Influenza Strain	ATCC #	Concentration
Flu A/Mal/302/54 (H1N1)	VR-98	10^2-10^6 $CEID_{50}$/ml
Flu A/Port Chalmers/1/73 (H3N2)	VR-810	10^2-10^6 $CEID_{50}$/ml
Flu A/Hong Kong/156/97 (H5N1)	–	1.3 x 10^2 $TCID_{50}$/ml
Flu A/Vietnam/1194/04 (H5N1)	–	1.0 x 10^4 $TCID_{50}$/ml
Flu A/California/04/2009 (H1N1) swl (swine lineage)	–	5.63 x 10^4 $TCID_{50}$/ml
Flu A/Auckland/1/2009 A (H1N1) swl	–	1.0 x 10^5 $TCID_{50}$/ml
Flu A/Auckland/3/2009 A (H1N1) swl	–	1.0 x 10^5 $TCID_{50}$/ml
Flu A/Chicken/NY/117228-7/01 (H5N2)	–	1.0 x 10^4 EID_{50}/ml
Flu A/Turkey/VA/SEP-66/02 (H7N2)	–	1.0 x 10^5 EID_{50}/ml
Flu B/Lee/40	VR-101	10^2-10^6 $CEID_{50}$/ml
Flu B/Brigit	VR-786	10^2-10^6 $CEID_{50}$/ml
Flu B/Russia/69	VR-790	10^2-10^6 $CEID_{50}$/ml
Flu B/Hong Kong/5/72	VR-791	10^2-10^6 $CEID_{50}$/ml
Flu B/R75	VR-789	10^2-10^6 $CEID_{50}$/ml

Although this test has been shown to detect the Flu A/California/04/2009 (H1N1) virus cultured from a positive human specimen, the performance characteristics of this card with human specimens infected with the 2009 H1N1 influenza virus have not been established. The Alere BinaxNOW® Influenza A & B Card can distinguish between influenza A and B viruses, but it does not differentiate seasonal influenza A virus from the novel influenza A (i.e. 2009 H1N1) and its ability to detect human infection with the 2009 H1N1 influenza virus in clinical specimens is unknown.

Analytical Specificity (Cross-Reactivity):

To determine the analytical specificity of the Alere BinaxNOW® Influenza A & B Card, 36 commensal and pathogenic microorganisms (27 bacteria, 8 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 10^4 to 10^8 $TCID_{50}$/ml (viruses), 10^7 to 10^8 organisms/ml (bacteria) and 10^6 organisms/ml (yeast).

Bacteria	Viruses	Yeast
Acinetobacter	Adenovirus	*Candida albicans*
Bordetella pertussis	Coronavirus	
Enterococcus faecalis	Coxsackie B4	
Escherichia coli	Cytomegalovirus (CMV)	
Gardnerella vaginalis	Parainfluenza 1	
Haemophilus influenzae	Parainfluenza 2	
Klebsiella pneumoniae	Parainfluenza 3	
Lactobacillus casei	Respiratory Syncytial Virus (RSV)	
Legionella pneumophila		
Listeria monocytogenes		
Moraxella catarrhalis		
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 mutans		
Streptococcus pneumoniae		

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 BinaxNOW® Influenza A&B Card at the concentrations listed and were found not to affect test performance. Whole blood (1%) did not interfere with the interpretation of negative Alere BinaxNOW® Influenza A & B Card results, but did interfere with the interpretation of Flu A LOD positive samples. Therefore, visibly bloody samples may not be appropriate for use in this test.

Substance	Concentration	Substance	Concentration
1 OTC mouthwash	20%	Diphenhydramine	5 mg/ml
3 OTC nasal sprays	15%	Guaiacol glycerol ether	20 mg/ml
3 OTC throat drops	15%	Oxymetazoline	0.05%
2 OTC throat sprays	20%	Phenylephrine	50 mg/ml
4-acetamidophenol	10 mg/ml	Rebetol®	500 ng/ml
Acetylsalicylic acid	15 mg/ml	Relenza®	20 mg/ml
Albuterol	20 mg/ml	Rimantadine	500 ng/ml
Chlorpheniramine	5 mg/ml	Synagis®	0.1 mg/ml
Dextromethorphan	10 mg/ml	Tamiflu®	50 mg/ml

Transport Media:

The following transport media were tested in the Alere BinaxNOW® Influenza A & B Card as negative samples (no virus present) and after inoculation with the LOD levels of Influenza A & B. Media did not impact Alere BinaxNOW® Influenza A & B Card performance, with the media alone testing negative in the Alere BinaxNOW® Influenza A & B Card and media inoculated with LOD Influenza A & B testing positive on the appropriate test line in Alere BinaxNOW® Influenza A & B Card.

