CEDARS-SINAI MARINA DEL REY HOSPITAL	**POLICY & PROCEDURE MANUAL**	CS-Marina del Rey Hospital Clinical Laboratory & Pathology Marina del Rey Hospital 4690 Lincoln Blvd. 90292
		Date Created: _____
		Last Revised: _____
	Approved by: **James Keefe, MD** **Medical Director, Clinical Laboratory**	Date Approved: _____
Title:	**Alere™ i Influenza A & B**	

I. PURPOSE

This document outlines policies and procedures that deal with the Alere™ i Influenza A & B. To be concise some information may be excluded from the manufacturer's recommended procedure. This procedure shall be used in conjunction with the **Alere-i Instrument User Manual.**

II. SCOPE

All Clinical Lab Scientists (CLS) in all shifts who have successfully completed the initial training and maintained the annual competencies.

III. CLIA COMPLEXITY: WAIVED

Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification

IV. INTENDED USE

The **Alere™ i** Influenza A & B 2 assay performed on the **Alere™ i** Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in direct nasal or nasopharyngeal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus.

ALERE-I INSTRUMENT

Alere™ i Influenza A & B 2 is a rapid (13 minutes or less), instrument-based isothermal test for the qualitative detection and differentiation of influenza A and influenza B from nasal swabs and nasopharyngeal swabs (direct and eluted in viral transport media). The Alere™ i Influenza A & B 2 kit contains all components required to carry out an assay for influenza A and B on the Alere™ i Instrument.

V. TEST PRINCIPLE

Alere™ i Influenza A & B 2 is an automated multiplex assay that utilizes isothermal nucleic acid amplification technology for the differential and qualitative detection of influenza A and influenza B viral nucleic acids. It is comprised of a Sample Receiver, containing elution buffer, comprising two sealed reaction tubes, each containing a lyophilized pellet, a Transfer Cartridge for transfer of the eluted sample to the Test Base, and the Alere™ i Instrument.

The reaction tubes in the Test Base contain the reagents required for amplification of Influenza A and Influenza B, respectively, as well as an internal control. The templates (similar to

primers) designed to target Influenza A RNA amplify a unique region of the PB2 segment while the templates designed to amplify Influenza B RNA target a unique region of the PA segment. Fluorescently-labeled molecular beacons are used to specifically identify each of the amplified RNA targets.

To perform the assay, the Sample Receiver and Test Base are inserted into the Alere™ i Instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base, initiating target amplification. Heating, mixing and detection are provided by the instrument, with results automatically reported.

To perform the assay, the Sample Receiver and Test Base are inserted into the Alere™ i Instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base, initiating target amplification. Heating, mixing and detection are provided by the instrument, with results automatically reported.

VI. SPECIMEN COLLECTION AND HANDLING

Specimen:	Use freshly collected specimens for optimal test performance. Inadequate specimen collection or improper sample handling/storage/transport may yield erroneous results. **Sample Type: Nasal Swab or Nasopharyngeal Swab**
Specimen Collection & Handling	**Nasal Swab:** For optimal test performance, use the swabs provided in the test kit. Alternatively, rayon, foam, HydraFlock® Flocked swab (standard tip), HydraFlock® Flocked swab (mini tip), Copan Mini Tip Flocked Swab, or Copan Standard Flocked swabs can be used to collect nasal swab samples. *Puritan PurFlock Standard Tip Ultra Flocked Swabs, Puritan PurFlock Mini Tip Ultra Flocked Swabs and Copan Standard Rayon Tip Swabs are not suitable for use in this assay.* To collect a nasal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the swab several times against the nasal wall then slowly remove from the nostril. **Nasopharyngeal Swab:** Use sterile rayon, foam, polyester or flocked flexible-shaft NP swabs to collect a nasopharyngeal sample. To collect a nasopharyngeal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Pass the swab directly backwards without tipping the swab head up or down. The nasal passage runs parallel to the floor, not parallel to the bridge of the nose. Using gentle rotation, insert the swab into the anterior nares parallel to the palate advancing the swab into the nasopharynx, leave in place for a few seconds, and then slowly rotate the swab as it is being withdrawn.

