510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K162642

B. Purpose for Submission:

New device

C. Measurand:

Influenza A and B virus nucleoprotein antigens in nasal and nasopharyngeal swab specimens

D. Type of Test:

Qualitative, *in vitro* immunochromatographic assay

E. Applicant:

Alere Scarborough, Inc.
10 Southgate Road
Scarborough, Maine 04074

F. Proprietary and Established Names:

Alere BinaxNOW® Influenza A & B Card 2
Alere™ Reader

G. Regulatory Information:

1. Regulation section:

   866.3328 Influenza virus antigen detection test system

2. Classification:

   Class II

3. Product code:

   PSZ Devices Detecting Influenza A, B, and C Virus Antigens

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Alere BinaxNOW® Influenza A & B Card 2 is an *in vitro* immunochromatographic assay for the qualitative detection of influenza A and B nucleoprotein antigens in nasopharyngeal (NP) swab and nasal swab specimens. 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 viral infection and should not be used as the sole basis for treatment or other patient management decisions. Alere BinaxNOW® Influenza A & B Card 2 must be read by the Alere™ Reader.

   Performance characteristics for influenza A were established during the 2015-2016 influenza season when influenza A/H3N2 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

   If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

2. Indication(s) for use:

   To aid in the differential diagnosis of influenza A and B viral infections in patients with signs and symptoms of respiratory tract infection.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Alere™ Reader

I. Device Description:

The Alere BinaxNOW® Influenza A & B Card 2 is an immunochromatographic membrane assay that detects influenza type A and B nucleoprotein antigens in respiratory specimens. Influenza specific antibodies and a control antibody are immobilized onto a membrane support as three distinct lines on the test strip. The 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 off the swab into Elution Solution supplied with the kit in 0.5mL vials (350 µl per vial). The sample is then added to the top of the test strip with a fixed volume transfer pipette (100 µL) and the test card is closed. After 15 minutes, the card assay is inserted into the Alere™ Reader for the interpretation of results.

The Alere™ Reader is a small bench top camera-based instrument to be used in conjunction with the Alere BinaxNOW® Influenza A & B Card 2 assay. The Reader analyzes the intensity of the test and control lines and displays the results (positive, negative or invalid) on a display screen. The screen is intended as a means of user interface informing the user how to operate the Reader and to display test results. The Reader is supplied with a Calibration Check Card. To ensure proper function, a calibration check is required to be performed at least every 30 days. The calibration check verifies that the Reader's internal camera is functioning correctly and that the Reader is working to specification. Operator ID and subject ID can be entered manually, through the touchscreen or via the provided barcode scanner. Results are stored in memory, and can be printed, if desired, on an optional printer connected via USB. In addition, patient test results can be uploaded to a compatible data management system via Ethernet. If connected to a data management system, results are uploaded automatically and are no longer available in memory.

The assay kit consists of 22 Test Cards (a cardboard, book-shaped hinged test card containing the test strip), 25 fixed volume (100 µl) transfer pipettes, 22 Elution Solution Vials, 22 nasal swabs, 1 Positive Control swab (inactivated influenza A and B viruses dried onto the swab), and 1 Negative Control swab (inactivated *Streptococcus* Group A dried onto the swab).

The kit may be stored at 2-30°C.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BD Veritor System for Rapid Detection of Flu A+B Assay

2. Predicate 510(k) number(s):

   K160161

3. Comparison with predicate:

Parameter	Alere BinaxNOW® Influenza A & B Card 2	BD Veritor™ System for Rapid Detection of Flu A+B (K160161)
Assay Target	Influenza A and B nucleoprotein antigens	Same
Intended Use	The Alere BinaxNOW® Influenza A & B Card 2 is an *in vitro* immunochromatographic assay for the qualitative detection of influenza A and B nucleoprotein antigens in nasopharyngeal (NP) swab and nasal swab specimens. 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 viral infection and should not be used as the sole basis for treatment or other patient management decisions. Alere BinaxNOW® Influenza A & B Card 2 test results must be read by the Alere™ Reader. Performance characteristics for influenza A were established during the 2015-2016 influenza season when influenza A/H3N2 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.	The BD Veritor™ System for Rapid Detection of Flu A+B is a rapid chromatographic immunoassay for the direct and qualitative detection of influenza A and B viral nucleoprotein antigens from nasal and nasopharyngeal swabs of symptomatic patients. The BD Veritor System for Rapid Detection of Flu A+B (also referred to as the BD Veritor System and BD Veritor System Flu A+B) is a differentiated test, such that influenza A viral antigens can be distinguished from influenza B viral antigens from a single processed sample using a single device. The test is to be used as an aid in the diagnosis of influenza A and B viral infections. A negative test is presumptive and it is recommended that these results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Outside the U.S., a negative test is presumptive and it is recommended that these results be confirmed by viral culture or a molecular assay cleared for diagnostic use in the country of use. FDA has not cleared this device for use outside of the U.S. Negative test results do not preclude influenza viral infection and should not be used as the sole basis for treatment or other patient management decisions. The test is not intended to detect influenza C antigens. Performance characteristics for influenza A and B were established