Amies Media	Brain Heart Infusion Broth
Dulbecco Medium	Hank's Balanced Salt Solution
M4 Media	M4-RT Media
M5 Media	M6 Media
Phosphate Buffer Solution	Saline
Stuart's Media	Tryptose Phosphate Broth
UTM-RT Media	Veal Infusion Broth

It has been determined that Sucrose-Phosphate Buffer may not be suitable for use with this test.

Reproducibility Study:

A blind study of the Alere BinaxNOW® Influenza A & B Card was conducted at 3 separate sites using panels of blind coded specimens containing negative, low positive, and moderate positive samples. Participants tested each sample multiple times on 3 different days.

There was 97% (242/250) agreement with expected test results, with no significant differences within run (replicates tested by one operator), between run (3 different days), between sites (3 sites), or between operators (6 operators).

Consumer Precision Study:

Swab Specimen

The Alere BinaxNOW® Influenza A & B Card was evaluated by twelve (12) adults with no professional laboratory experience (intended user) at three (3) intended use sites. Each operator at each site tested thirty-five (35) samples from a randomly coded panel of true positive, true negative, limit of detection (LOD), and below LOD for both influenza A and influenza B. In order to demonstrate equivalent performance among intended users and trained laboratorians, twenty-four (24) trained laboratorians, ran blind coded panels of Flu A and Flu B samples containing the same true negative, true positive, below LOD, and LOD samples described above.

As indicated by the overlapping 95% confidence intervals in the tables below, no significant differences were observed between the intended user and the expected results established by the trained laboratorians. These results demonstrate that users with no formal laboratory training can read the package insert and perform the test with the same precision as trained laboratorians.

Influenza A and Influenza B Sample Testing
– Intended Users vs. Trained Laboratorians - Overall Results

	Participant Type	Negative-% Negative (95% CI)	Below LOD % Detection (95% CI)	LOD % Detection (95% CI)	True Positive % Detection (95% CI)	% Invalid Tests
Flu A Samples	Intended User	97% (57/59) (88-99)	79% (46/58) (67-88)	95% (59/62) (87-98)	100% (60/60) (94-100)	1.6% (4/243)
	Trained Laboratorian	99% (179/180) (97-100)	84% (102/121) (77-90)	99% (179/180) (97-100)	100% (120/120) (97-100)	0% (0/601)
Flu B Samples	Intended User		72% (43/60) (59-81)	97% (59/61) (89-99)	98% (58/59) (97-100)	.6% (0/601)
	Trained Laboratorian		78% (93/120) (69-84)	99% (179/180) (97-100)	100% (120/120) (97-100)	0% (0/420)

Influenza A Sample Testing by Site - Intended Users (IU) and Trained Laboratorians (TL)

	Site #	Negative-% Negative (95% CI)	Below LOD % Detection (95% CI)	LOD % Detection (95% CI)	True Positive % Detection (95% CI)	% Invalid Tests
Intended User (IU) Results	1 IU	89% (17/19) (68-97)	63% (12/19) (41-84)	100% (20/20) (84-100)	100% (20/20) (84-100)	2.5% (2/80)
	2 IU	100% (20/20) (84-100)	79% (15/19) (56-91)	90% (19/21) (71-97)	100% (20/20) (84-100)	2.4% (2/82)
	3 IU	100% (20/20) (84-100)	95% (19/20) (76-99)	95% (20/21) (77-99)	100% (20/20) (84-100)	0% (0/81)
Trained Laboratorian (TL) Results	1 TL	100% (25/25) (87-100)	64% (16/25) (44-80)	100% (25/25) (87-100)	100% (25/25) (87-100)	0% (0/100)
	2 TL	100% (15/15) (79-100)	87% (13/15) (62-96)	100% (15/15) (79-100)	100% (15/15) (79-100)	0% (0/60)
	3 TL	100% (20/20) (84-100)	75% (15/20) (53-89)	100% (20/20) (84-100)	100% (20/20) (84-100)	0% (0/80)
	4 TL	99% (119/120) (95-100)	95% (58/61) (87-98)	99% (119/120) (95-100)	100% (60/60) (94-100)	0% (0/361)