	To ensure proper collection, the swab should be passed a distance that is halfway of that from the nose to the tip of the ear. This is about half the length of the swab. DO NOT USE FORCE while inserting the swab. The swab should travel smoothly with minimal resistance; if resistance is encountered, withdraw the swab a little bit without taking it out of the nostril. Then elevate the back of the swab and move it forward into the nasopharynx.	
Specimen Transport & Storage	Direct nasal or nasopharyngeal swabs	As soon as possible after collection
	@ room temperature	2 hours prior to testing
	If held longer	Specimen should be refrigerated. (2-8 C) and should be tested in 24 hours from the time of collection.
	If the transport of nasal or nasopharyngeal swab samples is required…	Elute the swab into 0.5 to 3.0 mL of saline or viral transport media by rotating the swab in the liquid for 10 seconds, within 1 hour of sample collection. Remove the swab and discard. If immediate testing is not possible, eluted swab samples can be held at room temperature (15-30°C) for up to eight (8) hours prior to testing. If the eluted swab sample will be held longer than eight (8) hours, it must be refrigerated at 2-8°C and tested within 72 hours from the time of sample collection. If needed, transport the sample at 2-8°C in a leak-proof container. Swirl eluted swab samples in transport media gently to mix before testing. Note: Minimal dilution of the sample is recommended as dilution may result in decreased test sensitivity.
	Acceptable Transport Media: Transport Media Amie's Media Dulbecco's Modified Eagles' Medium (D-MEM) Hank's Balanced Salt Solution M4 Media M4-RT Media M5 Media M6 Media Phosphate Buffered Saline Saline Stuart's Media Universal Transport Media Starplex Multitrans	

VII. REAGENTS AND MATERIALS

Component	Content
Test Bases BASE	Orange plastic components containing two reaction tubes of lyophilized reagents for the targeted amplification of Influenza A and B viral RNA.
Sample Receivers RCVR	Blue plastic components containing 2.5 mL of elution buffer.
Transfer Cartridges CARTRDG	White plastic components used to transfer 2 x 100 µL of sample extract from the Sample Receiver to the Test Base.
Nasal Swabs	Sterile swabs for use with the **Alere™ i** Influenza A & B 2 Test.
Positive Control Swab	The positive control swab is coated with inactivated influenza A and B viruses.
Negative Control Swab	The negative control swab is coated with inactivated Group C *Streptococcus.*
Plastic disposable pipettes capable of delivering 200µl VTM sample	
Product Insert	
Quick Reference Instructions	

VIII. STORAGE AND STABILITY

Store kit at 2-30°C. The **Alere™ i** Influenza A & B 2 kit is stable until the expiration date marked on the outer packaging and containers. Ensure all test components are at room temperature before use.

IX. REJECTION CRITERIA

Calcium alginate and Puritan Purflock® Ultra flocked swabs are not suitable for use in this assay.

Visibly bloody samples must not be used.

X. PRECAUTIONS

1. Treat all specimens as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
2. Proper sample collection, storage and transport are essential for correct results.
3. Leave test pieces sealed in their foil pouches until just before use
4. Do not tamper with test pieces prior to or after use.
5. Do not use kit past its expiration date.
6. Do not mix components from different kit lots or from other Alere™ i assays.
7. Solutions used to make the control swabs are inactivated using standard methods. However, patient samples, controls, and test pieces should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal.

8. If any assay components are dropped, cracked, found to be damaged or opened when received, DO NOT USE and discard. Do not use scissors or sharp objects to open foil pouches as damage to test pieces can occur.
9. Do not open the Sample Receiver before placing in the instrument. It will prohibit the Elution Buffer from reaching temperature and may impact test performance.
10. If the Sample Receiver is spilled while opening, clean the instrument per instructions provided in the instrument User Manual and cancel test. Repeat test with a new Sample Receiver.
11. All test pieces must be removed from the instrument according to removal instructions displayed on the instrument and disposed of according to country and local requirements. Pieces must not be separated once they are assembled.
12. Once reacted, the Test Base contains large amounts of amplified target (Amplicon). Do not disassemble the Test Base and Transfer Cartridge. In the case of a positive sample, this could lead to amplicon leakage and potential Alere™ i Influenza A & B 2 false positive test results.
13. Due to the high sensitivity of the assays run on the instrument, contamination of the work area with previous positive samples may cause false positive results. Handle samples according to standard laboratory practices. Clean instruments and surrounding surfaces according to instructions provided in the cleaning section of the instrument User Manual. Refer to Section 1.6, Maintenance & Cleaning, for further information.
14. Visibly bloody samples must not be used with Alere™ i Influenza A & B 2.
15. Do not touch the heads of the Control Swabs. Cross contamination with the Positive Control Swabs may occur due to the high sensitivity of the assays run on the instrument.

