Influenza Strain	Concentration
Flu A/Port Chalmers/1/73 (H3N2)	5.6 x 10^5 $TCID_{50}$/ml
Flu A/WS/33 (H1N1)	5.0 x 10^4 $TCID_{50}$/ml
Flu A/Aichi/2/68 (H3N2)	3.0 x 10^4 $TCID_{50}$/ml
Flu A/Malaya/302/54 (H1N1)	6.0 x 10^5 $TCID_{50}$/ml
Flu A/New Jersey/8/76 (H1N1)	2.8 x 10^5 $TCID_{50}$/ml
Flu A/Denver/1/57 (H1N1)	8.9 x 10^3 $TCID_{50}$/ml
Flu A/Victoria/3/75 (H3N2)	1.8 x 10^4 $TCID^{50}$/ml
Flu A/Solomon Islands/3/2006 (H1N1)	1.5 x 10^5 $TCID_{50}$/ml
Flu A/Brisbane/10/07 (H3N2)	2.5 x 10^6 EIU_{50}/ml
Flu A/PuertoRico/8/34 (H1N1)	5.6 x 10^5 $TCID_{50}$/ml
Flu A/Wisconsin/67/2005 (H3N2)	1.3 x 10^5 $TCID_{50}$/ml
Flu A/Hong Kong/8/68 (H3N2)	7.9 x 10^3 $TCID_{50}$/ml
Flu A/California/04/2009 (H1N1)	1.4 x 10^5 $TCID_{50}$/ml
Flu A/ANHUI/1/2013 (H7N9)	8.7 x 10^6 EID_{50}/ml
Flu B/Florida/02/2006	1.4 x 10^4 $TCID_{50}$/ml
Flu B/Florida/04/2006	7.1 x 10^4 $TCID_{50}$/ml
Flu B/Florida/07/04	8.5 x 10^4 $TCID_{50}$/ml
Flu B/Malaysia/2506/04	1.5 x 10^6 $TCID_{50}$/ml
Flu B/Panama/45/90	1.7 x 10^4 $TCID_{50}$/ml
Flu B/R75	5.0 x 10^5 $TCID_{50}$/ml
Flu B/Russia/69	2.2 x 10^6 $TCID_{50}$/ml
Flu B/Taiwan/2/62	1.0 x 10^5 $TCID_{50}$/ml
Flu B/Mass/3/66	1.5 x 10^5 $TCID_{50}$/ml
Flu B/Lee/40	1.8 x 10^5 $TCID_{50}$/ml

The Alere™ Influenza A & B Test was used to test 55 archived respiratory patient specimens, confirmed to be positive for the 2009 H1N1 influenza virus by an FDA cleared RT-PCR assay. Overall, the Alere™ Influenza A & B Test detected 45% (25/55) of the RT-PCR assay positive specimens. The detection rate was 94% (16/17) with the higher titer specimens and 24% (9/38) with the lower titer specimens.

Although this test has been shown to detect the Flu A/California/04/2009 (H1N1) and A/Anhui/1/2013 (H7N9) viruses cultured from positive human specimens, the performance characteristics of this device with clinical human specimens infected with these two influenza viruses have not been established. The Alere™ Influenza A & B test can distinguish between influenza A and B viruses, but it does not differentiate seasonal influenza A virus from influenza A 2009 H1N1 or influenza A H7N9. The test's ability to detect human infection with 2009 H1N1 or H7N9 influenza virus in clinical specimens is unknown.

Analytical Specificity (Cross-Reactivity):

To determine the analytical specificity of the Alere™ Influenza A & B Test, 54 commensal and pathogenic microorganisms (38 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), 10^5 to 10^8 $TCID_{50}$/ml or $CEID_{50}$/ml (viruses), and 10^9 cells/ml (yeast).

Bacteria	Viruses	Yeast
Acinetobacter calcoaceticus	Adenovirus type 1	Candida albicans
Bacteroides fragilis	Adenovirus type 7	
Bordetella pertussis	Coronavirus OC43	
Chlamydia pneumoniae	Coronavirus 229E	
Corynebacterium diphtheria	Coxsackievirus B4	
Enterococcus faecalis	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 catarrhalis	Rhinovirus type 1A	
Mycobacterium avium		
Mycobacterium intracellulare		
Mycobacterium tuberculosis		

Bacteria	Viruses	Yeast
Mycoplasma pneumoniae		
Neisseria gonorrhoeae		
Neisseria meningitidis		
Neisseria sicca		
Neisseria subflava		
Proteus vulgaris		
Pseudomonas aeruginosa		
Serratia marcescens		
Staphylococcus aureus		
Staphylococcus aureus (Cowan protein A producing strain)		
Staphylococcus epidermidis		
Streptococcus, Group A		
Streptococcus, Group B		
Streptococcus, Group C		
Streptococcus, Group F		
Streptococcus, Group G		
Streptococcus mutans		
Streptococcus pneumoniae		
Streptococcus salivaris		
Streptococcus sanguis		

Interfering Substances:

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated in the Alere™ Influenza A & B Test at the concentrations listed below and were found not to affect test performance. Whole blood (1%) did not interfere with the interpretation of negative Alere™ Influenza A & B test results, but did interfere with the interpretation of influenza A LOD (or C_{95}) positive samples. Therefore, visibly bloody samples may not be appropriate for use in this test.

Substance	Concentration
3 OTC nasal sprays	10%
3 OTC mouthwashes	10%
3 OTC throat drops	10%
4-acetamidophenol	10 mg/ml
Acetylsalicylic acid	20 mg/ml
Albuterol	20 mg/ml
Chlorpheniramine	5 mg/ml
Dexamethasone	5 mg/ml
Dextromethorphan	10 mg/ml
Diphenhydramine	5 mg/ml
Doxylamine succinate	1 mg/ml
Flunisolide	3 mg/ml
Guaiacol glycerol ether	20 mg/ml
Mucin	1%
Mupirocin	250 µg/ml
Oxymetazoline	10 mg/ml
Phenylephrine	10 mg/ml
Phenylpropanolamine	20 mg/ml
Rebetol® (ribavirin)	500 ng/ml
Relenza® (zanamivir)	20 mg/ml
Rimantadine	500 ng/ml
Tamiflu® (oseltamivir)	100 mg/ml
Tobramycin	40 mg/ml
Triamcinolone	14 mg/ml

Reproducibility Study

A reproducibility study of the Alere™ Influenza A & B Test was conducted by operators from 3 sites using panels of blind-coded randomized 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 99.2% (119/120), 94.2% (113/120) and 9.2% (11/120), respectively. The detection rates for the influenza B moderate positive, low positive, and high negative samples were 99.2% (119/120), 96.7% (116/120) and 7.5% (9/120), respectively. All of the negative samples (118) generated negative test results.

CLIA Waiver Studies:

The accuracy of the Alere™ Influenza A & B Test was evaluated when it was used by operators who had no laboratory experience and who were representative of CLIA waived testing sites (intended users). The study was conducted at nine CLIA waived sites with 27 intended users participating. No training on the use of the test was provided to the operators. The testing of 478 prospectively collected samples, described above in the section titled "Prospective Clinical Study - Alere™ Influenza A & B Test Performance vs. Viral Culture", 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 either influenza A or influenza B. A total of 134 swabs (98 swabs positive for influenza A and 36 swabs positive for influenza B) were tested. The supplemental samples were blind-coded and randomized.

Overall, 612 nasal swab specimens were tested by intended users at CLIA waived sites with the Alere™ Influenza A & B Test, and the results were compared to the results of viral culture, the comparator method for this study. The positive percent agreement (PPA) and the negative percent agreement (NPA) between the Alere™ Influenza A & B test results and the viral culture results, for all specimens combined, are presented in the tables below, including the 95% confidence intervals (95% CI). There were 9 samples that generated invalid results that are not included in the calculations presented below. The percent of invalid results was 1.5% (9/621) with 95% CI: 0.8%, 2.8%.

Positive and Negative Percent Agreements of the Alere™ Influenza A & B Test with Viral Culture for Influenza Type A

Number of samples	PPA	95% CI	NPA	95% CI
612	95.2% (139/146)	90.4%, 97.7%	96.1% (448/466)	94.0%, 97.5%

Positive and Negative Percent Agreements of the Alere™ Influenza A & B Test with Viral Culture for Influenza Type B

Number of samples	PPA	95% CI	NPA	95% CI
612	82.5% (99/120)	74.7%, 88.3%	98.4% (484/492)	96.8%, 99.2%

A study was conducted to evaluate the performance of the Alere™ Influenza A & B Test 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™ Influenza A & B Test at 3 CLIA waived sites (60 tests in total per sample type). Nine untrained users at the CLIA waived sites participated in the study. The panel testing was conducted over a minimum of 3 days at each site, and the testing was integrated into the users' daily work flow. The performance of the Alere™ Influenza A & B Test 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)	93% (56/60)	84%, 97%
Influenza A High Negative (below LOD)	8% (5/60)	4%, 18%
Influenza B Low Positive (at LOD)	97% (58/60)	89%, 99%
Influenza B High Negative (below LOD)	7% (4/59*)	3%, 16%
Negative Sample	0% (0/58*)	0%, 6%

*Three invalid results were generated by the untrained users (1% (3/300) with 95% CI: 0.3%, 2.9%)

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

In support of the CLIA waiver, an additional reactivity study was performed at an independent laboratory to demonstrate reactivity of the Alere™ Influenza A & B Test with a broad range of contemporary influenza A and influenza B viruses. The Alere™ Influenza A & B Test yielded positive results with all 18 influenza A viruses and 7 influenza B viruses included in the test panel at acceptable viral load levels.

References

1. Williams, KM, Jackson MA, Hamilton M. Rapid Diagnostic Testing for URIs in Children: Impact on Physician Decision Making and Cost. *Infect. Med.* 19(3): 109-111, 2002.
2. "Updated Interim Guidance for Laboratory Testing of Persons with Suspected Infection with Avian Influenza A (H5N1) Virus in the United States" CDC Health Alert, June 7, 2006. http://www.phppo.cdc.gov/HAN/ArchiveSys/ViewMsgV.asp?AlertNum=00246
3. Anne Moscona. Neuraminidase Inhibitors for Influenza, 2005. The New England Journal of Medicine, 353 (13):1363-1373.
4. Dowdle, W.R, Kendal, A.P., and Noble, G.R. 1980. Influenza Virus, p 836-844. Manual of Clinical Microbiology, 3rd edition, in Lennette, *et. al* (ed.). American Society for Microbiology, Washington, D.C.

Symbols

Do Not Reuse	*In Vitro* Diagnostic Medical Device	Consult Instructions for Use
Batch Code	Catalog Number	Temperature Limitation
WARNING Causes Serious Eye Irritation	Contains Sufficient for <N> Tests	Prescription Only (Applies to U.S. only)
Manufacturer		

Ordering and Contact Information

Reorder number:

412-000: Alere™ Influenza A & B Test Kit

412-080: Alere™ Influenza A & B Control Swab Kit

Phone: 1-877-441-7440
Fax: 1-877-441-7441

Technical Support

Advice Line

Further information can be obtained from your distributor, or by contacting Alere™ Technical Support at 1-877-866-9340 or ts.scr@alere.com

Contact Information:

Alere Scarborough, Inc.
10 Southgate Road
Scarborough, ME 04074
Internet: www.alere.com

PN: IN412000 Rev. 5 2017/08

Alere™

Influenza A & B Test

For use with nasal swab specimens

For *in vitro* diagnostic use only.

CLIA Complexity: Waived

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.

Intended Use

The Alere™ Influenza A & B Test is an *in vitro* immunochromatographic assay for the qualitative detection of influenza A and B nucleoprotein antigens in nasal swab specimens collected from symptomatic patients. It is intended to aid in the rapid differential diagnosis of influenza A and B viral infections. Negative test results are presumptive and should be confirmed by cell culture or an FDA-cleared influenza A and B molecular assay.

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

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 influenza viruses are typically more prevalent than type B viruses and are associated with most serious influenza epidemics, while type B influenza infections are usually more mild.

Rapid diagnosis of influenza A and B has become more important due to the availability of effective antiviral therapy. Rapid diagnosis of influenza can lead to reduced hospital stays, antimicrobial use and cost of care.[1]

The Alere™ Influenza A & B Test provides a simple, rapid method for the diagnosis of influenza A and B using nasal swab specimens. The easy-to-use format and rapid results allow for its use in "STAT" testing where it can provide information to assist with treatment and patient management decisions.

Principles of the Test

The Alere™ Influenza A & B Test is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect influenza type A and B nucleoprotein antigens in nasal swab specimens. These antibodies and a control protein are immobilized onto a membrane support as three distinct lines and are combined with other reagents/pads to construct a Test Strip.

Nasal swab samples are added to a Coated Reaction Tube to which an extraction reagent has been added. An Alere™ Influenza A & B Test Strip is then placed in the Coated Reaction Tube holding the extracted liquid sample. Test results are interpreted at 10 minutes based on the presence or absence of pink-to-purple colored Sample Lines. The yellow Control Line turns blue in a valid test.

Reagents and Materials

Materials Provided

- **Test Strips:** A membrane support combined with other reagents/pads to construct a Test Strip. A/Texas/1/77 was the master influenza virus strain used to develop the monoclonal influenza A antibodies incorporated into the test device.
- **Coated Reaction Tubes:** Tubes containing a dried visualizing reagent.
- **Reagent 1 (R1) Vials:** Vials containing an extraction reagent.
- **Workstations:** Workstations that hold Coated Reaction Tubes and Test Strips during testing.
- **Influenza A & B Positive Control Swab:** Inactivated influenza A/Texas 1/77 (H3N2) or influenza A/T/W/66 (H9N2) virus and inactivated influenza B/Harbin or influenza B/Hong Kong 5/72 virus dried onto a swab. The influenza viruses are originally grown in embryonic eggs and are inactivated via gamma radiation or beta propiolactone. Viruses are tested for inactivation and non-infectivity by inoculating virus into embryonic eggs or by observing cytopathic effects (CPE) in culture. Viruses are considered inactivated when no viral propagation is seen in eggs or cells.
- **Viral Negative Control Swab:** Inactivated *Streptococcus* Group A dried onto swab. Organism used to inoculate the swab is heat inactivated, and then tested for inactivation and non-infectivity by standard culture. The organisms are determined to be inactivated when no growth is present on the plate.
- **Nasal Swabs:** Sterile swabs for use in the Alere™ Influenza A & B Test.

Materials Required, but Not Provided:

Clock, timer or stopwatch.

Precautions

1. For *in vitro* diagnostic use only.
2. The Test Strip and Coated Reaction Tubes are sealed in a protective pouch. Leave sealed in the foil pouch until just before use. Do not use if pouch is damaged or open.
3. Do not use kit past its expiration date.
4. Do not mix components from different kit lots.
5. The Coated Reaction Tube contains a test reagent in a purple colored coating at the bottom of the tube. If the purple coating is missing, discard all of the contents of the foil pouch and use a new pouch.
6. Handle the Test Strip carefully. Hold it only at the top, which is the end without the arrows.
7. Solutions used to make the control swabs are inactivated using standard methods. However, patient samples, controls, and used test devices should be handled as though they could transmit disease. Observe established precautions against microbial hazards. The use of lab coats, gloves and safety eye glasses is recommended.
8. 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.[2]
9. 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.

Storage and Stability

Store kit at 2-30°C. The Alere™ Influenza A & B Test kit and reagents are stable until the expiration date printed on the outer box.

Quality Control

Internal Procedural Controls:

Every Alere™ Influenza A & B Test Strip has internal (built-in) procedural controls.

A. An untested Test Strip has a yellow line at the "Control" position. If the test flows correctly and the reagents are working properly, this yellow line will always turn blue on the Test Strip.
B. The clearing of background color on the Test Strip is a negative background control. The background color should be light pink to white within 10 minutes. Background color should not interfere with the reading of the test result.

For each sample tested, the user should confirm that the BLUE internal control line is present before reading the result.

External Positive and Negative Controls:

Each Alere™ Influenza A & B Test kit contains external Influenza A & B Positive and Viral Negative Control Swabs. At a minimum, these control swabs should be run:

- once with each new shipment received,
- once with each new kit lot, and
- once by each new untrained operator before he/she tests patient samples.

Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working and that the test is correctly performed.

Other controls may be tested in order to conform with:

- local, state and/or federal regulations;
- accreditting groups, and/or;
- your laboratory's standard Quality Control procedures.

If the kit controls do not perform as expected, do not test patient samples. Contact Alere™ Technical Service during normal business hours at 877-866-9340.

Specimen Collection and Handling

Use freshly collected specimens for best test performance. Inadequate specimen collection or improper sample handling / transport may affect test performance.

The clinical performance of any rapid influenza test that targets the viral nucleoprotein is directly proportional to the titer of the virus and, therefore, the amount of nucleoprotein present in the sample being tested. There are several factors that contribute to the amount of viral nucleoprotein in the sample. One is duration of infection. It has been reported that the titer of influenza virus, and thus nucleoprotein, is higher earlier in the course of infection as compared to later in the course of infection.[3] It naturally follows that rapid tests will have more reliable clinical performance when performed early in the course of the infection.

Nasal Swabs

For optimal performance, use the swabs supplied in the test kit. Alternatively, sterile nylon flocked, foam or rayon nasal swabs can be used to collect samples. Do not use calcium alginate swabs.

To ensure optimal nasal swab specimen collection, insert the nasal swab 2 cm (approximately 3/4") into the nostril that exhibits the most visible drainage or the nostril that is most congested (if drainage is not visible). Leave the swab in place and gently rotate (roll) the swab a few times against the inside nasal wall. Slowly withdraw the swab while continuing with a rotating motion.

Test as soon as possible. The nasal swab may be stored at room temperature (15-30°C) in a clean, dry, tightly sealed plastic tube for up to 8 hours before testing.

Test Procedure for Specimens and for Alere™ Influenza A & B Postive and Viral Negative Control Swabs

For each specimen (or control swab), open a foil pouch just before testing and remove the Test Strip, the Coated Reaction Tube, and the Workstation. Label the Tube with the patient identification. Make sure the purple coating is in the bottom of the Reaction Tube. If the coating is missing, do NOT use the contents of the pouch; use a new pouch. Take the cap off the Reaction Tube, and put the Reaction Tube in the Workstation.

1. Twist off the top of a Reagent 1 (R1) Vial and squeeze **all** of its contents directly into the bottom of the Coated Reaction Tube.

2. Put the sample swab (or Control Swab) to be tested into the Reaction Tube. Mix the swab **in the liquid** for about 10 seconds. Press the swab head against the side of the tube as you mix it. This helps remove the sample from the swab. **Make sure all of the purple coating in the tube is mixed in well**.

3. While squeezing the tube around the swab head, remove the swab from the Reaction Tube. This removes sample from the swab. Discard the swab.

4. Put a Test Strip into the Reaction Tube so the arrows on the Test Strip point down. Start a timer for 10 minutes. Do **not** handle or move the Test Strip until the 10 minutes are up.

5. At 10 minutes, remove the Test Strip from the tube and read the result.

***Note**: When reading test results, tilt the Test Strip to reduce glare on the Test Strip, if necessary. Individuals with color-impaired vision may not be able to adequately interpret test results.*

Result Interpretation

Note: Do not read test results before 10 minutes.

See the actual size picture of a Test Strip on the Alere™ Influenza A & B Test Quick Reference Instructions for the locations of the **Control Line**, the **Flu A Line**, and the **Flu B Line**:

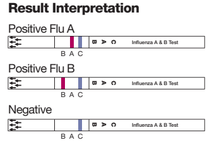
- The Control Line is at the top and is colored **BLUE** in a valid test.
- The Flu A line is just below the Control Line; it is colored pink-to-purple when positive.
- The Flu B line is at the bottom (below the Flu A line); it is colored pink-to-purple when positive.

***Note**: The letters "C", "A", and "B", that are printed on each Test Strip, are intended to remind the user of the order of the Control Line and the Sample Lines in the test result section of the Test Strip.*

After 10 minutes, hold the Test Strip next to the picture on the Quick Reference Instructions with the arrows pointing down. Line up the BLUE Control Line on the picture with the BLUE Control Line on the tested strip. Use the test lines on the picture as a guide to read the result on the Test Strip.

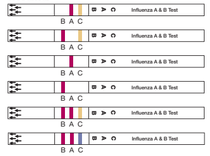
- For a **NEGATIVE SAMPLE**, the yellow Control Line turns BLUE. No other line appears.
- For a **FLU A POSITIVE SAMPLE**, the yellow Control Line turns BLUE AND a second pink-to-purple Sample Line appears in the section of the Test Strip that lines up with the FLU A line on the picture. Any Sample Line, even when very faint, is positive.
- For a **FLU B POSITIVE SAMPLE**, the yellow Control Line turns BLUE AND a second pink-to-purple Sample Line appears in the section of the Test Strip that lines up with the FLU B line on the picture. Any Sample Line, even when very faint, is positive.

Result Interpretation



- A test is **INVALID** if the Control Line remains **YELLOW** or is not present at all, even if a Sample Line is present. Repeat invalid tests with a new sample and new test reagents. Contact Alere™ Technical Service if the problem persists.

Invalid



***Note**: Co-infection with influenza A and B is very rare. A clinical specimen that generates positive results for both influenza A and B on the Alere™ Influenza A & B Test should be considered an invalid result, and another specimen should be collected and tested. If the test result is again positive for both influenza A and B, the specimen should be re-tested by another method prior to reporting the results.*

Reporting of Results

Result	Suggested Report
Positive for Flu A	Positive for Flu A protein antigen. This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
Positive for Flu B	Positive for Flu B protein antigen. This result does not rule out co-infections with other pathogens or identify any specific influenza B virus strain.
Negative	Negative for Flu A and Flu B protein antigens. Infection due to Flu A and Flu B cannot be ruled out. Flu A or Flu B antigen in the sample may be below the detection limit of the test. Alere recommends culture of negative samples or confirmation with an FDA cleared influenza A and B molecular assay.
Invalid	Do not report results. Collect another specimen and repeat the test.

Limitations

A negative test result does not exclude infection with influenza A or B. Therefore, the results obtained with the Alere™ Influenza A & B Test should be used in conjunction with clinical findings to make an accurate diagnosis.

The Alere™ Influenza A & B Test does not differentiate between the specific influenza A and B subtypes or strains. Additional testing is required to differentiate any specific influenza A and B subtypes or strains, in consultation with state or local public health departments.

The Alere™ Influenza A & B Test detects both viable (live) and non-viable influenza A and B. Test performance depends on the amount of virus (antigen) in the specimen and may or may not correlate with cell culture results performed on the same specimen.

Monoclonal antibodies may fail to detect, or detect with less sensitivity, influenza A and B viruses that have undergone minor amino acid changes in the target epitope region.

Performance of the Alere™ Influenza A & B Test has not been established for monitoring antiviral treatment of influenza.

Positive and negative predictive values of *in vitro* diagnostic tests are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low.

The performance of this assay has not been evaluated for use in patients without signs and symptoms of influenza infection.

Use of visibly bloody samples is not recommended with the Alere™ Influenza A & B Test.

Individuals who have received nasally administered influenza A vaccine may test positive in commercially available influenza rapid diagnostic tests for up to three (3) days after vaccination.

Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, *in vitro* diagnostic tests for influenza may have lower detectability in adults than in children.

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 by Alere in the U.S. during the 2008-2009 respiratory season, the average prevalence of influenza A (as determined by viral culture) was 10%. The average prevalence of influenza B was 18%.

Performance Characteristics

Clinical Study:

Prospective Clinical Study - Alere™ Influenza A & B Test Performance vs. Viral Culture

The prospective clinical study was conducted at seven geographically diverse sites during the 2008-2009 respiratory season. The sites were located in the northeast, midwest, southeast, and southwest regions of the U.S.

A total of 478 prospective nasal swab specimens, collected from patients of all ages presenting with influenza-like symptoms, were evaluated in the Alere™ Influenza A & B Test and compared to viral culture. Forty-four percent (44%) of the population tested was < 5 years of age, 31% was 5 - < 18 years of age, and 25% was ≥ 18 years. A/H3 and A/H1 were the predominant influenza A subtypes observed during the times the specimens were collected.

