Alere™ i

Influenza A & B Package Insert

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For use with the **Alere™ i** Instrument
For use with nasal or nasopharyngeal specimens
For *in vitro* use only
Rx Only

CLIA COMPLEXITY: WAIVED - For Direct Nasal Swabs (Not Eluted in Viral Transport Media) Only

A Certificate of Waiver is required to perform this test in a CLIA Waived setting. To obtain CLIA waiver information and a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA.

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.

CLIA COMPLEXITY: MODERATE – For Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media

INTENDED USE

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 direct nasal 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.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

Performance characteristics for influenza A were established during the 2012-2013 and the 2014-2015 influenza seasons 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.

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 department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

SUMMARY AND EXPLANATION OF THE TEST

Influenza is a highly contagious, acute, viral infection of the respiratory tract. It is a communicable disease that is easily transmitted through the coughing and sneezing of aerosolized droplets containing live virus. Influenza outbreaks occur each year during the fall and winter months.[1] Type A viruses are typically more prevalent than type B viruses and are associated with most serious influenza epidemics, while Type B infections are usually milder[1].

Rapid diagnostics with increased sensitivity are essential for the reliable detection of influenza A and B, allowing immediate, effective treatment decisions. Rapid diagnosis of influenza can lead to reduced hospital stays, reduced secondary complications and reduced cost of hospital care, and allow effective implementation of infection control measures.[1, 2]

Alere™ i Influenza A & B is a rapid (less than 15 minutes), instrument-based isothermal test for the qualitative detection and differentiation of influenza A and influenza B from nasal swabs and nasal or nasopharyngeal swabs eluted in viral transport media. The **Alere™ i** Instrument has a small footprint and easy to use graphical user interface for convenience within a busy hospital or point-of-care environment. The **Alere™ i** Influenza A & B kit contains all components required to carry out an assay for influenza A and B on the **Alere™ i** Instrument.

PRINCIPLES OF THE PROCEDURE

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 are provided by the instrument, with results automatically reported.

REAGENTS AND MATERIALS

Materials Provided

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 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*.
Package Insert	
Quick Reference Guide	

Materials Required but not Provided

Alere™ i Instrument

Precision pipette capable of delivering 200µL of sample with disposable tips (for VTM samples only)

Nasopharyngeal Swabs

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 or after use.
8. Do not use kit past its expiration date.
9. Do not mix components from different kit lots.
10. 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.
11. **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.**
12. 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.
13. 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.
14. 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.**

15. 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.
16. All test pieces are single use items. Do not use with multiple specimens.
17. 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.
18. 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.
19. At a low frequency, clinical samples can contain inhibitors that may generate invalid results.
20. 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.
21. Visibly bloody samples must not be used with **Alere™ i** Influenza A & B.
22. 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.

STORAGE AND STABILITY

For convenience, the entire kit may be refrigerated at 2-8°C. **The orange Test Base kit must be stored at 2-8°C.** The remainder of the kit can be stored at room temperature (15-30°C) if preferred. **The blue Sample Receiver must be allowed to reach room temperature prior to use**, if stored at 2-8°C. Do not freeze. The orange Test Base stored at 2-8°C 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.

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.

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.

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 once for each untrained operator. Further controls may be tested in order to conform with local, state and/or federal regulations, accrediting groups, or your lab's standard Quality Control procedures.

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 Quality Control Swab 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 during normal business hours before testing patient specimens.

SPECIMEN COLLECTION AND HANDLING

Use freshly collected specimens for optimal test performance. Inadequate specimen collection or improper sample handling/storage/transport may yield erroneous results.

Nasal Swab

For optimal performance, use the swabs provided in the test kit. Alternatively, rayon, foam, HydraFlock® Flocked swab (standard tip), Copan Standard Flocked swab, or polyester nasal swabs can be used to collect nasal swab samples.

Calcium alginate and Puritan Purflock® Ultra flocked 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 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

nare 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 AND STORAGE

Direct nasal swabs should be tested as soon as possible after collection. If immediate testing is not possible, a direct 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 a direct nasal swab specimen will be held longer than two (2) hours, it must be refrigerated at 2-8°C and tested within 24 hours from the time of sample collection.

If the transport of nasal or nasopharyngeal swab samples is required, the transport media listed below were tested and are acceptable for use in **Alere™ i** Influenza A & B. 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 (18-22°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 24 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.

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
Tryptose Phosphate Broth
Veal Infusion Broth
Universal Transport Media
Starplex Multitrans
Vircell

It has been determined that Brain Heart Infusion Broth is **NOT** suitable for use with this test.

TEST PROCEDURE

Before testing with **Alere™ i** Influenza A & B:

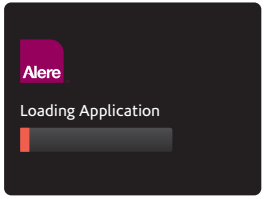
- Allow all samples to reach room temperature.
- **If stored at 2-8°C, allow the blue Sample Receiver to reach room temperature.**
- The orange Test Base can be tested without the need to warm to room temperature.

To Perform a Test:

Step 1

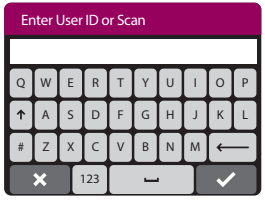
Turn on the **Alere™ i** Instrument - press the power button ⏻ on the side of the instrument.

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.



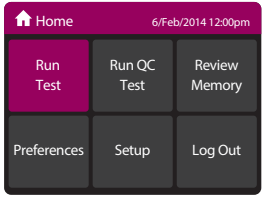
Enter User ID

Press '✓' after entry.



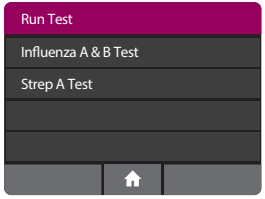
Touch 'Run Test'

This will begin the test process.



Touch 'Influenza A & B Test'

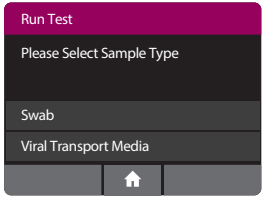
This starts an Influenza A & B test.



To Perform a Test:

Select Sample Type (if prompted)

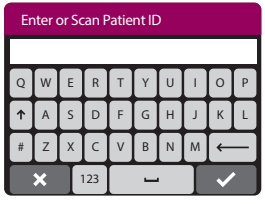
If the sample type has already been specified by the Admin, the instrument will automatically advance to the next step.



Enter Patient ID using on screen keyboard or barcode scanner

Touch '✔'.

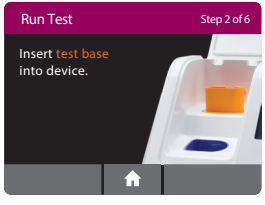
Verify that the ID was entered correctly, then touch '✔' to confirm entry.



Step 2

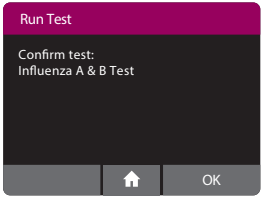
Open the Lid and Insert Orange Test Base into Orange Test Base holder

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



To Perform a Test:

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

Insert Blue Sample Receiver into the Blue Sample Receiver holder

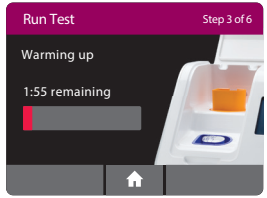
⚠ **Caution: Do not apply excessive force. Excessive force could damage the instrument.**

⚠ **Caution: Confirm that the foil seal on the Sample Receiver indicates that it is for use with Alere™ i Influenza A & B. If not, then remove the Sample Receiver and replace it with a new Sample Receiver for Alere™ i Influenza A & B.**

⚠ **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.**

To Perform a Test:

Wait for the Sample Receiver to Warm Up.

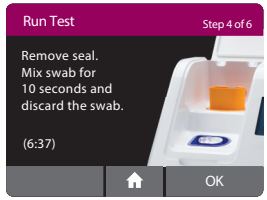


⚠ **Caution: DO NOT REMOVE THE FOIL SEAL UNTIL PROMPTED BY THE INSTRUMENT. DO NOT** close the lid or insert the sample until prompted by the instrument.

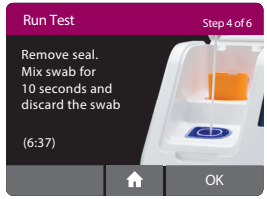
Step 4

Direct Nasal 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 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.

To Perform a Test:

Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media Test Procedure

When prompted, remove the foil seal and add 0.2mL of sample to the Sample Receiver using a precision pipette with a disposable tip.

Vigorously mix the sample in the liquid for 10 seconds. Pipette the sample up and down while swirling the pipette tip in the liquid. Once the sample is mixed and the pipette is removed, 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.**

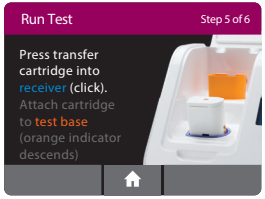
To Perform a Test:

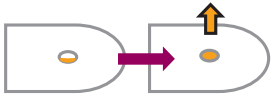
Step 5a

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.



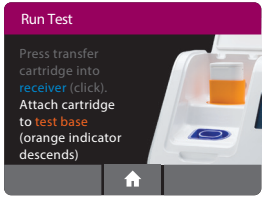


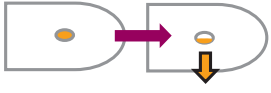
⚠ **Caution: The orange indicator should be observed closely. If the orange indicator does not fully rise, the Transfer Cartridge may not collect enough sample.**

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.**

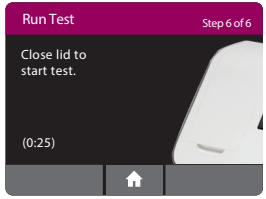
To Perform a Test:

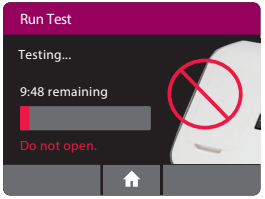
Step 6

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.

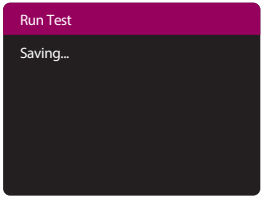




⚠ **Caution: This screen will be displayed for up to 30 seconds once the Transfer Cartridge is detected. If the instrument does not detect that the lid has been closed by then, it will time out and all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded. The 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.**

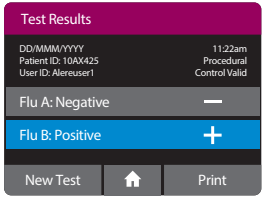
When amplification and detection is complete, the instrument will automatically save the data before advancing to the results screen.



⚠ **Caution: The test is not saved until the completed result is displayed. Do not open the lid until the results are displayed.**

To Perform a Test:

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.



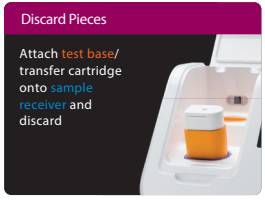
Press Print to print test results, press New Test to run another test, Press Home to return to the Home screen

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.



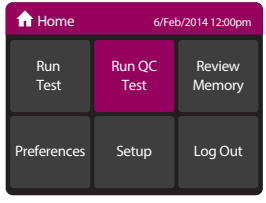
⚠ **Caution: DO NOT disassemble the Transfer Cartridge and the Test Base before disposal.**

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.

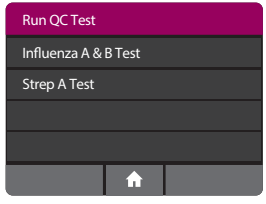
Quality Control Swab Test Procedure

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.

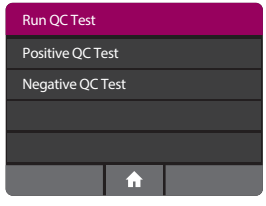
1 Touch 'Run QC Test'



2 Touch 'Influenza A & B Test'



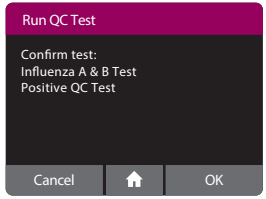
3 Select the QC Test to be Run



4 Confirm Test

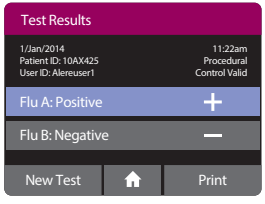
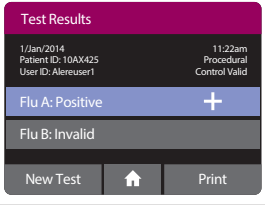
Confirm the test type to match the QC sample intended for testing by touching ‘OK’ and following the on screen prompts to complete testing.

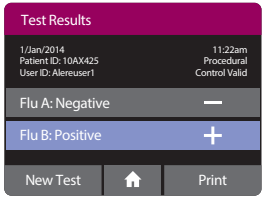
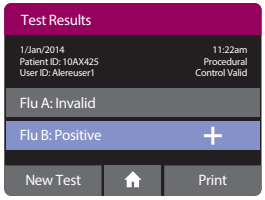
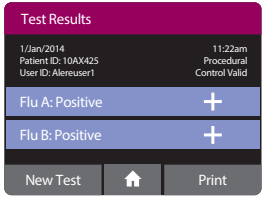
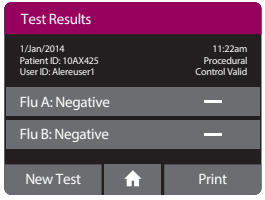
*Note: The QC test is run in the same manner as a Patient Test. See the **To Perform a Test** section above for step by step instructions.*

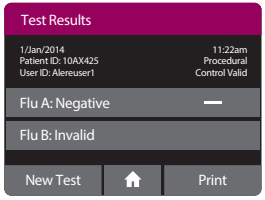
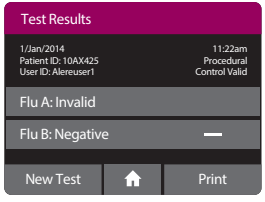
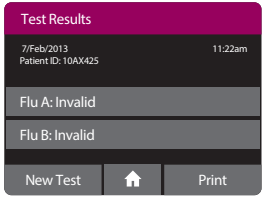


RESULT INTERPRETATION

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.

Instrument Display	Interpretation of Results and Follow-up Actions
	**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.**

Instrument Display	Interpretation of Results and Follow-up Actions
	**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.

LIMITATIONS

- 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.
- **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 virus(es) are infectious, or are the causative agents for clinical symptoms.
- Performance of **Alere™ i** Influenza A & B has not been established for monitoring antiviral treatment of influenza.
- 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.
- 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.
- 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.
- 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 2012 to 2013 influenza season. 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 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.

EXPECTED VALUES

The prevalence of influenza varies from year to year, with outbreaks typically occurring during the fall and winter months.[2] The rate of positivity found in influenza testing is dependent on many factors including the method of specimen collection, the test method used, geographic location, and the disease prevalence in specific localities. In the **Alere™ i** Influenza A & B multi center prospective clinical studies (described in the "Clinical Study" section below), a total of 585 direct nasal swab specimens (2012/2013 influenza season) and 1243 nasal or nasopharyngeal swab specimens eluted in viral transport media (2014/2015 influenza season) were determined to be evaluable. The number and percentage of influenza A and influenza B positive cases per specified age group, as determined by the **Alere™ i** Influenza A & B assay, are presented in the two tables below:

Influenza A Positives by the Alere™ i Influenza A & B Assay per Age Group

	Prospective Clinical Study During the 2012/2013 Influenza Season			Prospective Clinical Study During the 2014/2015 Influenza Season		
Age Group	**Number of Direct Nasal Swab Specimens**	**Number of Influenza A Positives**	**Influenza A Positivity Rate**	**Number of Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media Specimens**	**Number of Influenza A Positives**	**Influenza A Positivity Rate**
<1 year	121	25	20.1%	173	21	12.1%
1 to 5 years	219	63	28.8%	533	95	17.8%
6 to 10 years	102	35	34.3%	238	75	31.5%
11 to 15 years	41	12	29.3%	115	30	26.1%
16 to 21 years	22	3	13.6%	35	5	14.3%
>21 to 60 years	71	18	25.4%	85	19	22.4%
>60 years	9	2	22.2%	64	11	17.2%
Total	585	158	27.0%	1243	256	20.6%

Influenza B Positives by the Alere™ i Influenza A & B Assay per Age Group

	Prospective Clinical Study During the 2012/2013 Influenza Season			Prospective Clinical Study During the 2014/2015 Influenza Season		
Age Group	**Number of Direct Nasal Swab Specimens**	**Number of Influenza B Positives**	**Influenza B Positivity Rate**	**Number of Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media Specimens**	**Number of Influenza B Positives**	**Influenza B Positivity Rate**
<1 year	121	12	9.9%	173	10	5.8%
1 to 5 years	219	26	11.9%	533	41	7.7%
6 to 10 years	102	26	25.5%	238	37	15.5%
11 to 15 years	41	7	17.1%	115	15	13.0%
16 to 21 years	22	5	22.7%	35	2	5.7%
>21 to 60 years	71	14	19.7%	85	5	5.9%
>60 years	9	1	11.1%	64	1	1.7%
Total	585	91	15.6%	1243	111	8.9%

PERFORMANCE CHARACTERISTICS

Clinical Study:

Direct Nasal Swabs (Without Elution in Viral Transport Media)

Clinical performance characteristics of **Alere™ i** Influenza A & B were evaluated in a multi-site prospective study during the 2012-2013 flu season in the U.S. A total of eight investigational sites throughout the U.S. participated in the study. To be enrolled in the study, patients had to be presenting at the participating study centers with flu-like symptoms. Direct nasal swab specimens from patients with flu-like symptoms were collected and tested using the **Alere™ i** Influenza A & B at the eight study sites. Viral culture performed according to standard virology culture procedures, was utilized as the reference method for this study.

Two nasal swabs were collected from one nostril from each subject using standard collection methods. At all sites, one nasal swab was tested directly on **Alere™ i** Influenza A & B, according to product instructions. The other nasal swab was eluted in 3-mL of viral transport media (VTM). Six of the eight sites (Site 1, Site 4, Site 8, Site 10, Site 11, and Site 12) shipped nasal swab samples in VTM to a central testing laboratory for viral culture testing. This central testing laboratory was located at Site 1, which also participated as a sample collection and **Alere™ i** Influenza A & B testing site. The nasal swab samples in VTM from Site 2 and Site 9 were cultured on site by a local laboratory.

External control testing, using **Alere™ i** Influenza A & B Positive and Negative Controls, was performed prior to sample testing each day and on each **Alere™ i** instrument the testing was performed, at all study sites.

All specimens generating discrepant **Alere™ i** Influenza A & B and viral culture results were investigated by testing using an FDA-cleared Influenza RT-PCR assay at a central testing laboratory located at Site 1.

A total of 612 nasal swab specimens were enrolled in this study. Of those, 27 nasal swab specimens did not meet eligibility criteria. A total of 585 direct nasal swab specimens were considered evaluable. Patient age and gender distribution for all the evaluable specimens is presented in the table below.

Age and Gender Distribution – Direct Nasal Swab Study

Age Group	Female	Male
<1 year	56	59
1 to 5 years	108	117
6 to 10 years	55	47
11 to 15 years	21	20
16 to 21 years	14	8
>21 to 60 years	51	20
>60 years	5	4
Total	310	275

Of the evaluable 585 specimens, **Alere™ i** Influenza A & B generated influenza A invalid results for 14 specimens and influenza B invalid results for 16 specimens, resulting in a total of 571 specimens for influenza A performance analysis and 569 specimens for influenza B performance analysis.

Compared to the viral culture reference method, the performance of **Alere™ i** Influenza A & B for influenza A and influenza B are presented in the two tables below.

Direct Nasal Swab - Performance Obtained for Influenza A with Alere™ i Influenza A & B against Viral Culture

Alere™ i Influenza A & B – Flu A	**Culture**		
	Positive	**Negative**	**Total**
Positive	92	66[a]	158
Negative	2[b]	411	413
Total	94	477	571
Sensitivity: 92/94 97.9% (95%CI: 92.6%-99.4%)			
Specificity: 411/477 86.2% (95%CI: 82.8%-89.0%)			

a Flu A nucleic acid was detected in 58/66 False Positive specimens using an FDA-cleared molecular test
b Flu A nucleic acid was not detected in 1/2 False Negative specimens using an FDA-cleared molecular test

Direct Nasal Swab - Performance Obtained for Influenza B with Alere™ i Influenza A & B against Viral Culture

Alere™ i Influenza A & B – Flu B	**Culture**		
	Positive	**Negative**	**Total**
Positive	74	17[a]	91
Negative	6[b]	472	478
Total	80	489	569
Sensitivity: 74/80 92.5% (95%CI: 84.6%-96.5%)			
Specificity: 472/489 96.5% (95%CI: 94.5%-97.8%)			

a Flu B nucleic acid was detected in 15/17 False Positive specimens using an FDA-cleared molecular test
b Flu B nucleic acid was not detected in 4/6 False Negative specimens using an FDA-cleared molecular test

Performance of **Alere™ i** Influenza A & B for the detection of influenza A and influenza B versus culture is presented in the table below stratified by patient age.

Direct Nasal Swab - Performance Obtained for Influenza A and Influenza B with Alere™ i Influenza A & B in Comparison to Viral Culture – Stratified by Patient Age

	≤ 5 Years of Age (n = 332)		6 - ≤ 21 Years of Age (n = 162)		≥ 22 Years of Age (n = 77)	
Influenza Type	**Sensitivity 95% CI**	**Specificity 95% CI**	**Sensitivity 95% CI**	**Specificity 95% CI**	**Sensitivity 95% CI**	**Specificity 95% CI**
Flu A	98.3% (58/59) 91.0% - 99.7%	89.0% (243/273) 84.7% - 92.2%	100% (31/31) 89.0% - 100%	85.5% (112/131) 78.5% - 90.5%	75.0% (3/4) 30.1% - 95.4%	76.7% (56/73) 65.8% - 84.9%
Flu B	88.9% (32/36) 74.7% - 95.6%	98.0% (288/294) 95.6% - 99.1%	94.4% (34/36) 81.9% - 98.5%	96.8% (122/126) 92.1% - 98.8%	100% (8/8) 67.6% - 100%	89.9% (62/69) 80.5% - 95.0%

Alere™ i Influenza A & B detected one mixed influenza A and B infection in the prospective clinical evaluation. This sample tested positive for influenza B only by viral culture, but tested positive for influenza A only by an FDA cleared Influenza RT-PCR assay.

During the prospective clinical study, the initial invalid rate (before repeat testing per the product instructions) was 5.8% (34/585) (95% CI: 4.2% to 8.0%) for Flu A, and 3.6% (21/585) (95% CI: 2.4% to 5.4%) for Flu B. After repeat testing per the product instructions, the invalid rate was 2.4% (14/585) (95% CI: 1.4%, 4.0%) for Flu A, and 2.7% (16/585) (95% CI: 1.7%, 4.4%) for Flu B.

Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media

Clinical performance characteristics of **Alere™ i** Influenza A & B were evaluated in a multi-site prospective study during the 2014-2015 flu season in the U.S. A total of three investigational sites across the U.S. participated in the study. To be enrolled in the study, patients had to be presenting at the participating study centers with flu-like symptoms. Nasal or nasopharyngeal swab specimens were collected from patients with flu-like symptoms and were placed in viral transport media. The samples were processed and tested using the **Alere™ i** Influenza A & B assay according to the test procedure for **Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media**. An FDA-cleared influenza real-time Polymerase Chain Reaction (RT-PCR) test was utilized as the comparator method for this study. All discrepant samples were tested on a different FDA-cleared influenza real-time RT-PCR assay at Alere Scarborough Inc. to confirm influenza status. External control testing, using **Alere™ i** Influenza A & B Positive and Negative Controls, was performed prior to sample testing each day and on each **Alere™ i** instrument for the duration of the clinical study. A total of 1,273 nasal or nasopharyngeal swabs eluted in viral transport media were enrolled in this study. Of those, 3 specimens did not meet eligibility criteria. A total of 1,270 viral transport media specimens were tested with the **Alere™ i** Influenza A & B assay. Patient age and gender distribution for all included specimens in this study is presented in the table below.

Age and Gender Distribution - Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media Study

Age Group	Female	Male
<1 year	92	84
1 to 5 years	258	282
6 to 10 years	113	131
11 to 15 years	63	56
16 to 21 years	16	20
>21 to 60 years	52	37
>60 years	42	24
Total	636	634

Of the 1,270 specimens, **Alere™ i** Influenza A & B generated invalid results for 27 specimens after repeat testing per the product instructions, resulting in a total of 1,243 specimens for performance analysis.

Compared to the comparator method, the performance of **Alere™ i** Influenza A & B for influenza A and influenza B are presented in the two tables below.

Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media - Performance Obtained for Influenza A with Alere™ i Influenza A & B against the Comparator Method

Alere™ i Influenza A & B – Flu A	**Comparator Method**		
	Positive	**Negative**	**Total**
Positive	221	35[a]	256
Negative	5	982	987
Total	226	1017	1243
Sensitivity: 221/226	97.8% (95%CI: 94.9%-99.1%)		
Specificity: 982/1017	96.6% (95%CI: 95.3%-97.5%)		

[a] Flu A nucleic acid was detected in 31/35 False Positive specimens using an alternative FDA-cleared molecular test

Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media - Performance Obtained for Influenza B with Alere™ i Influenza A & B against the Comparator Method

Alere™ i Influenza A & B – Flu B	**Comparator Method**		
	Positive	**Negative**	**Total**
Positive	92	19[a]	111
Negative	7	1125	1132
Total	99	1144	1243
Sensitivity: 92/99	92.9% (95%CI: 86.1%-96.5%)		
Specificity: 1125/1141	98.3% (95%CI: 97.4%-98.9%)		

[a] Flu B nucleic acid was detected in 3/19 False Positive specimens using an alternative FDA-cleared molecular test

Performance of **Alere™ i** Influenza A & B for the detection of influenza A and influenza B versus the comparator method in this study is presented in the table below stratified by patient age.

Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media - Performance Obtained for Influenza A and Influenza B with Alere™ i Influenza A & B in Comparison to the Comparator Method - Stratified by Patient Age

	≤ 5 Years of Age (n = 706)		6 - ≤ 21 Years of Age (n = 388)		≥ 22 Years of Age (n = 149)	
Influenza Type	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
Flu A	99.1% (105/106) 94.8%, 99.8%	98.2% (589/600) 96.7%, 99.0%	97.0% (98/101) 91.6%, 99.0%	95.8% (275/287) 92.8%, 97.6%	94.7% (18/19) 75.4%, 99.1%	90.8% (118/130) 84.6%, 94.6%
Flu B	100% (39/39) 91.0%, 100%	98.2% (655/667) 96.9%, 99.0%	94.2% (49/52) 84.4%, 98.0%	98.5% (331/336) 96.6%, 99.4%	50% (4/8) 21.5%, 78.5%	98.6% (139/141) 95.0%, 99.6%

During this prospective clinical study, the initial invalid rate (before repeat testing per the product instructions) was 4.3% (54/1270) (95% CI: 3.3% to 5.5%). After repeat testing per the product instructions, the invalid rate was 2.1% (27/1270) (95% CI: 1.5%, 3.1%).

ANALYTICAL STUDIES:

Reproducibility

A reproducibility study of **Alere™ i** Influenza A & B was conducted by operators from three sites using panels of blind coded specimens containing negative, high negative (below the limit of detection), low positive (at the limit of detection), and moderate positive (above the limit of detection) influenza A and B viral samples.

Virus dilutions were prepared using one influenza A strain and one influenza B strain in Universal Transport Medium (UTM). The concentrations of the viral stocks (in $TCID_{50}$/mL) were determined by standard virologic method prior to inactivation by the vendors. The concentration for each dilution (in genome equivalents/mL) was also assessed using laboratory developed and validated influenza A and influenza B quantitative real-time PCR assays.

Contrived nasal swab specimens were prepared by coating 10 microliters of each virus dilution onto the swab. The contrived swab samples were tested without further elution in viral transport media according to product instructions.

Participants tested each sample multiple times on five different days. The percent agreement with expected results for the influenza A moderate positive, low positive, and high negative samples were 100% (90/90), 100 % (90/90) and 70% (63/90), respectfully. The percent agreement with expected result for the influenza B moderate positive, low positive, and high negative samples were 100% (90/90), 92% (83/90) and 90% (81/90), respectfully. All of the true negative samples (90) generated negative test results. There were no significant differences observed within run (replicates tested by one operator), between run (five different days), between sites (three sites), or between operators (six operators).

The Reproducibility Study site-to-site qualitative results (agreements with expected results) are presented in the table below:

Reproducibility Study Site-To-Site Qualitative Results

Sample Category	Site						Overall Percent Agreement and 95% CI	
	Site 1		Site 2		Site 3			
	Percent Agreement	Count	Percent Agreement	Count	Percent Agreement	Count		
HN[1] Influenza A	66.7%	20/30	80.0%	24/30	63.3%	19/30	70.0% (63/90)	(59.9%, 78.5%)
LP Influenza A	100%	30/30	100%	30/30	100%	30/30	100% (90/90)	(95.9%, 100%)
MP Influenza A	100%	30/30	100%	30/30	100%	30/30	100% (90/90)	(95.9%, 100%)
HN[1] Influenza B	86.7%	26/30	100%	30/30	83.3%	25/30	90.0% (81/90)	(82.1%, 94.6%)
LP Influenza B	93.3%	28/30	86.7%	26/30	96.7%	29/30	92.2% (83/90)	(84.8%, 96.2%)
MP Influenza B	100%	30/30	100%	30/30	100%	30/30	100% (90/90)	(95.9%, 100.0%)
TN	100%	30/30	100%	30/30	100%	30/30	100% (90/90)	(95.9%, 100%)

[1] *Percent Agreement correlates to the percent of negative results.*

Analytical Sensitivity (Limit of Detection)

Alere™ i Influenza A & B limit of detection (LOD) in natural nasal swab matrix was determined by evaluating different concentrations of 3 strains of influenza A and 2 strains of influenza B virus in **Alere™ i** Influenza A & B. Three strains of influenza A virus representing each of the three common currently or recently circulating influenza A subtypes (i.e., A/H1N1, A/H3N2 seasonal, and A/H1N1 pandemic (pdm)) and two strains of influenza B virus representing each of the two influenza B genetic lineages (i.e., Victoria and Yamagata) were included in this study.

Presumed negative natural nasal swab specimens were eluted in UTM. Swab elutes were combined and mixed thoroughly to create a clinical matrix pool to be used as the diluent. Each influenza virus strain was diluted in this natural nasal swab matrix pool to generate virus dilutions for testing. The vendor provided virus strains were re-titered and the concentrations (in $TCID_{50}$/mL) were determined by standard virologic method. The concentration for each dilution (in genome equivalents/mL) was also assessed using laboratory developed and validated influenza A and influenza B quantitative real-time PCR assays.

Contrived nasal swab samples were prepared by coating 10 microliters of each virus dilution onto the swab. The contrived swab samples were tested without further elution in viral transport media according to the test procedure for Direct Nasal Swab.

An additional LOD study was conducted with contrived swab samples eluted into VTM and tested according to the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media. The LOD for each influenza strain tested was determined as the lowest virus concentration that was detected ≥ 95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive).

The confirmed LODs in natural nasal swab matrix for both direct swab and swab eluted in VTM for each influenza strain tested are presented in the tables below:

Limit of Detection (LOD) Study Results – Natural Nasal Swab Matrix (Direct Swab Testing)

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	LOD ($TCID_{50}$/mL)	LOD ($TCID_{50}$/Swab)*	LOD (Genome Equivalents/mL)	LOD (Genome Equivalents/Swab)*
A/Puerto Rico/8/34	A/H1N1	1.88×10^5	1.88×10^3	4.22×10^6	4.22×10^4
A/Perth/16/2009	A/H3N2	8.60×10^2	8.60×10^0	7.91×10^4	7.91×10^2
A/California/7/2009	A/2009 H1N1 pdm	1.25×10^4	1.25×10^2	5.20×10^6	5.20×10^4
B/Malaysia/2506/2004	B Victoria lineage	1.90×10^3	1.90×10^1	7.24×10^4	7.24×10^2
B/Bangladesh/3333/2007	B Yamagata lineage	5.55×10^2	5.55×10^0	7.36×10^4	7.36×10^2

**Note: 10 µL of each virus dilution was coated onto a swab*

Limit of Detection (LOD) Study Results - Natural Nasal Swab Matrix (Swab Eluted in VTM Testing)

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	LOD ($TCID_{50}$/mL)	LOD ($TCID_{50}$/Swab)*	LOD (Genome Equivalents/mL)	LOD (Genome Equivalents/Swab)*
A/Puerto Rico/8/34	A/H1N1	4.20×10^5	4.20×10^3	4.59×10^6	4.59×10^4
A/Perth/16/2009	A/H3N2	9.82×10^3	9.82×10^1	1.25×10^6	1.25×10^4
A/California/7/2009	A/2009 H1N1 pdm	5.20×10^5	5.20×10^3	7.77×10^6	7.77×10^4
B/Malaysia/2506/2004	B Victoria lineage	1.05×10^5	1.05×10^3	2.29×10^6	2.29×10^4
B/Bangladesh/3333/2007	B Yamagata lineage	1.34×10^4	1.34×10^2	1.98×10^6	1.98×10^4

**Note: 10 µL of each virus dilution was coated onto a swab; each contrived swab was further diluted into 3 mL of UTM*

Analytical Reactivity

An analytical reactivity (inclusivity) study was performed to determine whether the **Alere™ i** Influenza A & B assay is able to detect a variety of influenza A and B strains that represent temporal and geographic diversity.

Vendor provided stocks of influenza A and B strains were diluted in UTM to generate virus dilutions for testing. The concentration (in $TCID_{50}$/mL, $CEID_{50}$/mL, or EID_{50}/mL) for each strain was determined by standard virologic method. The concentration for each dilution (in genome equivalents/mL) was also assessed using laboratory developed and validated influenza A and influenza B quantitative real-time PCR assays.

Contrived swab samples were prepared by coating 10 microliters of virus dilution onto each swab. The contrived swab samples were tested without further elution in viral transport media according to the test procedure for Direct Nasal Swab.

The starting dilution concentration selected for testing in this study was higher than the established LoDs in the Limit of Detection study. Each starting dilution per virus strain was tested in triplicates initially. If the initial testing concentration tested positive for all three replicates, the strain was further diluted 10-fold and tested in triplicates until at least one out three replicates generated a negative result. When a negative result was obtained, additional 2-fold dilutions were tested, starting from the highest dilution that produced 100% (3/3) positive results. A concentration level was considered “reactive/positive” in this study for all but one strain tested (i.e., B/Texas/06/2011 – see footnote “c” under the table below) if all three replicates generated a positive result for the expected influenza virus.

The **Alere™ i** Influenza A & B assay detected all strains tested at the concentrations indicated in the table below:

Analytical Reactivity Study Results

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	Test Concentration (in $TCID_{50}$ or Genome Equivalents, unless indicated otherwise)				Flu A Result (n=3, unless indicate otherwise)	Flu B Result (n=3, unless indicate otherwise)
		$TCID_{50}$/mL	$TCID_{50}$/Swab*	Genome Equivalents/mL	Genome Equivalents/ Swab*		
A/New Caledonia/20/1999 [a]	A/H1N1	9.19×10^5	9.19×10^3	4.09×10^6	4.09×10^4	+	-
A/New Jersey/8/76 [a]	A/H1N1	3.41×10^1	3.41×10^{-1}	1.52×10^5	1.52×10^3	+	-
A/Brisbane/59/2007 [a]	A/H1N1	2.11×10^4	2.11×10^2	3.39×10^5	3.39×10^3	+	-
A/WSN/33 [a]	A/H1N1	2.11×10^2	2.11×10^0	2.43×10^5	2.43×10^3	+	-
A/Port Chalmers/1/73	A/H3N2	4.22×10^4	4.22×10^2	1.31×10^6	1.31×10^4	+	-
A/Hong Kong/8/68	A/H3N2	7.03×10^0	7.03×10^{-2}	2.70×10^5	2.70×10^3	+	-
A/Aichi/2/68	A/H3N2	2.08×10^5	2.08×10^3	7.47×10^6	7.47×10^4	+	-
A/Victoria/3/75	A/H3N2	3.68×10^5	3.68×10^3	3.39×10^6	3.39×10^4	+	-
A/Wisconsin/67/2005	A/H3N2	6.81×10^4	6.81×10^2	2.57×10^6	2.57×10^4	+	-
A/Brisbane/10/2007	A/H3N2	3.16×10^2	3.16×10^0	3.37×10^5	3.37×10^3	+	-
A/Texas/50/2012	A/H3N2	2.5×10^0	2.50×10^{-2}	6.35×10^3	6.35×10^1	+	-
A/Victoria/361/2011	A/H3N2	1.56×10^1	1.56×10^{-1}	3.53×10^5	3.53×10^3	+	-
A/California/4/2009	A/H1N1 (pdm)	1.47×10^4	1.47×10^2	1.07×10^6	1.07×10^4	+	-
A/Maryland/04/2011	A/H1N1 (pdm)	7.88×10^4	7.88×10^2	3.81×10^6	3.81×10^4	+	-
A/New York/18/2009	A/H1N1 (pdm)	1.25×10^2	1.25×10^0	9.16×10^5	9.16×10^3	+	-

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	Test Concentration (in $TCID_{50}$ or Genome Equivalents, unless indicated otherwise)				Flu A Result (n=3, unless indicate otherwise)	Flu B Result (n=3, unless indicate otherwise)
		$TCID_{50}$/mL	$TCID_{50}$/Swab*	Genome Equivalents/mL	Genome Equivalents/ Swab*		
A/Anhui/1/2013 (Inactivated) [a]	A/H7N9 (Detected in China in 2013)	4.00×10^6 EID_{50}/mL	4.00×10^4 EID_{50}/Swab	1.72×10^6	1.72×10^4	+	-
A/Indiana/10/2011[a]	A/H3N2v	2.00×10^8 EID_{50}/mL	2.00×10^6 EID_{50}/Swab	5.94×10^4	5.94×10^2	+	-
B/Lee/40	Victoria Lineage	5.00×10^1 $CEID_{50}$/mL	5.00×10^{-1} $CEID_{50}$/Swab	5.40×10^4	5.40×10^2	-	+
B/Victoria/504/2000	Victoria Lineage	1.19×10^3	1.19×10^1	6.24×10^4	6.24×10^2	-	+
B/Nevada/03/2011	Victoria Lineage	1.75×10^3	1.75×10^1	8.29×10^4	8.29×10^2	-	+
B/Montana/05/2012	Victoria Lineage	9.00×10^1	9.00×10^{-1}	2.55×10^4	2.55×10^2	-	+
B/Maryland/1/59	Yamagata Lineage	8.51×10^2	8.51×10^0	1.13×10^5	1.13×10^3	-	+
B/Russia/69 [b]	Yamagata Lineage	4.44×10^1	4.44×10^{-1}	2.96×10^6	2.96×10^4	-	+

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	Test Concentration (in $TCID_{50}$ or Genome Equivalents, unless indicated otherwise)				Flu A Result (n=3, unless indicate otherwise)	Flu B Result (n=3, unless indicate otherwise)
		$TCID_{50}$/mL	$TCID_{50}$/Swab*	Genome Equivalents/mL	Genome Equivalents/ Swab*		
B/Wisconsin/01/2010[c]	Yamagata Lineage	3.68×10^4	3.68×10^2	1.16×10^6	1.16×10^4	-	+
B/Massachusetts/2/2012	Yamagata Lineage	6.25×10^1	6.25×10^{-1}	2.28×10^5	2.28×10^3	-	+
B/Texas/06/2011[c]	Yamagata Lineage	2.89×10^5	6.25×10^3	2.00×10^6	2.00×10^4	-	+

** Note: 10 µL of each virus dilution was coated onto a swab*

a 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.

b Influenza B/Russia/69 lowest level in which 3/3 replicates were positive is approximately 40 to 150 x the LoD (as comparing to the Genome Equivalents/Swab values generated in the LoD with simulated clinical matrix study testing B/Malaysia/2506/2004 and B/Brisbane/60/2008, respectively). A polymorphism within segment PA of the Influenza B genome was identified at a position which is 4 nucleotides from the 3'-end of template 2. This G to A polymorphism results in a G/C (product/template) match to an A/C (product/template) mismatch. An A/C mismatch is determined to be moderately destabilizing, and coupled to its position only 4 nucleotides from the 3'-end of the template 2 recognition region, its impact on annealing is potentially great. The frequency of this G to A polymorphism is determined to be very low. In analyzing the strains present in the NCBI Influenza Virus Resource database from 2/2005 to 3/2014 (N=986), no strains contained this polymorphism, suggesting that it has not been circulating for an extended period of time.

c Influenza B/Wisconsin/01/2010 lowest level in which 3/3 replicates were positive is approximately 15 to 60 x the LoD, and Influenza B/Texas/06/2011 lowest level in which at least 1/3 replicates were positive is approximately 25 to 100 x the LoD (as comparing to the Genome Equivalents/Swab values generated in the LoD with simulated clinical matrix study testing B/Malaysia/2506/2004 and B/Brisbane/60/2008, respectively). A single G to A polymorphism within segment PA of the Influenza B genome was identified at a position which is 5 nucleotides from the 3'-end of the molecular beacon annealing region in both strains. The G to A polymorphism results in a C/G match to a C/A mismatch between the molecular beacon and product 1. The C/A mismatch is determined to be moderately destabilizing that can significantly reduce assay sensitivity. An assessment of what impact this polymorphism would have on the melting temperature (Tm) of the molecular beacon/product 1 annealing was performed and the results showed a Tm drop from 62.3°C to 55.6°C, just below the assay running temperature. This suggests that annealing would occur, but at a greatly reduced level, with a concomitant loss of assay sensitivity. The frequency of this G to A polymorphism is found at a frequency of approximately 5% within the NCBI Influenza Virus Resource database covering the time frame from 2/2005 through 3/2014.

An additional analytical reactivity study was also performed testing the same set of influenza A and B strains as described in the table above following the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media, and demonstrated equivalent analytical reactivity performance to that of testing direct swab samples.

Analytical Specificity (Cross Reactivity)

To determine the analytical specificity of **Alere™ i** Influenza A & B, 53 commensal and pathogenic microorganisms (37 bacteria, 15 viruses and 1 yeast) that may be present in the nasal cavity or nasopharynx were tested. All of the following microorganisms were negative when tested at concentrations ranging from 10^8 to 10^{10} cells/mL, CFU/mL or IFU/mL (bacteria), 10^4 to 10^8 $TCID_{50}$/mL or $CEID_{50}$/mL (viruses), and 10^8 cells/mL (yeast).

Bacteria	Viruses	Yeast
Acinetobacter calcoaceticus	Adenovirus type 1	*Candida albicans*
Bacteroides fragilis	Adenovirus type 7	
Bordetella pertussis	Human Coronavirus OC43	
Chlamydia pneumoniae	Human Coronavirus 229E	
Corynebacterium diphtheriae	Enterovirus/Coxsackievirus B4	
Enterococcus faecalis	Human Cytomegalovirus (CMV) (Herpes V)	
Escherichia coli	Epstein Barr Virus	
Gardnerella vaginalis	Human metapneumovirus	
Haemophilus influenzae	Measles (Edmonston)	
Klebsiella pneumoniae	Mumps (Enders)	
Lactobacillus casei	Parainfluenza 1	
Lactobacillus plantarum	Parainfluenza 2	
Legionella pneumophila	Parainfluenza 3	
Listeria monocytogenes	Respiratory Syncytial Virus type B	
Moraxella/Branhamella catarrhalis	Rhinovirus type 1A	
Mycobacterium avium		

Bacteria	Viruses	Yeast
Mycobacterium intracellulare		
Mycobacterium tuberculosis		
Mycoplasma pneumoniae		
Neisseria gonorrhoeae		
Neisseria meningitidis		
Neisseria sicca		
Neisseria subflava		
Proteus vulgaris		
Pseudomonas aeruginosa		
Serratia marcescens		
Staphylococcus aureus		
Staphylococcus epidermidis		
Streptococcus, Group A		
Streptococcus, Group B		
Streptococcus, Group C		
Streptococcus, Group F		
Streptococcus, Group G		
Streptococcus mutans		
Streptococcus pneumoniae		
Streptococcus salivarius		
Streptococcus sanguinis		

Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated with **Alere™ i** Influenza A & B at the concentrations listed below and were found not to affect test performance.

Substance	Concentration
Mucin	20 µg/mL
Whole Blood	50 µL/mL
Sinus Buster Nasal Spray	200 µL/mL
NeoSynephrine Cold & Sinus Extra Strength Spray	200 µL/mL
Zicam Extreme Congestion Relief	200 µL/mL
Afrin PumpMist Original	200 µL/mL
4-acetamidophenol	200 µg/mL
Acetylsalicylic acid	650 µg/mL
Albuterol	400 ng/mL
Chlorpheniramine	145 ng/mL
Dexamethasone	0.80 mg/mL
Dextromethorphan	1 µL/mL
Diphenhydramine	5 µg/mL
Doxylamine Succinate	236 ng/mL
Ephedrine	237 ng/mL
Flunisolide	6.8 ng/mL
Guaiacol glycerol ether	3.5 ng/mL
Mupirocin	12 mg/mL

Substance	Concentration
Oxymetazoline	0.6 mg/mL
Phenylephrine	12 mg/mL
Rebetol	4.5 µg/mL
Relenza	282 ng/mL
Rimatadine	282 ng/mL
Tamiflu	1.1 µg/mL
Tobramycin	2.43 mg/mL
Triamcinolone	40 µg/mL

Inhibition by other Microorganisms

Alere™ i Influenza A & B test performance in the presence of non-influenza respiratory pathogens was evaluated. Vendor provided stocks of influenza A and B strains were diluted in UTM to approximately 2 to 3 times the limit of detection. Contrived influenza A and B positive swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. The following panel of non-influenza viruses were tested at the concentration provided in the table below and was found not to affect test performance.

Virus Panel	Concentration ($TCID_{50}$/mL)
Adenovirus Type 1	1.58×10^7
Rhinovirus Type 1A	1.58×10^7
Respiratory Syncytial Virus, Type B, Strain 18537	8.89×10^5

In an additional study, contrived influenza A and B positive swab specimens were also eluted into UTM and tested according the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media using the same panel of non-influenza respiratory viruses as described in the table above. None of the non-influenza respiratory viruses tested was found to affect test performance at the concentrations evaluated.

Inhibition by High Levels of Influenza A and B

Alere™ i Influenza A & B test performance in the presence of high levels of influenza A and B was evaluated. Vendor provided stocks of influenza A and B strains were diluted in UTM to approximately 2 to 3 times the limit of detection. Contrived influenza A and B positive swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. To create the co-infection swabs, diluted influenza A (at a concentration approximately 5 times the LoD) was added to the near LoD Flu B swab. Likewise, diluted influenza B (at a concentration approximately 40 times the LoD) was added to the near LoD Flu A swab. No impact on test performance was observed.

Alere™ i Influenza A & B test performance in the presence of high levels of influenza A and B was also evaluated in an additional study following the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media. No impact on test performance was observed at the concentrations tested.

Carry-Over Contamination

An analytical carry-over study was performed to demonstrate that when recommended laboratory practices are followed, there is little risk of false positive results caused by carryover or cross-contamination in the **Alere™ i** Influenza A & B test. Vendor provided stocks of influenza A and B strains were diluted in UTM to a minimum of 10^6 $TCID_{50}$/mL. Contrived influenza A and B positive swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. Testing of the contrived positive swabs was alternated with testing of a negative swab sample for a total of 15 rounds. There were no false positive results obtained.

An additional analytical carry-over study was performed testing contrived positive VTM samples alternated with negative VTM samples following the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media for a total of 30 rounds. No false positive results were observed in this study except for one Flu B false positive result.

CLIA Waiver Studies:

As part of the prospective study testing direct nasal swabs (without elution in Viral Transport Media) (as described in the Performance Characteristics section above) the accuracy of **Alere™ i** Influenza A & B was evaluated when used by operators who had no laboratory experience and who were representative of CLIA waived testing sites (intended users). The study was conducted at eight (8) CLIA waived sites with 26 intended users participating. No training on the use of the test was provided to the operators. The testing of 571 prospectively collected samples, described above in the sub-section titled “Direct Nasal Swabs (Without Elution in Viral Transport

Media) Samples" under the "Clinical Study" section, was supplemented with testing swab samples prepared with archived respiratory specimens that were obtained from patients with influenza-like symptoms and that were positive by viral culture for influenza B or negative for both influenza A and influenza B. A total of 60 swabs (35 swabs positive for influenza B and 25 swabs negative for both influenza A and influenza B) were tested. The supplemental samples were blind-coded and randomized.

Overall, 630 nasal swab specimens were tested by intended users at CLIA waived sites with **Alere™ i** Influenza A & B, and the results were compared to the results of an FDA cleared Real-Time reverse transcriptase PCR (RT-PCR) assay, the comparator method for this study. One of the prospectively collected specimens did not contain a sufficient volume to perform RT-PCR and thus the numbers of prospective and retrospective specimens in this study were 570 and 60, respectively. The performance of **Alere™ i** Influenza A & B compared to PCR for all specimens combined, are presented in the tables below, including the 95% confidence intervals (95% CI).

Alere™ i Influenza A & B Performance against RT-PCR for Influenza A

Alere™ i Influenza A & B – Flu A	**RT-PCR**		
	Positive	**Negative**	**Total**
Positive	147	11	158
Negative	8	464	472
Total	155	475	630
Positive Percent Agreement: 147/155	94.8%	(95%CI: 90.1%-97.4%)	
Negative Percent Agreement: 464/475	97.7%	(95%CI: 95.9%-98.7%)	

Alere™ i Influenza A & B Performance against RT-PCR for Influenza B

Alere™ i Influenza A & B – Flu B	**RT-PCR**		
	Positive	**Negative**	**Total**
Positive	123	3	126
Negative	2	500	502
Total	125	503	628
Positive Percent Agreement: 123/125	98.4%	(95%CI: 94.4%-99.6%)	
Negative Percent Agreement: 500/503	99.4%	(95%CI: 98.3%-99.8%)	

A study was conducted to evaluate the performance of **Alere™ i** Influenza A & B with weakly reactive samples when used by untrained users. Randomized blind-coded panels, containing negative, low positive (at the limit of detection {LOD} or assay cutoff), and high negative (below the LOD) influenza A and influenza B specimens, were tested with the **Alere™ i** Influenza A & B Test at 3 CLIA waived sites (60 tests in total). Six untrained users at the CLIA waived sites participated in the study. The panel testing was conducted over a minimum of 6 days at each site, and the testing was integrated into the users' daily work flow. The performance of **Alere™ i** Influenza A & B with samples near the assay cutoff was acceptable when used by untrained users.

The table below shows performance of the test with samples near the cutoff of the assay for influenza A and influenza B in the hands of untrained users.

Influenza A and B Testing of Samples near the Assay Cutoff (LOD)

	Untrained Users	
Sample Type	**% Detection**	**95% CI**
Influenza A Low Positive (at LOD)	100% (60/60)	94.0%, 100%
Influenza A High Negative (below LOD)	53% (32/60)	40.9%, 65.4%
Influenza B Low Positive (at LOD)	97% (58/60)	88.6%, 99.1%
Influenza B High Negative (below LOD)	8% (5/60)	3.6%, 18.1%
Negative Sample	0% (0/60)	0%, 6.0%

**10 invalid results were generated by the untrained users (3% (10/300) with 95% CI: 1.8%, 6.0%)*

Using risk analysis as a guide, analytical flex studies were conducted on **Alere™ i** Influenza A & B. The studies demonstrated that the test is insensitive to stresses of environmental conditions and potential user errors.

SYMBOLS

 Fragile, handle with care	BASE Test Base	CARTRDG Transfer Cartridge	RCVR Sample Receiver	℞ Only Prescription Only (Applies to U.S. only)

ORDERING AND CONTACT INFORMATION

Reorder numbers:

425-024: Alere™ i Influenza A & B 24 Test Kit US/OUS

NAT-024: Alere™ i Instrument US

NAT-000: Alere™ i Instrument OUS

425-080: Alere™ i Influenza A & B Control Swab Kit US/OUS

US +1 877 441 7440
OUS +1 321 441 7200

Technical Support Advice Line

Further information can be obtained from your distributor, or by contacting Technical Support on:

US
+1 855 731 2288 ts.scr@alere.com

Africa, Russia, CIS
+972 8 9429 683 ARCISproductsupport@alere.com

Asia Pacific
+61 7 3363 7711 APproductsupport@alere.com

Canada
+1 800 818 8335 CANproductsupport@alere.com

Europe & Middle East
+44 161 483 9032 EMEproductsupport@alere.com

Latin America
+57 2 6618797 LAproductsupport@alere.com

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Alere Scarborough, Inc.
10 Southgate Road
Scarborough, Maine 04074 USA
www.alere.com

EMERGO EUROPE
Molenstraat 15
2513 BH, The Hague
The Netherlands

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