Alere™ i

Influenza A & B Package Insert

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INTENDED USE

Alere™ i Influenza A & B is a rapid, instrument-based, molecular *in vitro* diagnostic test utilizing isothermal nucleic acid amplification technology for the qualitative detection of influenza A and B viral nucleic acid in nasal swab and viral transport media specimens. It is intended to aid in the rapid differential diagnosis of influenza A and B viral infections.

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.

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, instrument-based isothermal test for the qualitative detection and differentiation of influenza A and influenza B from nasal swab and viral transport media specimens. 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 the target nucleic acid and an internal control. **Alere™ i** Influenza A & B utilizes a pair of templates (similar to primers) for the specific amplification of RNA from influenza A and B and a fluorescently-labelled molecular beacon designed to specifically identify 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, 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

PRECAUTIONS

1. For *in vitro* diagnostic use.
2. To be used in conjunction with the **Alere™ i** Instrument.
3. Leave test pieces sealed in their foil pouches until just before use.
4. Do not tamper with test pieces prior to use.
5. Do not use kit past its expiration date.
6. Do not mix components from different kit lots.
7. Solutions used to make the control swabs are inactivated using standard methods. However, patient samples, controls, and test pieces should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal.
8. **If any assay components are dropped, cracked, found to be damaged or opened when received, DO NOT USE and discard. Do not use scissors or sharp objects to open foil pouches as damage to test pieces can occur.**
9. Do not open the Sample Receiver before placing in the instrument. It will prohibit the Elution Buffer from reaching temperature and may impact test performance.
10. If the Sample Receiver is spilled while opening, clean the instrument per instructions provided in the instrument User Manual and cancel test. Repeat test with a new Sample Receiver.
11. All test pieces must be removed from the instrument according to removal instructions displayed on the instrument, and disposed of according to country and local requirements. **Pieces must not be separated once they are assembled.**
12. 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.
13. All test pieces are single use items. Do not use with multiple specimens.
14. Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.
15. 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, H3N2 (variant) and H7N9 influenza subtypes but the utility of the assay for detection of these subtypes in a clinical setting has not been established due to the lack of sufficient numbers of samples.

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 very 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.

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 Test Procedure or Instrument User Manual for further details.

*Note: The **Alere™ i** Instrument reports QC results as Pass or Fail. Flu A/B Positve 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, flocked 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. Other flocked swabs have been validated for use.

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.

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 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°C and tested within 24 hours from the time of sample collection.

SPECIMEN TRANSPORT AND STORAGE

If the transport of samples is required, the transport medias listed below were tested and are acceptable for use in the **Alere™ i** Influenza A & B system. Minimal dilution of the sample is recommended as dilution may result in decreased test sensitivity. 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. Eluted swab samples can be held at 2-8°C for up to 24 hours prior to testing with **Alere™ i** Influenza A & B. If needed, transport sample at 2-8°C in a leak-proof container.

Swirl eluted viral transport media samples gently to mix before testing.

Transport Media:

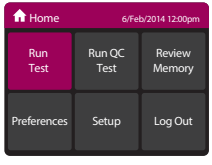
Hank's Balanced Salt Solution	Stuart's Media
M4 Media	Tryptose Phosphate Broth
M4-RT Media	Veal Infusion Broth
M5 Media	Universal Transport Media
M6 Media	Starplex Multitrans
Phosphate Buffered Saline	Vircell
Saline	

It has been determined that Amie's Media, Brain Heart Infusion Broth, and Dulbecco's Modified Eagles' Medium are 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.
- Turn on the **Alere™ i** Instrument and log in, so that you can see the Home screen



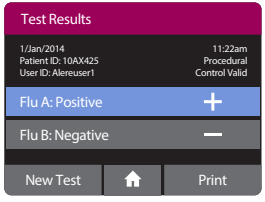
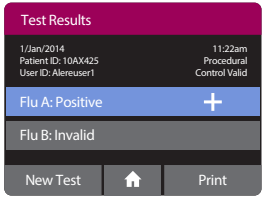
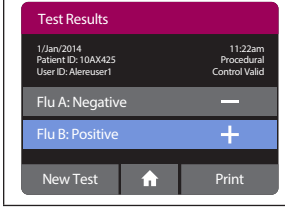
To Perform Test:

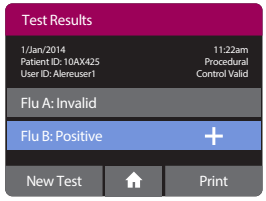
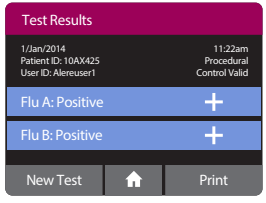
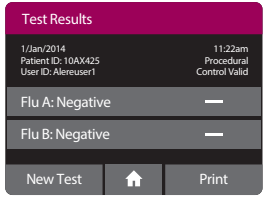
1. Select **Run Test**.
2. Scan or input Patient ID
3. Follow the step-by-step instructions shown on the instrument screen. Refer to the Running a Test in the **Alere™ i** Instrument User Manual for further details.

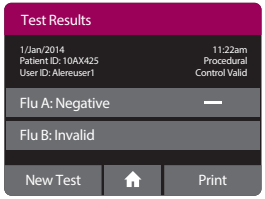
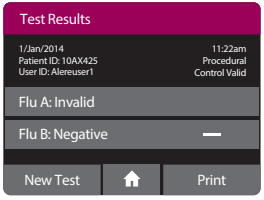
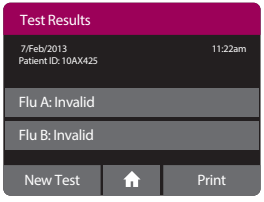
For QC testing, select Run QC Test on the Home screen, and follow the displayed instructions. Refer to Running a QC Test in the **Alere™ i** Instrument User Manual for further details.

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	Suggested Report
	**Positive for Flu A viral RNA.** This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
	**Positive for Flu A viral RNA.** This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
	**Positive for Flu B viral RNA.** This result does not rule out co-infections with other pathogens or identify any specific influenza B virus subtype.

Instrument Display	Suggested Report
	**Positive for Flu B viral RNA.** This result does not rule out co-infections with other pathogens or identify any specific influenza B virus subtype.
	**Positive for Flu A and Flu B viral RNA.** Co-infection with influenza A and B is rare. This result does not rule out co-infections with other pathogens or identify any specific influenza A or influenza B virus subtype.
	**Negative for Flu A and Flu B viral RNA.**

Instrument Display	Suggested Report
	**Negative for Flu A viral RNA.** Infection due to Flu B cannot be ruled out. If infection with Flu B is suspected, 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.
	**Negative for Flu B viral RNA.** Infection due to Flu A cannot be ruled out. If infection with Flu A is suspected, 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.
	**Invalid.** Repeat the test using new test components. If repeated Invalid results are obtained, repeat test with a new patient sample and new test components.

LIMITATIONS

- **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 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.
- Visibly bloody samples must not be used with **Alere™ i** Influenza A & B.
- 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.
- If differentiation of specific influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.

EXPECTED VALUES

The prevalence of influenza varies from year to year, with outbreaks typically occurring during the fall and winter months.[1] 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. Type A viruses are typically associated with most serious influenza epidemics, while Type B are typically milder. In multi-center clinical studies conducted in the U.S. during the 2012-2013 respiratory season, the average prevalence of influenza A (as determined by viral cell culture) was 16%. The average prevalence of influenza B was 14%.

PERFORMANCE CHARACTERISTICS

Clinical Study:

The clinical performance of **Alere™ i** Influenza A & B was established in a multi-center, prospective clinical study conducted at 8 US trial sites during the 2012-2013 respiratory seasons.

A total of 571 prospective nasal swab specimens, collected from patients of all ages presenting with influenza-like symptoms, were evaluated with **Alere™ i** Influenza A & B, and compared to viral culture. Fifty-eight percent (58%) of the population tested was ≤ 5 years of age, 28% was 6 - 21 years of age, and 13% was > 21 years. A/H3 and A/H1 were the predominant influenza A subtypes observed during the times the specimens were collected.

In this study, two (2) nasal swabs were collected from each of a total of 571 patients. One nasal swab from each patient was tested with **Alere™ i** Influenza A & B. The other nasal swab was placed in 3 mL of VTM, eluted and used for viral culture. At four (4) designated sites the eluted sample was also tested with **Alere™ i** Influenza A & B.

Alere™ i Influenza A & B performance, including 95% confidence intervals, versus viral culture, presented by sample type and stratified by age group is provided below. **Alere™ i** Influenza A & B sensitivity versus positive culture results for influenza A or influenza B is also presented by the duration of time that passed between the onset of symptoms and when the sample was collected.

NASAL SWAB DIRECT – WITHOUT DILUTION IN VIRAL TRANSPORT MEDIA

Alere™ i Influenza A & B Performance vs. Culture (All Age Groups Combined)

Influenza Type A

	Culture +	Culture -	
Alere™ i +	92	66	158
Alere™ i -	2	411	413
	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%)

Influenza Type B

	Culture +	Culture -	
Alere™ i +	74	17	91
Alere™ i -	6	472	478
	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%)

Two percent (2%) (14/585) of the total number of specimens tested generated invalid results for Flu A (95% CI: 1.4%, 4.0%). Three percent (3%) (16/585) of the total number of specimens tested generated invalid results for Flu B (95% CI: 1.7%, 4.4%).

There were a large number of samples with discrepant **Alere™ i** Influenza A & B and culture results. In most cases **Alere™ i** Influenza A & B was positive and culture was negative, indicating that **Alere™ i** Influenza A & B is more sensitive than viral culture for the detection of influenza A and/or B. All discrepant samples were tested on an FDA cleared RT-PCR assay at a central testing laboratory to confirm influenza status. Performance of **Alere™ i** Influenza A & B for the detection of Flu A and Flu B when the results of PCR are incorporated for all age groups combined is presented in the table below

Alere™ i Influenza A & B Performance vs. Culture (All Age Groups Combined) – Discrepant Results Resolved by RT-PCR

Influenza Type A

	Culture or PCR +	Culture or PCR -	
Alere™ i +	150	8	158
Alere™ i -	1	412	413
	151	420	571

Sensitivity: 150/151 = 99.3% (95% CI: 96.3%, 99.9%)
Specificity: 412/420 = 98.1% (95% CI: 96.3%, 99.0%)

Influenza Type B

	Culture or PCR +	Culture or PCR -	
Alere™ i +	89	2	91
Alere™ i -	2	476	478
	91	478	569

Sensitivity: 89/91 = 97.8% (95% CI: 92.3%, 99.4%)
Specificity: 476/478 = 99.6% (95% CI: 98.5%, 99.9%)

Alere™ i Influenza A & B Performance vs. Culture (By Age Group) – Discrepant Results Resolved by RT-PCR

≤ 5 Years of Age (n = 332)	Influenza Type	
	Type A	Type B
Sens (95% CI)	98.8% (85/86) 93.7%, 99.8%	100% (38/38) 90.8%, 100%
Spec (95% CI)	98.8% (243/246) 96.5%, 99.6%	100% (292/292) 98.7%, 100%
6 - ≤ 21 Years of Age (n = 162)	**Influenza Type**	
	Type A	**Type B**
Sens (95% CI)	100% (47/47) 92.4%, 100%	95.0% (38/40) 83.5%, 98.6%
Spec (95% CI)	97.4% (112/115) 92.6%, 99.1%	100% (122/122) 96.7%, 100%
≥ 22 Years of Age (n = 77)	**Influenza Type**	
	Type A	**Type B**
Sens (95% CI)	100% (18/18) 82.4%, 100%	100% (13/13) 77.2%, 100%
Spec (95% CI)	96.6% (57/59) 88.5%, 99.1%	96.9% (62/64) 89.3%, 99.1%

Alere™ i Influenza A & B Sensitivity vs. Culture (By Time Between Onset of Symptoms and Sample Collection) – Discrepant Results Resolved by RT-PCR

	Influenza Type	
< 2 Days Between Onset & Sample Collection (95% CI)	**Type A**	**Type B**
	112/113 = 99.1% (95.2%, 99.8%)	44/46 = 95.7% (85.5%, 98.8%)
2 - 4 Days Between Onset & Sample Collection (95% CI)	**Type A**	**Type B**
	13/13 = 100% (77.2%, 100%)	19/19 = 100% (83.2%, 100%)
> 4 Days Between Onset & Sample Collection (95% CI)	**Type A**	**Type B**
	1/1 = 100% (20.7%, 100%)	1/1 = 100% (20.7%, 100%)

Note: The patients' symptom information was self-reported and was not colleted using clinical study controls.