Influenza B Sample Testing by Site - Intended Users (IU) and Trained Laboratorians (TL)

	Site #	Below LOD % Detection (95% CI)	LOD % Detection (95% CI)	True Positive % Detection (95% CI)	% Invalid Tests
Intended User (IU) Results	1 IU	70% (14/20) (48-85)	95% (19/20) (76-99)	100% (19/19) (83-100)	1.7% (1/60)
	2 IU	65% (13/20) (43-82)	95% (20/21) (77-99)	100% (20/20) (84-100)	0% (0/61)
	3 IU	80% (16/20) (58-92)	100% (20/20) (84-100)	95% (19/20) (76-99)	0% (0/60)
Trained Laboratorian (TL) Results	1 TL	60% (15/25) (41-77)	100% (25/25) (87-100)	100% (25/25) (87-100)	0% (0/75)
	2 TL	73% (11/15) (48-89)	100% (15/15) (79-100)	100% (15/15) (79-100)	0% (0/45)
	3 TL	65% (13/20) (43-82)	100% (20/20) (84-100)	100% (20/20) (84-100)	0% (0/60)
	4 TL	90% (54/60) (80-95)	99% (119/120) (95-100)	100% (60/60) (94-100)	0% (0/240)

Liquid Specimen

According to the 1995 CLIA Rule, Alere conducted their Consumer Precision testing of the Alere BinaxNOW® Influenza A & B Card with a total of 120 lay users at 6 sites. Participants tested proficiency panels consisting of high negative, assay cutoff, and limit of detection (LOD) samples for both influenza A and influenza B, as well as true negative samples.

Expected results for each sample type were generated by trained laboratorians. Six percent (6%) of the total tests run by the lay users and 0.4% of the tests run by the trained laboratorians resulted in invalid tests. The tables below detail the number of invalid tests generated by each site.

As indicated by the overlapping 95% confidence intervals in the tables below, no significant differences were observed between the lay users and the expected results established by the trained laboratorians. These results demonstrate that users with no formal laboratory training can read the package insert and perform the Alere BinaxNOW® Influenza A & B Card with a relatively high level of precision.

Influenza A and Influenza B Sample Testing
– Lay Users vs. Trained Laboratorians - Overall Results.

	Participant Type	Negative-% Negative (95% CI)	High Negative*- % Detection (95% CI)	Assay Cutoff*- % Detection (95% CI)	LOD-% Detection	% Invalid Tests
Flu A Samples	Lay User	96% (54/56) (88-99)	32% (18/57) (21-44)	78% (46/59) (66-87)	95% (57/60) (86-98)	3.3% (8/240)
	Trained Laboratorian	100% (8/8) (66-100)	22% (8/36) (12-38)	64% (23/36) (47-77)	94% (34/36) (82-98)	0.9% (1/117)
Flu B Samples	Lay User	96% (53/55) (88-99)	4% (2/52) (1-13)	27% (15/56) (17-40)	82% (45/55) (70-90)	9.2% (22/240)
	Trained Laboratorian	100% (9/9) (69-100)	11% (4/36) (4-25)	49% (17/35) (33-64)	92% (33/36) (78-97)	0.0% (0/116)

* These levels are below the detection limit of the test.

Influenza A Sample Testing by Site - Lay Users (LU) and Trained Laboratorians (TL)

	Site #	Negative-% Negative (95% CI)	High Negative*- % Detection (95% CI)	Assay Cutoff*- % Detection (95% CI)	LOD-% Detection	% Invalid Tests
Lay User (LU) Results	2 LU	100% (18/18) (82-100)	33% (6/18) (16-57)	70% (14/20) (48-85)	90% (18/20) (70-97)	5.0% (4/80)
	4 LU	90% (18/20) (70-97)	45% (9/20) (26-66)	85% (17/20) (64-94)	100% (20/20) (84-100)	0% (0/80)
	6 LU	100% (18/18) (82-100)	16% (3/19) (6-38)	79% (15/19) (56-91)	95% (19/20) (76-99)	5.0% (4/80)
Trained Laboratorian (TL) Results	1 TL	100% (3/3) (40-99)	0% (0/12) (0-25)	25% (3/12) (9-54)	100% (12/12) (75-100)	0% (0/39)
	2 TL	100% (2/2) (29-99)	33% (4/12) (14-61)	92% (11/12) (64-98)	83% (10/12) (54-95)	3% (1/39)
	3 TL	100% (3/3) (40-99)	33% (4/12) (14-61)	75% (9/12) (46-90)	100% (12/12) (75-100)	0% (0/39)