XI. QUALITY CONTROL

Alere™ i Influenza A & B 2 has built-in procedural controls. The result of the Procedural Control is displayed on the screen and is automatically stored in the instrument with each test result. This can be reviewed later by selecting Review.

PROCEDURAL CONTROLS:

Alere™ i Influenza A & B 2 contains an internal control that has been designed to control for sample inhibition, amplification and assay reagent function. In positive samples where target amplification is strong, the internal control is ignored, and the target amplification serves as the ‘control’ to confirm that the clinical sample was not inhibitory, and that assay reagent performance was robust. At a very low frequency, clinical samples can contain inhibitors that may generate invalid results.

External Positive and Negative Controls

Use of positive and negative controls -to ensure that test reagents are working and that the test is correctly performed. Alere™ i Influenza A & B 2 kits contain Positive and Negative Control Swabs. These swabs will monitor the entire assay.

Control Swab Procedure:

External Positive and Negative Control swabs are provided and should be tested following the Run QC Test instructions on the Alere™ i Instrument.

Note: The Alere™ i Instrument reports QC results as Pass or Fail. Flu A/B Positive QC pass indicates a positive result for both influenza A and influenza B.

If the correct control results are not obtained, do not perform patient tests or report patient results. Contact Technical Support during normal business hours before testing patient specimens.

FREQUENCY:

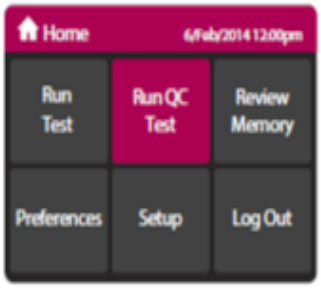
1. **Run QC for new shipment**
2. **Run QC for new operators**
3. **The first time an assay is run on an instrument**
4. **After Alere™ i instrument software upgrade**
5. **When required by local, state, and/or federal regulations, accrediting groups, or your lab's Quality Control procedures**

Run QC Test:

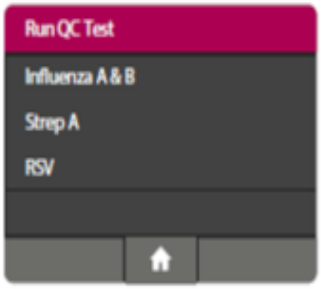
Quality Control Swab Test Procedure

For QC testing, select Run QC Test on the Home screen, and follow the displayed instructions. Refer to Running a QC Test in the **Alere™ i Instrument User Manual** for further details.

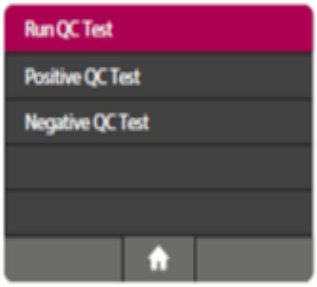
1 Touch 'Run QC Test'



2 Touch 'Influenza A & B'



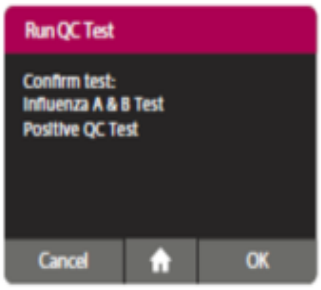
3 Select the QC Test to be Run



4 Confirm Test

Confirm the test type to match the QC sample intended for testing by touching 'OK' and following the on screen prompts to complete testing.

Note: *The QC test is run in the same manner as a Direct Nasal/Nasopharyngeal Swab Patient Test. See the* ***To Perform a***

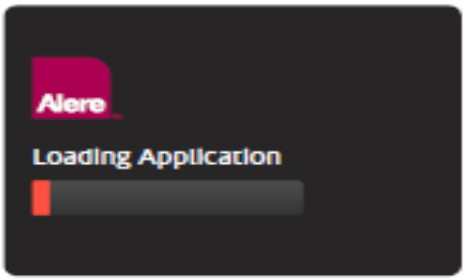


XII. TEST PROCEDURE

STEP 1

Turn on the **Alere™ i** Instrument - press the power button ⏻ on the side of the instrument.

Note: *If the unit is unattended for one hour, the instrument will go to a black screen power save mode. Touch the screen to return the unit to active display operation.*

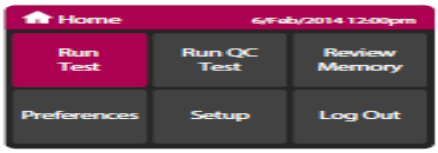


Enter User ID

Press ✓ after entry

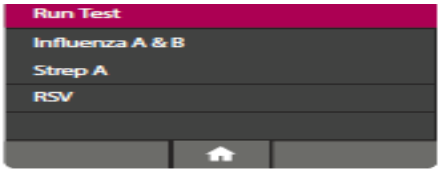
Touch 'Run Test'

This will begin the test process.



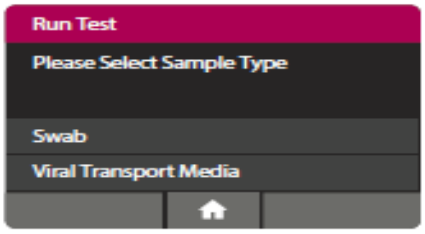
Touch 'Influenza A & B Test'

This starts an Influenza A & B test.



Select Sample Type (if prompted)

If the sample type has already been specified by the Admin, the instrument will automatically advance to the next step.



STEP 2

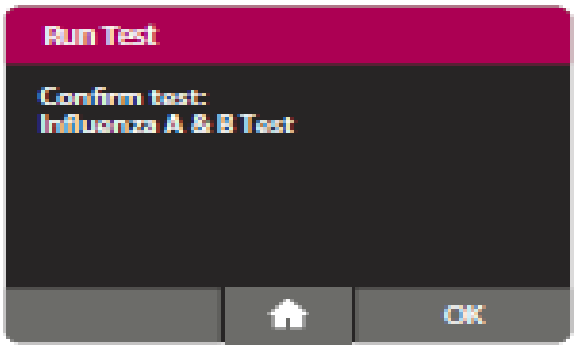
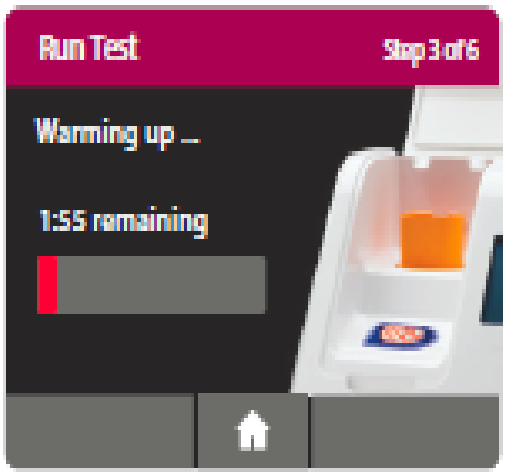
Open the Lid and Insert Orange Test Base into Orange Test.

⚠

Caution: Do not apply excessive force. Excessive force could damage the instrument.

Confirm that the correct test is displayed on the screen.

Touch 'OK' to proceed

	⚠ Caution: Once the Test Base has been placed in the holder, the user will have 10 minutes to confirm the test. If the test is not confirmed within 10 minutes, the instrument will time out and the Test Base must be removed and discarded. If the incorrect Test Base has been inserted, remove and dispose of the incorrect Test Base. Close the lid. The instrument will then run a self-test before proceeding to the Home screen. Press Run Test and restart the test using the correct Test Base.	
STEP 3	⚠ Caution: Confirm that the Sample Receiver foil pouch indicates Influenza A & B 2 (not another Alere™ i assay). Confirm that the foil seal on the Sample Receiver is for the Influenza A & B assay. If not, then remove the Sample Receiver and replace it with a new Sample Receiver for Alere™ i Influenza A & B 2. Insert Blue Sample Receiver into the Blue Sample Receiver holder ⚠ Do not apply excessive force. Excessive force could damage the instrument. ⚠ **Caution**: Once the Sample Receiver has been placed in the holder, the user will have 10 minutes to start the test (Steps 3 through 5). If the test is not started within 10 minutes, the instrument will time out and all test pieces (Test Base and Sample Receiver) must be removed and discarded. The instrument will proceed to the Home screen. Press Run Test and restart the test using a new Test Base and Sample Receiver. Wait for the Sample Receiver to Warm Up. Do not remove the Sample Receiver from the instrument once the Warm Up begins. ⚠ **Caution: DO NOT REMOVE THE FOIL SEAL UNTIL PROMPTED BY THE INSTRUMENT. DO NOT** close the lid or insert the sample until prompted by the instrument.	 

STEP 4

Direct Nasal or Nasopharyngeal Swab Test Procedure

When prompted, remove the foil seal and place the patient swab to be tested into the Sample Receiver**.**

Vigorously mix the swab in the liquid for 10 seconds. Press the swab head against the side of the Sample Receiver as you mix it. This helps remove the sample from the swab. Once the swab is removed, touch 'OK' to proceed.

⚠ Caution: To ensure that the Sample Receiver remains in the instrument while removing the foil seal, place two fingers along the outer edge of the Sample Receiver to hold it in place. If the Sample Receiver spills after warm up, cancel the test by pressing the Home button. Remove and discard the test pieces (Sample Receiver and Test Base) and clean the instrument. Press Run Test to start a new test using a new Test Base and Sample Receiver**.**

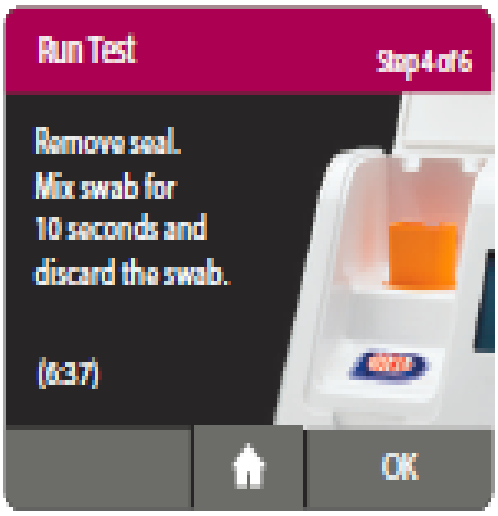
Discard the swab.

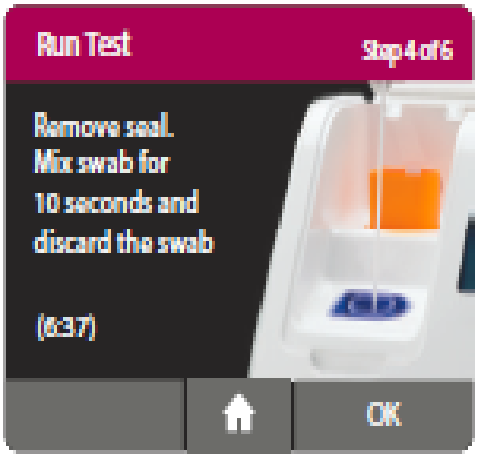
Skip to Step 5a

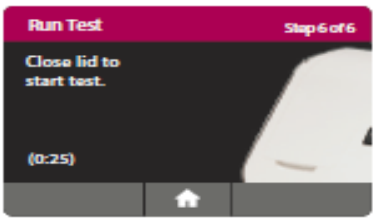
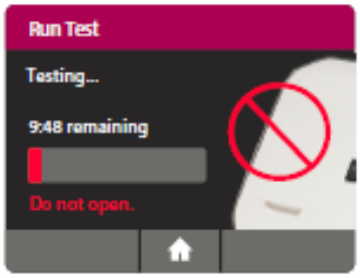
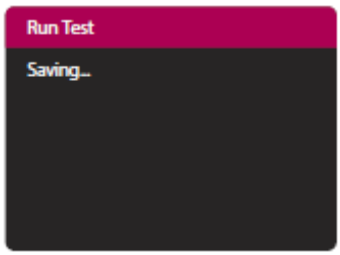
⚠Caution: To ensure the Sample Receiver remains in the instrument while removing the foil seal, place two fingers Vigorously mix the sample in the liquid for 10 seconds. Use the pipette tip to swirl the liquid.