Alere™ Influenza A & B Test performance versus viral culture, for all patients combined and stratified by age group, is detailed below, including the 95% confidence intervals (95% CI). Differences in performance are expected when this test is used on specimens from adults versus from children, but specific differences are not known.

The Alere™ Influenza A & B Test 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.

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

	Influenza Type A		
	Culture +	**Culture -**	
Alere +	45	18	63
Alere -	3	412	415
	48	430	478

Sensitivity: 93.8% (45/48) (95% CI: 83.2%, 97.9%)
Specificity: 95.8% (412/430) (95% CI: 93.5%, 97.3%)

	Influenza Type B		
	Culture +	**Culture -**	
Alere +	65	8	73
Alere -	19*	386	405
	84	394	478

Sensitivity: 77.4% (65/84) (95% CI: 67.4%, 85.0%)
Specificity: 98.0% (386/394) (95% CI: 96.1%, 99.0%)

* The nineteen samples that tested positive on culture for influenza B, but were negative on the Alere™ Influenza A & B Test, were also tested on an investigational RT-PCR assay. Ten (10) of these samples were negative for influenza B by PCR.

The rate of invalid results was 1.9% (9/487) with 95% CI: 1.0%, 3.5%.

Alere™ Influenza A & B Test Performance vs. Culture
(By Age Group)

< 5 Years of Age (n = 209)		
Influenza Type	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Type A	100% (23/23) (86%, 100%)	97% (180/186) (93%, 99%)
Type B	71% (24/34) (54%, 83%)	97% (170/175) (93%, 99%)

5 - < 18 Years of Age (n = 146)		
Influenza Type	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Type A	95% (18/19) (75%, 99%)	94% (119/127) (88%, 97%)
Type B	81% (35/43) (67%, 90%)	98% (101/103) (93%, 99%)

≥ 18 Years of Age (n = 123)		
Influenza Type	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Type A	67% (4/6) (30%, 90%)	97% (113/117) (92%, 99%)
Type B	86% (6/7) (49%, 97%)	99% (115/116) (95%, 100%)

Alere™ Influenza A & B Test Sensitivity vs. Culture
(By Time Between Onset of Symptoms and Sample Collection)

Influenza Type	< 2 Days Between Onset & Sample Collection	2 - 4 Days Between Onset & Sample Collection	> 4 Days Between Onset & Sample Collection
Type A	96% (25/26) (95% CI: 81%, 99%)	95% (18/19) (95% CI: 75%, 99%)	67% (2/3) (95% CI: 21%, 94%)
Type B	78% (21/27) (95% CI: 59%, 89%)	78% (36/46) (95% CI: 64%, 88%)	73% (8/11) (95% CI: 43%, 90%)

***Note**: The patients' symptom information was self reported.*

Analytical Studies

Analytical Sensitivity:

The Alere™ Influenza A & B Test limit of detection (LOD or C_{95}), defined as the concentration of influenza virus that produces positive Alere™ Influenza A & B test results approximately 95% of the time, was identified by evaluating different concentrations of 2 subtypes of live influenza A and 2 strains of live influenza B in the Alere™ Influenza A & B Test. Multiple operators tested each concentration of the four influenza strains multiple times. 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
Influenza A/HongKong/8/68	2.37×10^4	64/66	97%
Influenza A/PuertoRico/8/34	3.16×10^5	37/42	88%
Influenza B/ Malaysia/2506/2004	3.00×10^6	19/20	95%
Influenza B/Lee/40	4.20×10^5	19/20	95%

Analytical Reactivity

The influenza A and B strains listed tested positive in the Alere™ Influenza A & B Test at the concentrations specified. Although the specific influenza strains causing infection in humans can vary year to year, all contain the conserved nucleoproteins targeted by the Alere™ Influenza A & B Test.[4] Performance characteristics of the Alere™ Influenza A & B Test 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.

Annual analytical reactivity testing data can be found at:
www.alere.com/en/home/products-services/infectious/influenza.html