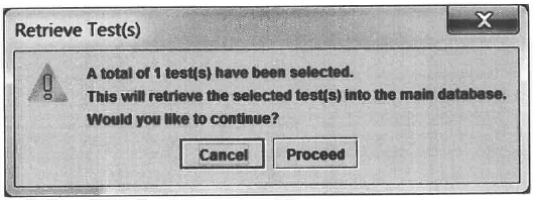
9. Locate file that you exported (Note, part of the file name consists of the date and time file was created.). Highlight the file and click on Open.

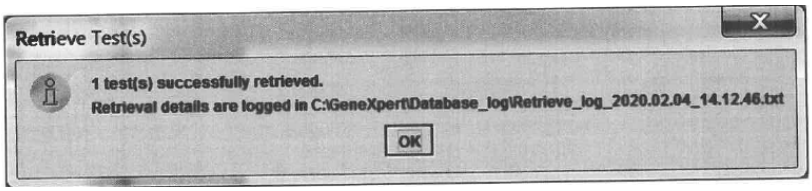


10. To the left of the Sample ID, check off the Sample ID (SQ acc #) that you want to retrieve to edit. Then click on OK.

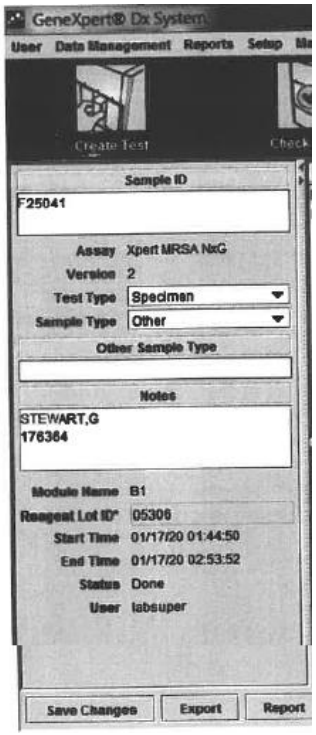


11. Retrieve prompt displays. Click on **Proceed**. Retrieve Test(s) confirm displays. Click on **OK**.

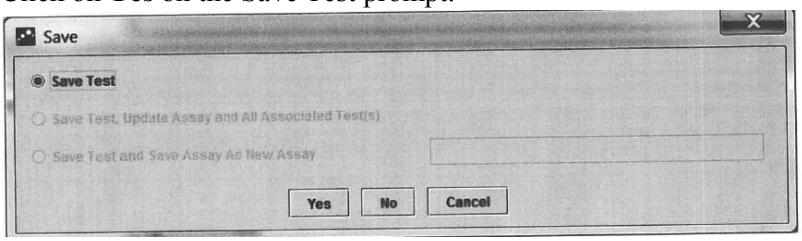




12. Proceed to edit Sample ID (SQ Accession #). Click on Save when you are done.



13. Click on **Yes** on the Save Test prompt.



14. Follow the steps in part C above to upload the results to Sunquest.

FLU A / B / RSV PCR QUALITY CONTROL LOG

☐ Shady Grove Medical Center
☐ White Oak Medical Center

Last external QC performed (date): _____ **Next external QC is due = *Month*** _____ *Circle day below*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

1. **External Positive and Negative Controls** are tested and documented ~~with each day of testing and~~ with each new kit lot number or shipment or every 31 days, whichever is more frequent.
2. **Internal controls** must be documented each time the test is performed.
3. If QC results are not acceptable, document corrective action. Do not accept patient results before reviewing QC results for proper reactions.

Date	Patient Name / MR#	Patient Result			Kit	Internal Controls	External Pos Control (+ / + / +) = Positive		External Neg Control (- / - / -) = Negative		Tech Code
		FLU A DET/ NTD / INVLD	FLU B DET/ NTD / INVLD	RSV DET/ NTD / INVLD	Lot # / Expire	Pass / Fail	Lot # / Expire	Result	Lot # / Expire	Result	

Weekly review:	Weekly review:	Weekly review:
Weekly review:	Weekly review:	Monthly review: