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<tr><td rowspan="5">UnityPoint Health
PEKIN

SEROLOGY
LABORATORY</td><td>Page 1 of 14</td><td>Section: Sero</td><td>Policy #: SER-0642</td></tr>
<tr><td colspan="2">Approved by: see signature block at end of document</td><td>Date:02/06/19
Review by:</td></tr>
<tr><td colspan="3">Supersedes:
Date Revised:</td></tr>
<tr><td colspan="3">Primary Responsible Parties: Angie Guppy Secondary Responsible Parties:</td></tr>
<tr><td colspan="3">CAP Standard:</td></tr>
<tr><td>SUBJECT:</td><td colspan="3">ALERE™ I INFLUENZA A & B LABORATORY PROCEDURE</td></tr>
</table>

I. PRINCIPLE

Alere™ i Influenza A & B 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, a Test Base, 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 is provided by the instrument.

II. CLINICAL SIGNIFICANCE

The **Alere™** i Influenza A & B 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 nasal swabs 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.

III. POLICY SCOPE:

The scope of this policy applies to all Laboratory staff that prepares or performs testing on laboratory specimens at UnityPoint Methodist.

IV. SPECIMEN

A. Specimen:	For optimal performance, use the swabs provided in the test kit. Alternatively polyester nasal swabs can be used to collect nasal swab samples. These swabs are available in Microbiology. Calcium alginate is 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. Visibly bloody samples must NOT be used with **Alere™** i Influenza A & B.
B. Specimen Storage:	Swab specimens should be tested as soon as possible after collection. If immediate testing is not possible, the nasal swab can be held in its original package at room temperature (18-22°C) for up to two (2) hours prior to testing. If the swab will be held longer than two (2) hours, it must be refrigerated at 2-8^{o}C and tested within 24 hours from the time of sample collection.
C. Handling Precautions:	Patient samples, controls, and test devices should be handled as though they could transmit disease. Observe established precautions against microbial hazards.

V. REAGENTS

A.

Component	Content	Quantity
Test Bases	Orange plastic components containing two reaction tubes of lyophilized reagents for the targeted amplification of Influenza A and B viral RNA. Must store at 2-8°C. Can be used for testing without warming to room temperature.	24
Sample Receivers	Blue plastic components containing 2.5 mL	24

	of elution buffer. May store at room temperature. If refrigerated must reach room temperature before testing.	
Transfer Cartridge	White plastic components used to transfer 2 x 100 µL of sample extract from the Sample Receiver to the Test Base.	24
Nasal Swabs	Sterile swabs for use with the **Alere™** i Influenza A & B Test.	26
Positive Control Swab	The positive control swab is coated with inactivated influenza A and B viruses.	1
Negative Control Swab	The negative control swab is coated with inactivated Group C *Streptococcus*	1

B. Storage and Stability

For convenience, the entire kit may be refrigerated at 2-8ºC. **The orange Test Base kit must be stored at 2-8ºC.** The remainder of the kit can be stored at room temperature (15-30ºC) if preferred. **The blue Sample Receiver must be allowed to reach room temperature prior to use**, if stored at 2-8ºC. Do not freeze. The orange Test Base stored at 2-8ºC can be tested without the need to warm to room temperature

Alere™ i Influenza A & B kits are stable until the expiration dates marked on their outer packaging and containers.

VI. INSTRUMENTATION/EQUIPMENT

Alere™ i Instrument

The Alere™ i Instrument is maintenance-free and has no serviceable parts. In the case of instrument failure or damage, contact Alere Technical Support 1-855-731-2288.

The Alere™ i Instrument will be cleaned each day of use using 10% bleach solution, on a damp, lint free cloth. Record cleaning on the monthly Maintenance form. Do not spray or pour solution directly onto instrument when cleaning. Ensure no excess liquid is used when cleaning as it may damage the instrument.

The exterior instrument surfaces and the surfaces visible under the open lid are cleaned daily. Clean surrounding bench area. Clean instrument and surrounding areas immediately after possible patient sample contamination.

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VII. QUALITY CONTROL

Alere™ i Influenza A & B 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 Memory on the instrument.

A. **Procedural Controls:**
Alere™ i Influenza A & B 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.

Procedural Control Valid displayed on the instrument screen indicates that the assay reagents maintained their functional integrity and the sample did not significantly inhibit assay performance.

B. **External Positive and Negative Controls:**
Good laboratory practice suggests the 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 kits contain Positive and Negative Control Swabs. These swabs will monitor the entire assay. Test these swabs once with each new shipment received and monthly thereafter. Enter results of Quality Control on appropriate log.

C. 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. Refer to Test Procedure or Instrument User Manual for further details.

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 (1-855-731-2288) during normal business hours before testing patient specimens.

1. Frequency of QC = Every new lot/shipment andevery 30 days.
2. A Positive and Negative External and Internal QC
    a. Internal and External QC:
        i. Document all QC on the appropriate log in the Serology Worksheets (P1MAN)binder.

If the correct control results are not obtained, do not perform patient tests or report patient results.

VIII. PROCEDURE:

A. PRECAUTIONS

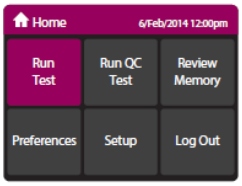
1. For *in vitro* diagnostic use.

2. Federal Law restricts this device to sale by or on the order of a licensed practitioner.
3. To be used in conjunction with the **Alere™** i Instrument.
4. Performance characteristics of this test have been established with the specimen type listed in the **Intended Use Section** only. The performance of this assay with other specimen types or samples has not been evaluated.
5. Treat all specimens as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
6. Proper sample collection, storage and transport are essential for correct results. Leave test pieces sealed in their foil pouches until just before use.
7. Do not tamper with test pieces prior to use. Do not use kit past its expiration date. Do not mix components from different kit lots.
8. 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.
9. **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.**
10. 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.
11. 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.
12. All test pieces must be removed from the instrument according to removal instructions displayed on the instrument, and disposed of in a biohazard waste container. **Pieces must not be separated once they are assembled.**
13. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
14. All test pieces are single use items. Do not use with multiple specimens.
15. Performance characteristics for influenza A were established when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.
16. 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 false positive test results.

17. At a low frequency, clinical samples can contain inhibitors that may generate invalid results.
18. 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.

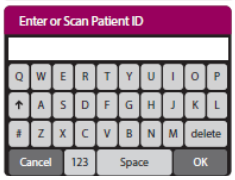
1. **Touch 'Run Test'**
   This will begin the test process.



2. **Enter Patient ID or QC lot number** using on screen keyboard (no spaces or special characters) or barcode scanner

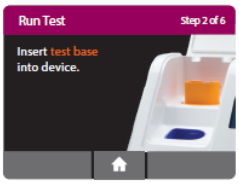
   Touch 'OK'.

   Verify that the ID was entered correctly, then touch 'OK' to confirm entry.



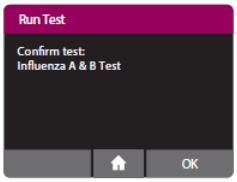
3. **Open the Lid and Insert Orange Test Base into Orange Test Base holder**

   Caution: Do not apply excessive force.

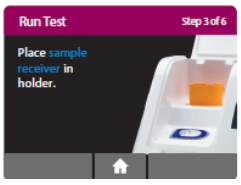


4. **Confirm that the correct test is displayed on the screen.**

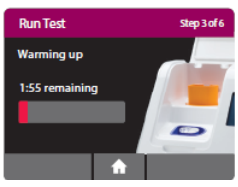
   Touch 'OK' to proceed.



5. **Insert Blue Sample Receiver into the Blue Sample Receiver holder**

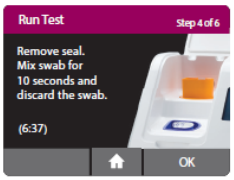


6. **Wait for the Sample Receiver to Warm Up.**
   Caution: DO NOT REMOVE THE FOIL SEAL UNTIL PROMPTED BY THE



INSTRUMENT. DO NOT close the lid or insert the swab sample until prompted by the instrument.

7. **When prompted, remove the foil seal and place the QCswab or 0.2 (Plastic disposable pipettes provided in kit) VTM liquid to be tested into the Sample Receiver.**

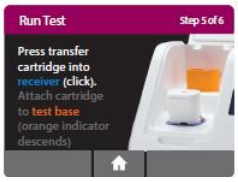


Note: To ensure that the Sample Receiver stays in the holder while removing the seal place two fingers along the outer edge of the Sample Receiver to keep it in place.

8. **Vigorously mix the swab/liquid (use disposable pipet)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.

Discard the swab.

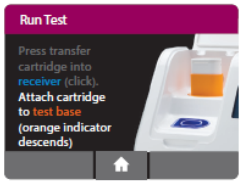
9. **Press the White Transfer Cartridge into the Blue Sample Receiver**



Listen for a click

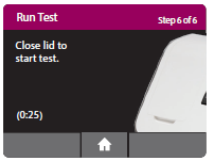
When the Transfer Cartridge is properly attached to the Sample Receiver, the orange indicator on the Transfer Cartridge will rise. If the orange indicator does not rise, continue pushing onto the Sample Receiver until it does.

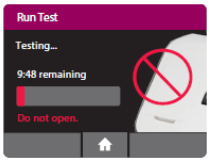
10. **Lift and then connect the Transfer Cartridge to the Test Base**



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.

11. **Close the Lid.**
DO NOT OPEN THE LID until the **Test Complete** message appears on the screen.



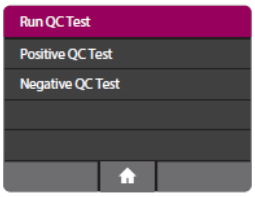


Note: The test will be cancelled if the lid is opened.

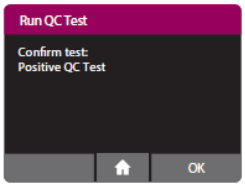
For QC testing, select Run QC Test on the Home screen, and follow the displayed instructions. Refer to Running a QC Test in the **Alere™** i Instrument User Manual for further details.

1. **Touch 'Run QC Test'**

2. **Select the QC Test to be Run**



3. **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.

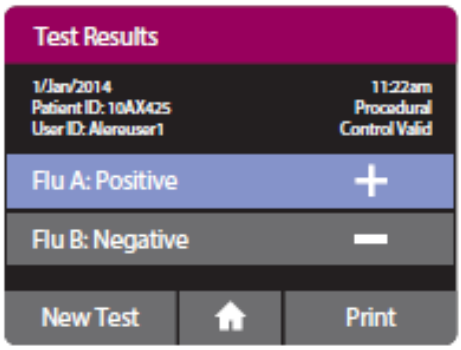
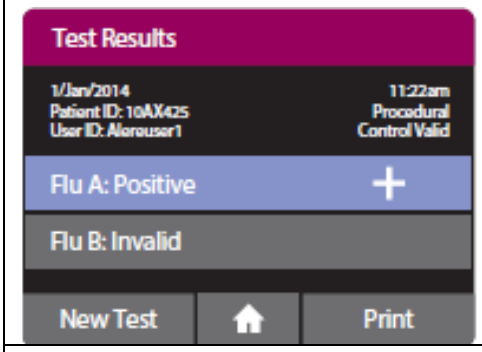
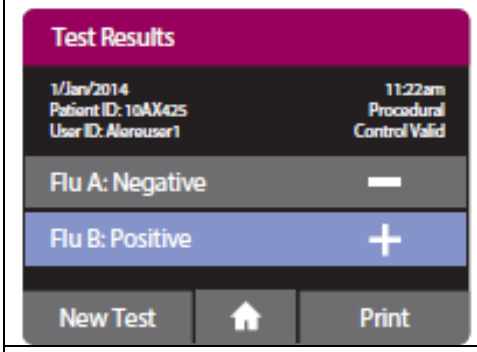
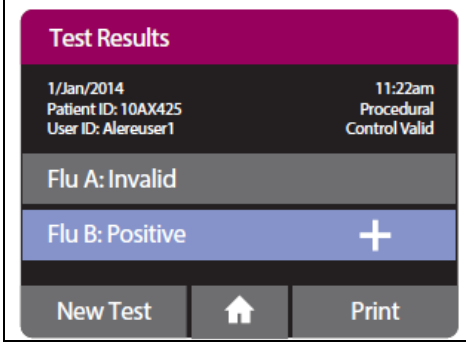


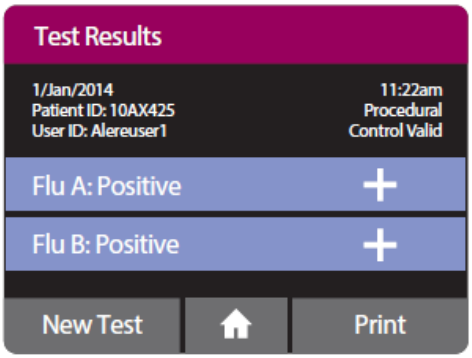
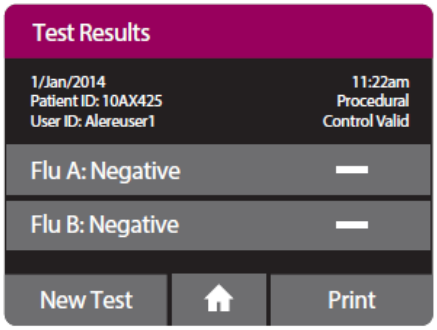
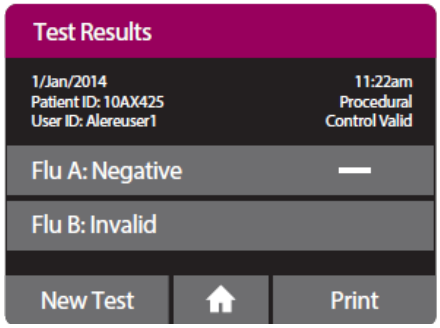
*Note: The QC test is run in the same manner as a Patient Test. See the **To Perform a Test** section above for step by step instructions.*

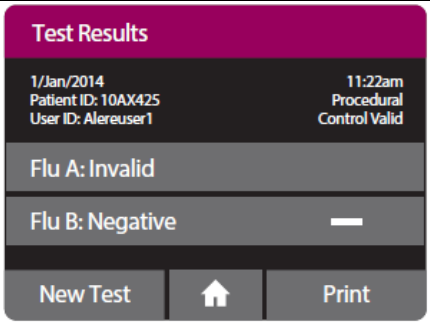
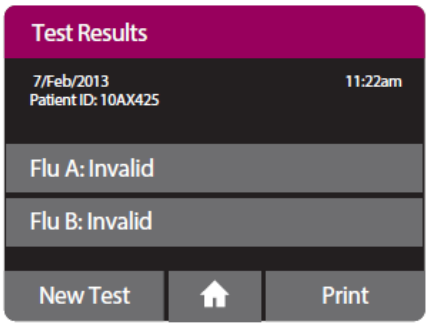
IX. REPORTING RESULTS

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 B Viral RNA Detected; The presence or absence of Flu A Viral RNA cannot be determined.** 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.
	**Flu A Viral RNA Not Detected; Flu B Viral RNA Not Detected.**
	**Flu A Viral RNA Not Detected; The presence or absence of Flu B Viral RNA cannot be determined.** Infection due to Flu B cannot be ruled out. Repeat testing of the sample using new test components. If repeated Flu B Invalid results are obtained, results should be confirmed by another method prior to reporting the results.
	Flu B Viral RNA Not Detected; The presence or absence of Flu A Viral RNA cannot be determined. Infection due to Flu A cannot be ruled out. Repeat testing of the sample using new test components. If

	repeated Flu A Invalid results are obtained, results should be confirmed by another method prior to reporting the results.
	The presence or absence of Flu A and Flu B Viral RNAs cannot be determined. Repeat testing of the sample using new test components. If repeated Flu A and Flu B Invalid results are obtained, results should be confirmed by another method prior to reporting the results.

Enter the results in the LIS as Positive or Negative. Invalid results should be repeated.
If an invalid result is obtained upon repeat testing a new specimen must be obtained for testing by another method. Report the Alere™ i Influenza test as invalid. ARUP test 2002643 Influenza Virus A and B DFA with Reflex to Respiratory Virus Mini Panel by PCR may be sent out (specimen is nasopharyngeal swab in viral transport media stored at 2-8°C after collection).

The result will be appended with the following:
'This test does not subtype Influenza beyond type A and type B. Individuals vaccinated with FluMist may test positive for up to three days after receiving vaccine.'

X. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

1. The performance of the **Alere™** i Influenza A & B was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
2. **Alere™** i Influenza A & B 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 viruses(es) are infectious, or are the causative agents for clinical symptoms.
3. Performance of **Alere™** i Influenza A & B has not been established for monitoring antiviral treatment of influenza.

4. 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.
5. 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.
6. 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.
7. Potential interference effects from FluMist™ have not been evaluated. Individuals who have received nasally administered influenza vaccine may test positive in commercially available influenza rapid diagnostic tests for up to three days after vaccination.
8. 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.
9. Negative results do not preclude infection with influenza virus and should not be the sole basis of a patient treatment decision.
10. Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2012 to 2013 influenza season. The positive and negative predictive values may vary depending on the prevalence and population tested.
11. This test has not been evaluated for patients without signs and symptoms of influenza infection.
12. The test is a qualitative test and does not provide the quantitative value of detected organism present.
13. Cross-reactivity with respiratory tract organisms other than those tested in the Analytical Specificity Study may lead to erroneous results.
14. This assay has not been evaluated for immunocompromised individuals.
15. 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).
16. Laboratory testing has shown that **Alere™** i Influenza A & B 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.

XI. REFERENCES

1. Hay, A, Gregory, V, Douglas, A, Lin Y. The evolution of human influenza viruses. The Royal Society of London. B (2001) 356, 1861-1870.
2. 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.
3. 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.
4. **Alere™** i Influenza A & B package insert

POLICY CREATION :	***Date***
Author: ***Angie Guppy, MLT (ASCP)***	***02/06/19***
Medical Director: ***Lori Racsa, DO***	***02/06/19***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
SECTION MEDICAL DIRECTOR		

REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date

Reviewed by:

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date