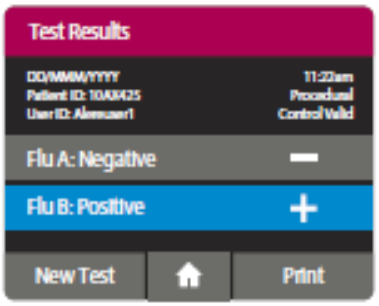
Once the sample is mixed and the pipette is removed, immediately touch 'OK' to proceed. Continue to Step 5a.

⚠Caution: To ensure the Sample Receiver remains in the instrument while removing the foil seal, place two fingers along the outer edge of the Sample Receiver to hold it in place. If the Sample Receiver spills after warm up, cancel the test by pressing the Home button. Remove and discard the test pieces (Sample Receiver and Test Base) and clean the instrument. Press Run Test to start a new test using a new Test Base and Sample Receiver**.**



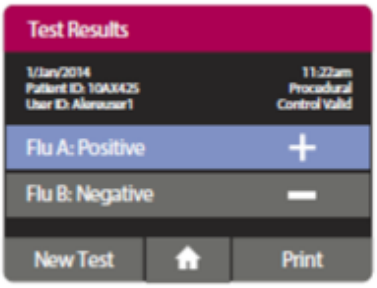
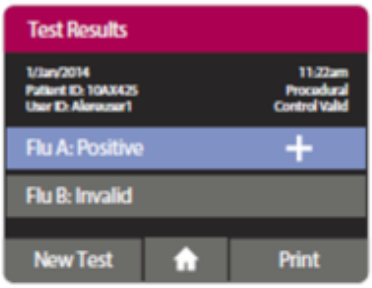
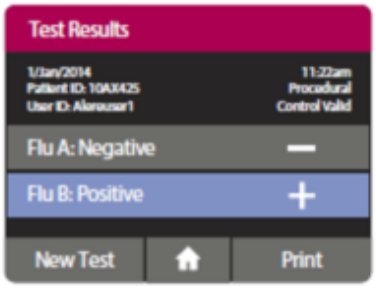