NASAL SWAB – SAMPLES IN VIRAL TRANSPORT MEDIA

Alere™ i Influenza A & B Performance vs. Culture (All Age Groups Combined)

Influenza Type A

	Culture +	Culture -	
Alere™ i +	86	37	123
Alere™ i -	3	316	319
	89	353	442

Sensitivity: 86/89 = 96.6% (95% CI: 90.6%, 98.8%)
Specificity: 316/353 = 89.5% (95% CI: 85.9%, 92.3%)

Influenza Type B

	Culture +	Culture -	
Alere™ i +	53	13	66
Alere™ i -	10	365	375
	63	378	441

Sensitivity: 53/63 = 84.1% (95% CI: 73.2%, 91.1%)
Specificity: 365/378 = 96.6% (95% CI: 94.2%, 98.0%)

Less than one percent (0.5%) (2/444) of the total number of specimens tested generated invalid results for Flu A (95% CI: 0.1%, 1.6%). Less than one percent (0.7%) (3/444) of the total number of specimens tested generated invalid results for Flu B (95% CI: 0.2%, 2.0%).

There were a large number of samples with discrepant **Alere™ i** Influenza A & B and culture results. In most cases **Alere™ i** Influenza A & B was positive and culture was negative, indicating that **Alere™ i** Influenza A & B is more sensitive than viral culture for the detection of influenza A and/or B. All discrepant samples were tested on an FDA cleared RT-PCR assay at a central testing laboratory to confirm influenza status. Performance of **Alere™ i** Influenza A & B for the detection of Flu A and Flu B when the results of PCR are incorporated for all age groups combined is presented in the table below.

Alere™ i Influenza A & B Performance vs. Culture (All Age Groups Combined) – Discrepant Results Resolved by RT-PCR

Influenza Type A

	Culture or PCR +	Culture or PCR -	
Alere™ i +	119	4	123
Alere™ i -	3	316	319
	122	320	442

Sensitivity: 119/122 = 97.5% (95% CI: 93.0%, 99.2%)
Specificity: 316/320 = 98.8% (95% CI: 96.8%, 99.5%)

Influenza Type B

	Culture or PCR +	Culture or PCR -	
Alere™ i +	57	9	66
Alere™ i -	6	369	375
	63	378	441

Sensitivity: 57/63 = 90.5% (95% CI: 80.7%, 95.6%)
Specificity: 369/378 = 97.6% (95% CI: 95.5%, 98.7%)

Alere™ i Influenza A & B Performance vs. Culture (By Age Group) - Discrepant Results Resolved by RT-PCR

≤ 5 Years of Age (n = 302)	**Influenza Type**	
	Type A	**Type B**
Sens (95% CI)	97.5% (79/81) 91.4%, 99.3%	90.6% (29/32) 75.8%, 96.8%
Spec (95% CI)	99.1% (219/221) 96.8%, 99.8%	99.6% (268/269) 97.9%, 99.9%
6 - ≤ 21 Years of Age (n = 122)	**Influenza Type**	
	Type A	**Type B**
Sens (95% CI)	97.4% (38/39) 86.8%, 99.5%	89.3% (25/28) 72.8%, 96.3%
Spec (95% CI)	97.6% (81/83) 91.6%, 99.3%	96.8% (91/94) 91.0%, 98.9%
≥ 22 Years of Age (n = 18)	**Influenza Type**	
	Type A	**Type B**
Sens (95% CI)	100% (2/2) 34.2%, 100%	100% (3/3) 43.9%, 100%
Spec (95% CI)	100% (16/16) 80.6%, 100%	66.7% (10/15) 41.7%, 84.8%

Alere™ i Influenza A & B Sensitivity vs. Culture (By Time Between Onset of Symptoms and Sample Collection) – Discrepant Results Resolved by RT-PCR

	Influenza Type	
< 2 Days Between Onset & Sample Collection (95% CI)	**Type A**	**Type B**
	93/96 = 96.9% (91.2%, 98.9%)	36/39 = 92.3% (79.7%, 97.4%)
2 - 4 Days Between Onset & Sample Collection (95% CI)	**Type A**	**Type B**
	5/5 = 100% (56.6%, 100%)	5/5 = 100% (56.6%, 100%)
> 4 Days Between Onset & Sample Collection (95% CI)	**Type A**	**Type B**
	1/1 = 100% (20.7%, 100%)	1/1 = 100% (20.7%, 100%)

Note: The patients' symptom information was self-reported and was not collected using clinical study controls.

ANALYTICAL STUDIES:

Reproducibility

A reproducibility study of **Alere™ i** Influenza A & B was conducted by operators from 3 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. Participants tested each sample multiple times on 5 different days. The detection rates for the influenza A moderate positive, low positive, and high negative samples were 100% (90/90), 100% (90/90) and 30% (27/90), respectfully. The detection rates for the influenza B moderate positive, low positive, and high negative samples were 100% (90/90), 92% (83/90) and 10% (9/90), respectfully. All of the negative samples (90) generated negative test results. There were no significant differences within run (replicates tested by one operator), between run (5 different days), between sites (3 sites), or between operators (6 operators).

Analytical Sensitivity (Limit of Detection)

Alere™ i Influenza A & B limit of detection (LOD or C_{95}), defined as the concentration of influenza virus that produces positive **Alere™ i** Influenza A & B results approximately 95% of the time, was identified by evaluating different concentrations of 2 strains of influenza A and 2 strains of influenza B virus in **Alere™ i** Influenza A & B . The concentrations identified as the LOD (or C_{95}) levels for each strain tested are listed below.

Influenza Strain	Concentration ($TCID_{50}$/mL)	# Detected per Total Tests	% Detected
A/Solomon Islands/3/2006 (H1N1)	4.75×10^2	19/20	95%
A/Puerto Rico/8/34 (H1N1)	5.48×10^3	19/20	95%
B/Malaysia/ 2506/2004	7.5×10^1	19/20	95%
B/Brisbane/ 6/2008	5.00×10^1	19/20	95%

Analytical Reactivity

The influenza A and B strains listed tested positive with **Alere™ i** Influenza A & B at concentrations ranging from 10^1 to 10^6 $TCID_{50}$/mL, 10^7 EID_{50}/mL, or 10^3 $CEID_{50}$/mL. Although the specific influenza strains causing infection in humans can vary year to year, all contain the conserved nucleic acid sequence targeted by **Alere™ i** Influenza A & B. Performance characteristics of **Alere™ i** Influenza A & B for detecting influenza A virus from human specimens were established when H1 and H3 subtypes were prevalent. Performance characteristics of the test when other influenza A virus subtypes are emerging as human pathogens have not been established.

Influenza Strain
A/California/7/2009 (H1N1)
A/New Caledonia/20/1999 (H1N1)
A/New Jersey/8/76 (H1N1)
A/Brisbane/59/2007 (H1N1)
A/WSN/33 (H1N1)
A/Port Chalmers/1/73 (H3N2)
A/Hong Kong/8/68 (H3N2)
A/Aichi/2/68 (H3N2)
A/Victoria/3/75 (H3N2)
A/Wisconsin/67/2005 (H3N2)

Influenza Strain
A/Brisbane/10/2007 (H3N2)
A/Perth/16/2009 (H3N2)
A/Anhui/1/2013 (H7N9)
B/Lee/40
B/Russia/69
B/Bangladesh/3333/2007
B/Victoria/504/2000
B/Wisconsin/01/2010
B/Maryland/1/59

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	

Bacteria	Viruses	Yeast
Moraxella/Branhamella catarrhalis	Rhinovirus type 1A	
Mycobacterium avium		
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
4-acetamidophenol	200 µg/mL
Acetylsalicylic acid	650 µg/mL
Albuterol	400 ng/mL

Substance	Concentration
Chlorpheniramine	145 ng/mL
Dexamethasone	0.80 mg/mL
Dextromethorphan	1 µg/mL
Diphenhydramine	5 µg/mL
Doxylamine Succinate	236 ng/mL
Ephedrine	273 ng/mL
Flunisolide	6.8 ng/mL
Guaiacol glycerol ether	3.5 ng/mL
Mupirocin	12 mg/mL
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

SYMBOLS

 Fragile, handle with care	BASE Test Base
CARTRDG Transfer Cartridge	RCVR Sample Receiver

ORDERING AND CONTACT INFORMATION

Reorder numbers:

425-000: Alere™ i Influenza A & B 24 Test Kit

NAT-000: Alere™ i Instrument

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

OUS +1 321 441 7200

Technical Support Advice Line

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

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

EC REP

EMERGO EUROPE
Molenstraat 15
2513 BH, The Hague
The Netherlands

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Alere™ i Influenza A & B Product Insert

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