Influenza B Sample Testing by Site - Lay Users (LU) and Trained Laboratorians (TL)

	Site #	Negative-% Negative (95% CI)	High Negative*- % Detection (95% CI)	Assay Cutoff*- % Detection (95% CI)	LOD-% Detection	% Invalid Tests
Lay User (LU) Results	1 LU	100% (20/20) (84-100)	5% (1/19) (1-25)	25% (5/20) (11-47)	85% (17/20) (64-94)	1% (1/80)
	3 LU	100% (19/19) (83-100)	0% (0/18) (0-18)	25% (5/20) (11-47)	79% (15/19) (56-91)	5% (4/80)
	5 LU	87% (14/16) (63-96)	7% (1/15) (2-30)	31% (5/16) (14-56)	81% (13/16) (57-93)	21% (17/80)
Trained Laboratorian (TL) Results	1 TL	100% (3/3) (40-99)	0% (0/12) (0-25)	9% (1/11) (21-38)	75% (9/12) (46-91)	0% (0/38)
	2 TL	100% (3/3) (40-99)	33% (4/12) (14-61)	67% (8/12) (39-86)	100% (12/12) (75-100)	0% (0/39)
	3 TL	100% (3/3) (40-99)	0% (0/12) (0-25)	67% (8/12) (39-86)	100% (12/12) (75-100)	0% (0/39)

REFERENCES

1. Williams, KM, Jackson MA, Hamilton M. (2002) Rapid Diagnostic Testing for URIs in Children: Impact on Physician Decision Making and Cost. *Infect. Med.* 19(3): 109-111.
2. 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.
3. "Key Facts about Avian Influenza (Bird Flu) and Avian Influenza A (H5N1) Virus" CDC Publication, May 24, 2005. http://www.cdc.gov/flu/avian/gen-info/facts.htm
4. "Avian Influenza Infection in Humans" CDC Publication, May 24, 2005. http://www.cdc.gov/flu/avian/gen-info/avian-flu-humans.htm
5. "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

ORDERING and CONTACT INFORMATION

US: 1 877 441 7440

OUS: +1 321 441 7200

Reorder numbers:

#416-022: Alere BinaxNOW® Influenza A&B Card (22 Tests)

#416-110: Alere BinaxNOW® Influenza A&B Card (10 Tests)

#400-065: Alere BinaxNOW® Nasopharyngeal Swab Accessory Pack

#416-080: Alere BinaxNOW® Influenza A&B Control Swab Kit

Technical Support

Further information can be obtained from your distributor or by contacting Technical Support.

1 877 866 9340 TS.SCR@alere.com

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Alere™

BinaxNOW®
Influenza A & B Card
Product Instructions

CLIA Waived

COMPLEXITY: WAIVED

Any modifications by the laboratory to the test system or FDA cleared test system instructions will result in the test no longer meeting the requirements for waived categorization.

INTENDED USE

The Alere BinaxNOW® Influenza A & B Card is an *in vitro* immunochromatographic assay for the qualitative detection of influenza A and B nucleoprotein antigens in nasopharyngeal (NP) swab, nasal swab, and nasal wash/aspirate specimens. It is intended to aid in the rapid differential diagnosis of influenza A and B viral infections. Negative test results should be confirmed by cell culture.

Caution: Assay sensitivity for nasal wash/aspirate samples was determined primarily using archived specimens. Users may want to establish the sensitivity of these specimens on fresh samples.

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 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 hospital care.[1]

The Alere BinaxNOW® Influenza A&B Card provides a simple, rapid method for the diagnosis of influenza A and B using NP swab, nasal swab and nasal wash/aspirate 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 hospitalization decisions.

There are many different subtypes of type A influenza viruses, some of which can be found in birds.[3] Direct human infection by Avian influenza A (H5N1), an influenza virus subtype that occurs mainly in birds, was first reported in 1997. Since then there have been other cases of H5N1 infections among humans leading to a concern that H5N1 could mutate enabling it to spread more easily from one person to another.[4] Due to the small percentage of documented cases of patients infected with avian influenza, the utility of rapid tests in managing those patients is currently unknown.