Parameter	Alere BinaxNOW® Influenza A & B Card 2	BD Veritor™ System for Rapid Detection of Flu A+B (K160161)
	If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	during January through March of 2011 when influenza viruses A/2009 H1N1, A/H3N2, B/Victoria lineage, and B/Yamagata lineage were the predominant influenza viruses in circulation according to the Morbidity and Mortality Weekly Report from the CDC entitled "Update: Influenza Activity — United States, 2010-2011 Season, and Composition of the 2011-2012 Influenza Vaccine." Performance characteristics may vary against other emerging influenza viruses. If infection with a novel influenza 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 the state or local health department for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
Intended Environment for Use	Professional use, in a medical laboratory	Professional use, in a medical laboratory or in CLIA waived settings
Instrumentation	Alere™ Reader used in conjunction with device.	BD Veritor™ System Reader
Assay Information		
Sample Type	Nasopharyngeal and nasal swabs	Same
Technology	Immunochromatographic	Same
Detection Format	A camera-based reader is used to analyze the test and the control lines and display the results (positive, negative, or invalid) on a display screen.	An optoelectronic instrument that uses a reflectance-based measurement method to evaluate the line signal intensities at each of the spatially defined test and control line positions, interprets the results using

Parameter	Alere BinaxNOW® Influenza A & B Card 2	BD Veritor™ System for Rapid Detection of Flu A+B (K160161)
		a scoring algorithm, and reports a positive, negative, or invalid result on the LCD screen based on pre-set thresholds.
Internal Control	Yes	Yes
Assay Result	Qualitative	Same
Time to Result	15 minutes	10 minutes

K. Standard/Guidance Document Referenced (if applicable):

Guidance for Industry and FDA Staff: Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses (issued on: July 15, 2011).

L. Test Principle:

The test is based on the antigen-antibody binding properties characteristic of immunoassays. The Alere BinaxNOW® Influenza A & B Card 2 uses highly sensitive recombinant antibodies (rFabs) that specifically recognize influenza A and influenza B nucleoprotein antigens in respiratory specimens. A patient line (one for influenza A and one for influenza B) is formed by adsorbing purified viral antigen specific BSA-fab conjugate proteins on the test strip membrane. A control line is formed by adsorbing purified rabbit anti-mouse antibodies onto the test strip membrane. As the patient sample travels across the test strip, viral antigens, if present in the sample, react with the reagents on the membrane and are detected by the Alere™ Reader.

M. Performance Characteristics:

1. Analytical performance:

Analytical studies were conducted using pooled pre-screened negative nasal swab matrix (unless stated otherwise). The sample diluent was tested in triplicate with the Alere BinaxNOW® Influenza A & B Card 2 assay prior to use in preparing dilutions. The matrix was stored at 2-8°C for no more than 5 days.

The influenza virus strains used in the studies were purchased, then re-grown and re-titered to concentrations expressed in $TCID_{50}$/mL. Each influenza virus strain was serially diluted in the nasal swab matrix pool to generate the desired virus concentrations for testing.

a. Analytical Sensitivity (Limit of Detection)

The limit of detection (LoD) of the assay was determined by evaluating different concentrations of five (5) strains of influenza A and three (3) strains of influenza B virus for swabs eluted in Elution Solution. The study was conducted using pooled, pre-screened negative nasal swab matrix. Contrived nasal swab samples were prepared by coating 10 µL of each virus dilution onto the swab. The contrived swab samples were tested by elution in the elution solution according to the test procedure. The LoD for each influenza strain tested was determined by testing 20 replicates of each dilution. The LoD was defined as the concentration where at least 19 of the 20 replicates were positive for the given virus. The confirmed LoDs in natural nasal swab matrix for swabs eluted in Elution Solution for each influenza strain tested are presented in the table below:

Limit of Detection for the Alere BinaxNOW® Influenza A & B Card 2

Strain	LoD Concentration ($TCID_{50}$/mL)
A/Anhui/13 (H7N9) – Inactivated*	1:1500*
A/Indiana/10/11 (H3N2v)	3.67×10^{1}
A/California/7/2009 (H1N1)	5.94×10^{3}
A/Perth/16/2009 (H3N2)	1.68×10^{4}
A/Puerto Rico/8/34 (H1N1)	3.16×10^{4}
B/Massachusetts/02/12 (Yamagata)	1.47×10^{6}
B/Nevada/03/2011 (Victoria)	9.72×10^{3}
B/Malaysia/2506/2004	4.27×10^{3}

*The LoD is reported as a dilution factor from the stock of inactivated virus. The concentration of the virus stock prior to inactivation was $10^{10.9}$ EID_{50}/mL

b. Reproducibility

The reproducibility of the Alere BinaxNOW® Influenza A & B Card 2 assay was evaluated at three testing sites, with 6 operators (2 at each site), over five testing days, using two lots of reagents and five Readers. One strain of influenza A (Influenza A/California/7/2009) and one strain of influenza B (Influenza B/Nevada/03/2011), were diluted in Viral Transport Media (VTM) to targeted concentrations: high negative (just below the LoD), low positive (at the limit of detection), and moderate positive (3x LoD), for a total of 7 test samples. Contrived nasal swab samples were prepared by coating 10 µL of each virus dilution onto the swab. Dried swabs were packaged and assembled into blinded panels of 21 swabs. Each panel consisted of three swabs (individual replicates) for each of the test samples. Each test operator was provided with a panel of samples to be tested on each of the 5 days of testing. The swab samples were tested according to the product instructions with results read by the Alere™ Reader. There were six instances of invalid results, of which 5 were repeated and generated valid results. There was one sample that generated Flu A and B positive results, which was considered invalid and was not repeated.

One positive and one negative control swab was tested with the Alere BinaxNOW® Influenza A & B Card 2 test on each day of testing prior to performing the study testing.

The site-to-site reproducibility of the Alere BinaxNOW® Influenza A & B Card 2 assay is shown below.

% Agreement with Expected Results – by Sites

Sample Type		Site 1	Site 2	Site 3	Overall
Influenza A	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
	Low Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
	High Negative	100% (29/29[1])	93.3% (28/30)	96.7% (29/30)	96.7% (86/89)
Influenza B	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
	Low Positive	100% (30/30)[1]	100% (30/30)	100% (29/29[2])	100% (89/89)
	High Negative	100% (30/30)	93.3% (28/30)	100% (30/30)	97.8% (88/90)
True Negative		100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)

[1]One sample with invalid result was not re-tested
[2]One sample positive for Flu A and Flu B was considered invalid and was not re-tested

The operator-to-operator reproducibility of the Alere BinaxNOW® Influenza A & B Card 2 assay is shown below.

% Agreement with Expected Results – by Operator

		Site 1		Site 2		Site 3		
Sample Type		Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2	All
Influenza A	Moderate Positive	15/15	15/15	15/15	15/15	15/15	15/15	90/90
	Low Positive	15/15	15/15	15/15	15/15	15/15	15/15	90/90
	High Negative	15/15	14/14[1]	15/15	13/15	14/15	15/15	86/89
Influenza B	Moderate Positive	15/15	15/15	15/15	15/15	15/15	15/15	90/90
	Low Positive	15/15	15/15	15/15	15/15	15/15	14/14[2]	89/89
	High Negative	15/15	15/15	14/15	14/15	15/15	15/15	88/90
True Negative		15/15	15/15	15/15	15/15	15/15	15/15	90/90

[1]One sample with invalid result was not re-tested
[2]One sample positive for Flu A and Flu B was considered invalid and was not re-tested

The lot-to-lot reproducibility of the Alere BinaxNOW® Influenza A & B Card 2 assay is shown below.

% Agreement with Expected Results – Lot-to-Lot

Sample Type		Lot 1		Lot 2		Overall	
		% Agreement	Count	% Agreement	Count	% Agreement	95% CI
Influenza A	Moderate Positive	100%	48/48	100%	42/42	100%	95.9-100
	Low Positive	100%	47/47	100%	43/43	100%	95.9-100
	High Negative	95.7%	44/46	97.7%	42/43[1]	96.6%	90.6-98.8
Influenza B	Moderate Positive	100%	43/43	100%	43/43	100%	95.7-100
	Low Positive	100%	45/47[2]	100%	46/46	97.9%	92.5-99.4
	High Negative	95.7%	45/47	100%	43/43	97.8%	92.3-99.4
True Negative		100%	40/40	100%	50/50	100%	95.9-100