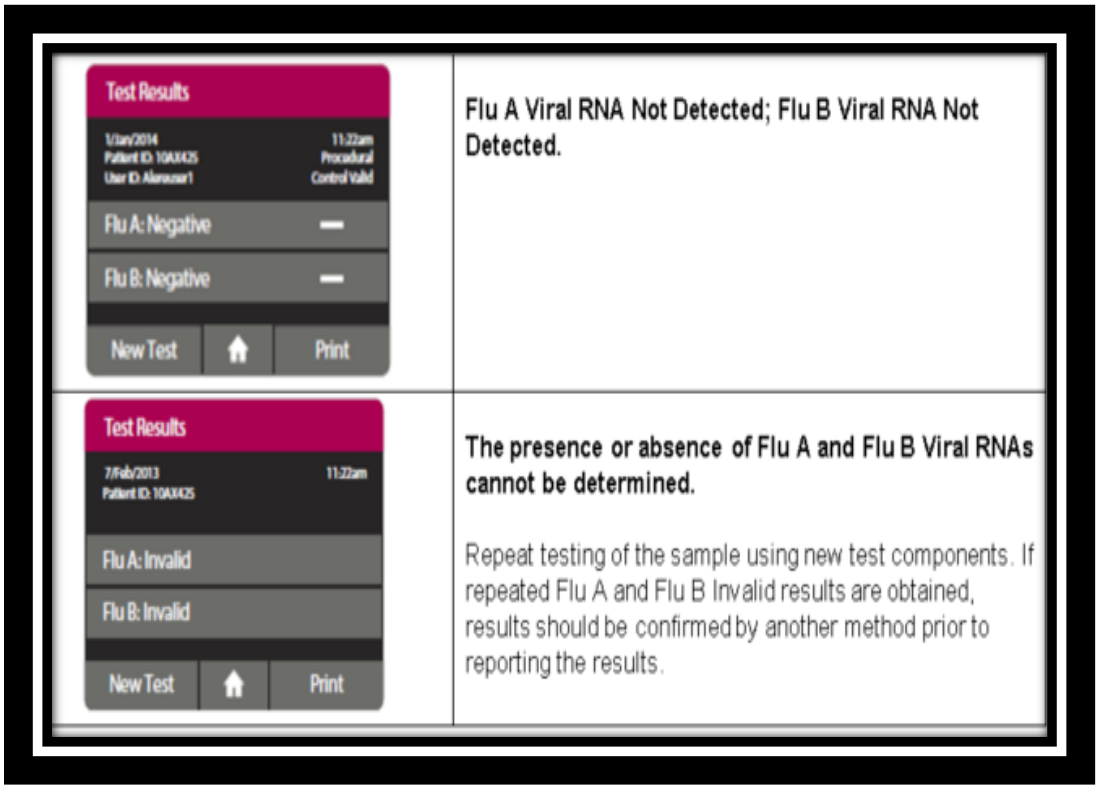
STEP 5A	Press the White Transfer Cartridge into the Blue Sample Receiver Listen for a click. When the Transfer Cartridge is properly attached to the Sample Receiver, the orange indicator on the Transfer Cartridge will rise. If the orange indicator does not rise, continue pushing onto the Sample Receiver until it does. ⚠ **Caution: The orange indicator should be observed closely. If the orange indicator does not fully rise, the Transfer Cartridge may not collect enough sample.**	 
STEP 5B	Lift and then connect the Transfer Cartridge to the Test Base When the Transfer Cartridge is properly attached to the Test Base, the orange indicator on the Transfer Cartridge will descend. If the orange indicator does not descend, continue pushing onto the Test Base until it does. ⚠ **Caution: If the orange indicator does not fully descend, not enough sample will be dispensed. This may potentially result in invalid or false negative results.**	
STEP 6	**Close the Lid.** **DO NOT OPEN THE LID** until the **Test Complete** message appears on the screen. *Note: The test will be cancelled if the lid is opened* Caution: This screen will be displayed for up to 30 seconds once the Transfer Cartridge is detected. If the instrument does not detect that the lid has been closed by then, it will time out and all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded. The instrument will proceed to the Home screen. Collect a new sample from the patient. Press Run Test and restart the test using a new Test Base and Sample Receiver. Caution: DO NOT OPEN THE LID. The test will be cancelled and all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded. A test result will not be reported or saved in the instrument memory. When amplification and detection is complete, the instrument will automatically save the data before advancing to the results screen. **Caution: The test is not saved until the completed result is displayed. Do not open the lid until the results are displayed**	  

	The **Test Results** screen displays either a Negative or Positive result for a successfully completed test. If a test error occurs, the display will read 'Invalid'. Refer to the Result Interpretation Section for Interpretation of Results. **Press Print to print test results, press New Test to run another test, Press Home to return to the Home screen**	
	After printing, or if New Test or Home are selected, the instrument will prompt to open the lid and discard the used test pieces. Remove test pieces by lifting the Transfer Cartridge attached to the Test Base, and clicking it into the Sample Receiver, by pressing into the Sample Receiver	
	⚠ **Caution: Do not try to remove the Sample Receiver by any other method as there is a risk of spilling the patient sample.** All test pieces will be connected and can now be removed from the instrument and disposed of according to federal, state and local regulations. **Caution: DO NOT disassemble the Transfer Cartridge and the Test Base before disposal**	
	Close the lid. The instrument will then run a Self-Test before showing the Home screen or Enter Patient ID screen, depending on the previous selection.	

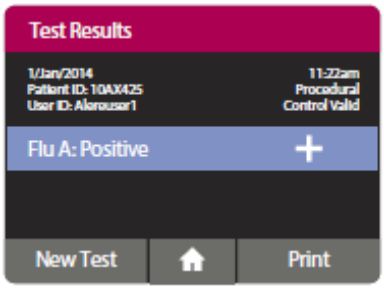
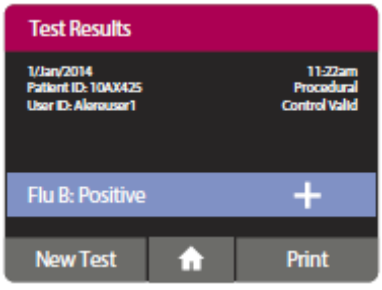
XIII. RESULT INTERPRETATION

When the test is complete, the results are clearly displayed on the instrument screen. An individual result for both influenza A and influenza B will be provided.

Instrument Display	Interpretation of Results and Follow-up Actions
	**Flu A Viral RNA Detected; Flu B Viral RNA Not Detected.** This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
	**Flu A Viral RNA Detected; The presence or absence of Flu B Viral RNA cannot be determined.** This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
	**Flu B Viral RNA Detected; Flu A Viral RNA Not Detected.** This result does not rule out co-infections with other pathogens or identify any specific influenza B virus lineage.
	**Flu A Viral RNA Detected; Flu B Viral RNA Detected.** Dual infections of Flu A and Flu B are rare. Repeat testing using new test components. Contact Technical Support during normal business hours if multiple samples provide this result. This result does not rule out co-infections with other pathogens or identify any specific influenza A or influenza B virus lineage.



If the Alere™ i Instrument is set to 'Early Detection' a positive result will be displayed for Influenza A or Influenza B immediately upon detection.

Instrument Display	Interpretation of Results and Follow-up Actions
	**Flu A Viral RNA Detected.**
	**Flu B Viral RNA Detected.**

If an Invalid result is received, one additional test may be run using the same Sample Receiver. The instructions below should be followed:

- Remove the connected Test Base and Transfer Cartridge from the instrument and connect the Test Base portion to an open, UNUSED Sample Receiver. The connected Test Base and Transfer Cartridge MUST be attached to a Sample Receiver prior to disposal. The Sample Receiver from a new Transfer Cartridge package may be used for this.

- Remove the blue Sample Receiver separately and carefully from the instrument. The Sample Receiver should be retained and kept upright to avoid spilling the liquid contents.
- From the Home Screen, start a new test. Follow the screen prompts; however, when asked to insert the Sample Receiver, reuse the Sample Receiver and DO NOT re-elute the swab.

XIV. LIMITATIONS

- Alere™ i Influenza A & B 2 performance depends on viral RNA load and may not correlate with cell culture performed on the same specimen. Viral nucleic acid may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- There is a risk of false negative results due to the presence of sequence variants in the viral targets of the assay. If the virus mutates in the target regions, influenza viruses A or B may not be detected or may be detected less efficiently. Additionally, if the sequence variant occurs in the target sequence recognized by the fluorescently-labeled molecular beacon an invalid assay may result.
- False negative results may occur if a specimen is improperly collected, transported or handled. False negative results may occur if inadequate levels of viruses are present in the specimen.
- False negative results may occur if mucin concentrations of 1% (w/v) or greater are present in the specimen.
- False negative results may occur if Respiratory Syncytial Virus is present as a co-infecting organism.
- 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.
- Negative results do not preclude infection with influenza virus and should not be the sole basis of a patient treatment decision.

- Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2016 to 2017 influenza seasons. The positive and negative predictive values may vary depending on the prevalence and population tested.
- This test has not been evaluated for patients without signs and symptoms of influenza infection.
- The test is a qualitative test and does not provide the quantitative value of detected organism present.
- Cross-reactivity with respiratory tract organisms other than those tested in the Analytical Specificity Study may lead to erroneous results.
- This assay has not been evaluated for immunocompromised individuals.
- This test cannot rule out diseases caused by other bacterial or viral pathogens. The regions selected for amplification are conserved among all known Influenza A and Influenza B subtypes and strains (where sequence data is available from public databases). Laboratory testing has shown that Alere™ i Influenza A & B 2 can readily amplify and detect H1N1 (pre-2009 pandemic), H3N2 (variant) and H7N9 (detected in China in 2013) influenza subtypes but the performance of the assay for detection of these subtypes in a clinical setting has not been established due to the lack of clinical samples.

XV. REFERENCES

1. Williams, KM, Jackson MA, Hamilton M. Rapid Diagnostic Testing for URIs in Children: Impact on Physician Decision Making and Cost. Infect. Med. 19(3): 109-111, 2002.

2. Bonner, A.B. et al. Impact of the Rapid Diagnosis of Influenza on Physician Decision-Making and Patient Management in the Pediatric Emergency Department: Results of a Randomized, Prospective, Controlled Trial. Pediatrics. 2003 Vol. 112 No. 2.

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