PRINCIPLES of the PROCEDURE

The Alere BinaxNOW® Influenza A & B Card is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect influenza type A and B nucleoprotein antigens in respiratory specimens. These antibodies and a control antibody are immobilized onto a membrane support as three distinct lines and combined with other reagents/pads to construct a test strip. This test strip is mounted inside a cardboard, book-shaped, hinged, test card.

Swab specimens require a sample preparation step, in which the sample is eluted or washed off the swab into an elution solution, saline or transport media. Nasal wash/aspirate samples require no preparation. Sample is added to the top of the test strip and the test card is closed. Test results are interpreted at 15 minutes based on the presence or absence of pink-to-purple colored Sample Lines. The blue Control Line turns pink in a valid assay.

REAGENTS and MATERIALS

Materials Provided

Alere BinaxNOW® Influenza A & B Card

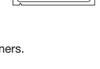
Test Cards: A cardboard, book-shaped, hinged, test card containing the test strip. A/Texas/1/77 was the master influenza virus strain used to develop the monoclonal antibodies incorporated into the test card to detect the influenza A virus.

Transfer Pipettes: Fixed volume (100 µl), transfer pipettes used to transfer sample to the test card. Use only pipettes provided by Alere.

Positive Control Swab: Inactivated influenza A/Beijing, 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 swab. The influenza viruses are originally grown in embryonic eggs and are Formalin or gamma radiation or Beta propiolactone inactivated. Viruses are tested for inactivation and non-infectiousness by re-growing virus in embryonic eggs or by cytopathic effect (CPE) in culture. Viruses are considered inactivated when no viral propagation is seen in eggs or cells.

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-infectiousness by standard culture. The organisms are determined to be inactivated when no growth is present on the plate.

Elution Solution Vials for Control Swabs/Swab Specimens: Vials containing elution solution used to prepare the Control Swabs/Swab Specimens for testing.



NP Swabs: Sterile swabs for use in the Alere BinaxNOW® Influenza A & B Card.

Materials Recommended But Not Provided

Clock, timer or stopwatch; nasal wash/aspirate collection containers.

PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Leave test sealed in its foil pouch until just before use.
3. Do not use kit past its expiration date.
4. Do not mix components from different kit lots.
5. The **WHITE** sample pad at the top of the test strip contains reagents that extract the target antigen from the virus. To ensure best performance, add the sample **SLOWLY** (drop-by-drop) to the **MIDDLE** of this pad, without touching with the pipette, such that all of the sample absorbs into the pad. **DO NOT** add sample to the pink/purple colored pad.
6. Solutions used to make the control swabs are inactivated using standard methods. However, patient samples, controls, and tests 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.
7. All transfer pipettes and test vials are single use items – do not use with more than one specimen.
8. The ability of this test to detect avian influenza was determined using cultured avian influenza viruses, the performance characteristics of this test with specimens collected from humans infected with H5N1 or other avian influenzas is unknown.
9. 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 culture specimens.
10. 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.

11. The elution solution packaged in this kit contains saline, detergents and preservatives that will inactivate cells and virus particles. Samples eluted in this solution are not suitable for culture.

STORAGE and STABILITY

Store kit at 2-30°C. The Alere BinaxNOW® Influenza A&B Card and reagents are stable until the expiration dates marked on their outer packaging and containers.

QUALITY CONTROL (QC)

Daily Quality Control:

The Alere BinaxNOW® Influenza A&B Card has built-in (internal) procedural controls. For daily quality control, Alere suggests that you record results of these controls for each test run.

Procedural Controls:

A. An untested strip has a blue line at the "Control" position. If the test flows correctly and the reagents work, this blue line will always turn pink on the strip.

B. The clearing of background color from the result window is a negative background control. The background color in the window should change from light pink to white within 15 minutes. Background color should not interfere with the reading of the test.

External Positive and Negative Controls:

Good laboratory practice suggests the use of positive and negative controls to ensure that:

- test reagents are working, and
- the test is correctly performed.

Alere BinaxNOW® Influenza A&B Card kits contain Positive and Negative Control Swabs. These swabs will verify the entire assay. Test these swabs once with each new shipment received. Other controls may be tested in order to conform with:

- local, state and/or federal regulations,
- accrediting groups, and/or,
- your lab's standard QC procedures.

Refer to 42 CFR 493.1256 for help on proper QC techniques (U.S. customers only).

If the correct control results are not obtained, do not report patient results. Contact Technical Service during normal business hours.

SPECIMEN COLLECTION and HANDLING

Use freshly collected specimens for best test performance. Inadequate specimen collection or improper sample handling/transport may yield a false-negative result.

Nasal Wash/Aspirates:

Collect nasal washes in standard containers. Test as soon as possible. Washes can be held at 36-46°F (2-8°C) for up to 24 hours prior to testing in the Alere BinaxNOW® Influenza A&B Card.

Nasopharyngeal and Nasal Swabs:

Use sterile cotton, rayon, foam, polyester or flocked swabs to collect NP sample. Use cotton, rayon, foam, polyester or flocked solid shaft swabs to collect nasal swab samples. Do not use calcium alginate swabs.

Elute swab samples within one hour of collection. Test as soon as possible. Eluted swab samples can be held at 36-46°F (2-8°C) for up to 24 hours before testing in the Alere BinaxNOW® Influenza A&B Card. If needed, transport sample at 36-46°F (2-8°C) in a leak-proof container.

Allow samples to warm to room temperature before testing in the Alere BinaxNOW® Influenza A &B Card. Swirl gently to mix (without creating bubbles) before testing.

Transport Media:

Transport media can be used in place of Alere BinaxNOW® Elution Solution to prepare patient swab samples for testing. The following transport media were tested and are acceptable for use in the Alere BinaxNOW® Influenza A&B Card.

Amies Media	Brain Heart Infusion Broth
Dulbecco Medium	Hank's Balanced Salt Solution
M4 Media	M4-RT Media
M5 Media	M6 Media
Phosphate Buffer Solution	Saline
Stuart's Media	Tryptose Phosphate Broth
UTM-RT Media	Veal Infusion Broth

It has been determined that Sucrose-Phosphate Buffer may not be suitable for use with this test.

SAMPLE PREPARATION PROCEDURE

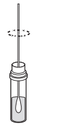
Nasal Wash/Aspirate:

Nasal wash/aspirates do not need preparation. Go to Test Procedure.

Precaution: When testing nasal wash/aspirate samples, avoid thick areas of the sample when drawing it into the transfer pipette. If the pipette becomes clogged, and the lower part of the pipette is not full, put the sample back into container by squeezing the top bulb. Redraw the sample into the pipette. Use a new pipette if needed.

Swab (Control & Patient) Elution using Alere BinaxNOW® Elution Solution:

1. Twist off the test vial cap.
2. Put the swab to be tested into test vial. Rotate the swab vigorously (without making a lot of bubbles) three (3) times **in the liquid**.
3. Press the swab against the side of the vial and turn as you remove it from the vial. This removes sample from the swab.
4. Discard the swab into a container intended for contagious material.
5. Test the liquid sample (from the test vial) in the Alere BinaxNOW® Influenza A&B Card as soon as possible. Go to Test Procedure.



Swab Elution Using Transport Media:

Remove sample from swab in 0.5 to 3.0 ml of saline or transport media/fluid by vigorously rotating the swab in the liquid. Refer to Specimen Collection and Handling section for acceptable transport media. Go to Test Procedure.

TEST PROCEDURE

WARNING: INVALID RESULTS can occur when too little sample is added to the test. Be sure that the lower part of the transfer pipette is full and does not have any air spaces before you add the sample to the Sample Pad. If there are air spaces, put the sample back into the container by squeezing the top bulb. Redraw the sample from the bottom of the container into the pipette. Use a new pipette if needed.

1. Remove card from the pouch just prior to testing and lay flat on work bench.
2. Fill pipette by firmly squeezing the top bulb and **then** placing pipette tip into sample. Slowly release bulb while tip is still in sample. This will pull liquid into the pipette. Make sure there are no air spaces in the lower part of the pipette.
3. See arrow on test card to find the **WHITE** sample pad at the top of the test strip. **SLOWLY** (drop by drop) add entire contents of the pipette (100 µl) to the **MIDDLE** of this pad by squeezing the top bulb such that all of the sample volume absorbs into this pad. **DO NOT** add sample to the pink/purple colored pad.
4. Immediately peel off adhesive liner from the test card. Close and securely seal the card. Read result in window 15 minutes after closing the card.

Note: *When reading test results, tilt the card to reduce glare on the result window 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 or after 15 minutes as they may not be correct.*

For a **NEGATIVE SAMPLE**, the BLUE Control Line in the **BOTTOM THIRD** of the window turns a pink to purple color. No other line appears.

For a **FLU A POSITIVE SAMPLE**, the BLUE Control Line turns a pink to purple color AND a SECOND pink to purple Sample Line appears above it in the **MIDDLE THIRD** of the window. Any shade of a pink to purple Sample Line, even when very faint, indicates a positive result.

For a **FLU B POSITIVE SAMPLE**, the BLUE Control Line turns a pink to purple color AND a SECOND pink to purple Sample Line appears above it in the **TOP THIRD** of the window. Any shade of a pink to purple Sample Line, even when very faint, indicates a positive result.

For a **FLU A and FLU B POSITIVE SAMPLE**, the BLUE Control Line turns a pink to purple color, AND two pink to purple Sample Lines appear above it in the **MIDDLE and TOP** thirds of the window. Any shade of pink to purple Sample Lines indicates positive results.

A test is **INVALID** if the Control Line remains BLUE or is not present at all, whether a Sample Line(s) is present or not. Repeat invalid tests. Contact Alere™ Technical Support if the problem continues.

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-infection with other pathogens or identify any specific influenza B virus subtype.
Positive for Flu A & B	Positive for both Flu A and B protein antigens. This result does not rule out co-infection with other pathogens or identify any specific influenza A or B virus subtype.
Negative	Negative for Flu A and Flu B protein antigens. Infection due to Flu A and Flu B cannot be ruled out. Flu A and/or Flu B antigen in the sample may be below the detection limit of the test. Alere suggests culture of negative samples.

Notify Alere of any performance (perceived or validated) that does not meet test specifications described in this insert.

LIMITATIONS

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

Inadequate specimen collection or improper sample handling/transport may yield a false-negative result.

Performance of the Alere BinaxNOW® Influenza A & B Card 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 results are more likely during periods of low influenza activity when prevalence is moderate to low.

Individuals who have received nasally administered influenza A vaccine may test positive in commercially available influenza rapid diagnostic tests for up to three 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 sensitivity in adults than in children.

Use of visibly bloody samples is not recommended with the Alere BinaxNOW® Influenza A&B Card.

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 outside the US during the 2004 respiratory season and in the U.S. during the 2004-2005 respiratory season, the average prevalence of influenza A (as determined by viral cell culture) was 18%. The average prevalence of influenza B was 3%.

PERFORMANCE CHARACTERISTICS

The clinical performance of the Alere BinaxNOW® Influenza A & B Card was established in multi-center, prospective, clinical studies conducted at a central testing laboratory outside the US during the 2004 respiratory season and at three US trial sites during the 2005-2006 respiratory season. Additional performance testing was conducted on retrospective frozen clinical samples collected from symptomatic patients at multiple physician offices, clinics and hospitals located in the Southern, Northeastern and Midwestern regions of the United States and from one hospital in Sweden.

Clinical Studies:

Alere BinaxNOW® Influenza A & B Card Performance vs. Cell Culture / DFA – Prospective Study

A total of 846 prospective specimens collected from children (less than 18 years of age) and adults (18 years or older) were evaluated in the Alere BinaxNOW® Influenza A & B Card and compared to culture/DFA. Evaluated specimens include nasopharyngeal, and nasal swabs collected from patients presenting with influenza-like symptoms. Forty-four percent (44%) of the population tested was male, 56% female, 54% pediatric (< 18 years), and 46% adult (≥ 18 years). No differences in test performance were observed based on patient age or gender. A/H3 and A/H1 were the predominant influenza subtypes observed during this time.

Alere BinaxNOW® Influenza A&B Card performance by sample type versus cell culture / DFA, including 95% confidence intervals, is listed below.

Alere BinaxNOW® Influenza A & B Card Performance vs. Cell Culture/DFA for Detection of Flu A

	Test Sensitivity				Test Specificity			
Sample	**+/+**	**-/+**	**% Sens**	**95% CI**	**-/-**	**+/-**	**% Spec**	**95% CI**
NP Swab	53	16	77%	65-86%	278	3	99%	97-100%
Nasal Swab	85	17	83%	74-90%	378	16	96%	93-98%
Overall	138	33	81%	74-86%	656	19	97%	96-98%

Alere BinaxNOW® Influenza A&B Card Performance vs. Cell Culture/DFA for Detection of Flu B

	Test Sensitivity				Test Specificity			
Sample	**+/+**	**-/+**	**% Sens**	**95% CI**	**-/-**	**+/-**	**% Spec**	**95% CI**
NP Swab	2	2	50%	9-91%	346	0	100%	99-100%
Nasal Swab	9	4	69%	39-90%	481	2	100%	98-100%
Overall	11	6	65%	39-85%	827	2	100%	99-100%

Alere BinaxNOW® Influenza A & B Card Performance vs. Cell Culture / DFA – Retrospective Study

A total of 293 retrospective frozen clinical samples were evaluated in the Alere BinaxNOW® Influenza A&B Card and compared to culture/DFA. All clinical samples were collected from symptomatic patients at multiple physician offices, clinics and hospitals located in the Southern, Northeastern and Midwestern regions of the United States and from one hospital in Sweden. Fifty-three percent (53%) of the population tested was male, 47% female, 62% pediatric (<18 years) and 38% adult (≥ 18 years). Nasal wash/aspirate specimens comprised approximately 61% of the samples tested, while NP swabs represented 39%. No differences in test performance were observed based on patient age and gender or based on sample type tested.

Alere BinaxNOW® Influenza A & B Card performance by sample type versus cell culture / DFA, including 95% confidence intervals, is listed below.

Alere BinaxNOW® Influenza A&B Card Performance vs. Cell Culture/DFA for Detection of Flu A.

	Test Sensitivity				Test Specificity			
Sample	**+/+**	**-/+**	**% Sens**	**95% CI**	**-/-**	**+/-**	**% Spec**	**95% CI**
NP Swab	19	8	70%	50-86%	77	9	90%	81-95%
Wash/ Aspirate	51	6	89%	78-96%	117	6	95%	89-98%
Overall	70	14	83%	73-90%	194	15	93%	88-96%

Alere BinaxNOW® Influenza A&B Card Performance vs. Cell Culture/DFA for Detection of Flu B.

	Test Sensitivity				Test Specificity			
Sample	**+/+**	**-/+**	**% Sens**	**95% CI**	**-/-**	**+/-**	**% Spec**	**95% CI**
NP Swab	0	0	N/A	N/A	111	2	98%	93-100%
Wash/ Aspirate	8	7	53%	27-78%	155	10	94%	89-97%
Overall	8	7	53%	27-78%	266	12	96%	92-98%

Analytical Sensitivity:

The Alere BinaxNOW® Influenza A&B Card limit of detection (LOD), defined as the concentration of influenza virus that produces positive Alere BinaxNOW® Influenza A &B Card results approximately 95% of the time, was identified by evaluating different concentrations of inactivated Flu A/Beijing and inactivated Flu B/Harbin in the Alere BinaxNOW® Influenza A&B Card.

Twelve (12) different operators each interpreted 2 tests run at each concentration for a total of 24 determinations per level. The following results identify a concentration of 1.03 x 10^2 ng/ml as the LOD for Flu A/Beijing and 6.05 x 10^1 ng/ml for Flu B/Harbin.

Flu A/Beijing		
Concentration (ng/ml)	**Detected #**	**Detected %**
1.03 x 10^2 (LOD)	23/24	96
5.60 x 10^1 (Cutoff)	*	50
3.27 x 10^1 (High Neg)	4/24	17
True Negative	0/24	0

Flu B/Harbin		
Concentration (ng/ml)	**Detected #**	**Detected %**
6.05 x 10^1 (LOD)	23/24	96
2.42 x 10^1 (Cutoff)	11/24	46
1.51 x 10^1 (High Neg)	6/24	25
True Negative	0/24	0

*Linear regression was used to calculate a line equation, which was then used to project the cutoff concentration of Flu A/Beijing.

Analytical Reactivity:

The Influenza A and B strains listed tested positive in the Alere BinaxNOW® Influenza A&B Card at 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 BinaxNOW® Influenza A&B Card.[2] Performance characteristics of the Alere BinaxNOW® Influenza A & B Card for detecting influenza A virus from human specimens was 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	ATCC #	Concentration
Flu A/WS/33 (H1N1)	VR-825	10^2-10^6 $CEID_{50}$/ml
Flu A/NWS/33 (H1N1)	VR-219	10^2-10^6 $CEID_{50}$/ml
Flu A/Hong Kong/8/68 (H3N2)	VR-544	10^2-10^6 $CEID_{50}$/ml
Flu A/Aichi/2/68 (H3N2)	VR-547	10^2-10^6 $CEID_{50}$/ml
Flu A/New Jersey/8/76 (Hsw1N1)	VR-897	10^2-10^6 $CEID_{50}$/ml