[1]One sample with invalid result was not re-tested
[2]One sample positive for Flu A and Flu B was considered invalid and was not re-tested

% Agreement with Expected Results –Reader-to-Reader, by Site

		Influenza A			Influenza B					
Site	Reader	Low Positive	Mod. Positive	High Negative	Low Positive	Mod. Positive	High Negative	True Negative	% Agreement	95% CI
1	1	15/15	15/15	14/14[1]	15/15	15/15	15/15	15/15	100% (104/104)	96.4-100
	2	15/15	15/15	15/15	15/15	15/15	15/15	15/15	100% (105/105)	96.5-100
2	3	30/30	30/30	28/30	30/30	30/30	28/30	30/30	98.1% (206/210)	95.2-99.3
3	4	15/15	15/15	14/15	15/15	15/15	15/15	15/15	99.0% (104/105)	94.8-99.8
	5	15/15	15/15	15/15	14/14[2]	15/15	15/15	15/15	100% (104/104)	96.4-100

[1]One sample with invalid result was not re-tested
[2]One sample positive for Flu A and Flu B was considered invalid and was not re-tested

c. Linearity/assay Reportable Range:

Not applicable.

d. Specimen Stability

Specimen stability was evaluated to support storage recommendations for swabs that are not tested immediately after collection. The study was conducted with weakly reactive samples for influenza A and influenza B, at concentrations approximately 2 times the

LoD. The influenza strains and the concentrations used in the sample stability study are shown below.

Strain	Target Concentration ($TCID_{50}$/mL)
A/California/7/2009	1.19×10^4
B/Nevada/03/2011	1.94×10^4

Swab samples were prepared by inoculating the swab head with 10µl of Flu A, Flu B or clinical matrix diluent sample (for negative samples).

The stability of the samples was evaluated both prior to and after processing with the Elution Solution.

Dry Swab Stability

Flu A and Flu B positive and negative swab samples, not eluted before storage, were tested at time=0 (soon after swab preparation) and at the following time points and temperatures of storage prior to testing:

Room Temperature (15-30°C): 1 hr, 2 hrs, 5 hrs, 9 hrs
Refrigerator (2-8°C): 1 hr, 9 hrs, 17 hrs, 25 hrs, 37 hrs

Samples were tested with the Alere BinaxNOW® Influenza A & B Card 2 in triplicate according to the product instructions.

All swabs generated expected results (i.e., influenza A swabs tested positive for Flu A, influenza B swabs tested positive for Flu B, and the negative swabs tested negative for Flu A and Flu B). The results support the sample stability of up to 4 hours after collection when stored at room temperature and up to 24 hours when stored in a refrigerator.

Swab Stability after Elution with the Elution Solution

Eluted samples were prepared by eluting swabs, prepared as above, in 350 µL of Elution Solution. Flu A and Flu B positive and negative swab samples eluted in the Elution Solution before storage were tested at time=0 and at the following time points when stored in a refrigerator prior to testing:

Refrigerator (2-8°C) 1 hr, 17 hrs, 25 hrs, 37 hrs

Samples were tested with the Alere BinaxNOW® Influenza A & B Card 2 in triplicate according to the product instructions.

All swabs generated expected results (i.e., influenza A swabs tested positive for Flu A, influenza B swabs tested positive for Flu B, and the negative swabs tested negative for

Flu A and Flu B). The results support the claim of stability up to 36 hours when samples eluted in the Elution Solution are stored in a refrigerator at 2-8°C before testing.

e. Swab Type Validation

The sponsor evaluated the performance of the Alere BinaxNOW® Influenza A & B Card 2 test when using various swab types for collection of nasal specimens. The study was conducted with swabs spiked with influenza A and influenza B viruses to approximately 2 times the established LoD. Clinical nasal matrix pool was used as a diluent. The following influenza strains and concentrations were used in this study.

Influenza Strain	**Target Concentration $TCID_{50}$/mL**
A/California/7/2009	5.54×10^4
B/Nevada/03/2011	7.9×10^4

For this study, 10 µL of the prepared virus dilution was spiked into 100 µL of the clinical matrix diluent. Positive swabs were prepared by dipping the swab into either the Flu A or Flu B spiked solution and twirling the swab three times to coat it. Negative swabs were prepared by dipping the swab into 110 µL of clinical matrix diluent and twirling the swab three times to coat it. Samples were tested with the Alere BinaxNOW® Influenza A & B Card 2 in 10 replicates according to the product instructions. The following swabs were evaluated:

- Puritan PurFlock® Ultra flocked swab – standard tip
- Puritan PurFlock® Ultra flocked swab – mini tip
- Puritan PurFlock® Ultra flocked swab - large tip
- Puritan HydraFlock® flocked swab – standard tip
- Puritan Nasopharyngeal foam tipped swab
- Puritan Foam tipped swab, polystyrene handle
- Puritan Polyester tipped applicator, plastic handle
- Puritan Rayon tipped applicator, plastic handle
- Puritan Calcium Alginate tipped applicator
- Copan Regular flocked swab, plastic applicator, sterile, individually wrapped 100 mm
- Copan Polyester, regular tip, plastic handle
- Copan, Rayon, regular tip, plastic handle

The data showed that of the swabs tested, Puritan Calcium Alginate and PurFlock Ultra Flocked Swab - large tip swabs are not appropriate for use with the Alere BinaxNOW® Influenza A & B Card 2 test. The following statement is included in the Sample Collection and Handling Section of the Product Insert: "Puritan PurFlock Ultra® Flocked

swabs – large tip and Puritan Calcium Alginate swabs are not suitable for use in this assay."

f. Assay Cutoff

The assay cutoff (threshold of detection) was determined based on testing of positive and negative samples of varying concentrations using 858 test devices, for a total 1716 test results [638 negative results (319 for Flu A and 319 for Flu B) and 1078 positive results (598 positive for Flu A and 480 positive for Flu B)]. The Reader analyzes the pixel intensity, and compares the pixel intensity of a "band" (i.e. Target Line), to its surrounding areas. The optical reading is calculated as follows:

Optical Reading = [(Background Intensity – Band Intensity)] / Background Intensity

The Optical Readings were analyzed to determine, statistically, what threshold represents a positive versus a negative result. The Mixed Effects Analysis of Variance method was employed to estimate and test for differences between diluents and lines (on the device) and also to estimate the standard deviation of the reading taking into account all known sources of variation. The statistical analysis was applied to all the negative results (lines with 0 signal) from:

a. 319 negative samples (2 lines with 0 signal per device, for a total of 638 results), and
b. 539 positive samples (1 line with 0 signal per device, (240 negative results for Flu A and 299 negative results for Flu B), for a total of 539 results).

The different sources of random variation considered included:

- Lot to lot differences
- Interaction between the Reader and diluent lots
- Differences between devices
- Differences with the devices (two lines on each device)

The cutoff was established based on the intensity of fluorescence of positive and negative results using the full set of data (1716 results).

g. Analytical Reactivity (inclusivity)

An analytical reactivity (inclusivity) study was performed to determine whether the Alere BinaxNOW® Influenza A & B Card 2 is able to detect a variety of influenza A and B strains that represent temporal and geographic diversity. Pre-screened negative natural nasal swab specimens were eluted in 1 X PBS. Swab elutes were combined and mixed thoroughly to create a clinical matrix pool to be used as the diluent. Each influenza virus strain was diluted in this natural nasal swab matrix pool to generate virus dilutions for testing. The vendor provided virus strains were re-titered and the concentrations (in

$TCID_{50}$/mL) were determined by standard virology methods.

Contrived swab samples were prepared by coating 10 µL of virus dilution onto each swab. The contrived swab samples were tested in triplicate in serial dilution series until the lowest concentration of each strain generated positive results in all three replicates. The Alere BinaxNOW® Influenza A & B Card 2 assay detected all strains tested (3/3) at the concentrations indicated in the table below.

Influenza Strain	**Influenza A Subtype or Influenza B Genetic Lineage**	**Concentration $TCID_{50}$/mL**
A/Brisbane/59/2007	A/H1N1	8.35×10^{1}
A/California/4/2009	A/H1N1 (pdm)	3.68×10^{3}
A/Maryland/04/2011	A/H1N1 (pdm)	1.07×10^{2}
A/New Caledonia/20/1999	A/H1N1	2.08×10^{2}
A/New Jersey/8/1976	A/H1N1	1.04×10^{1}
A/New York/18/2009	A/H1N1 (pdm)	1.41×10^{2}
A/Solomon Islands/3/2006	A/H1N1	5.28×10^{1}
A/WSN/33	A/H1N1	5.00×10^{2}
A/Texas/018/2014	A/H1N1	7.90×10^{3}
A/Texas/002/2014	A/H1N1	1.70×10^{3}
A/Aichi/2/68	H3N2	7.90×10^{3}
A/Brisbane/10/2007	H3N2	3.68×10^{0}
A/Hong Kong/8/68	H3N2	2.41×10^{1}
A/Port Chalmers/1/73	H3N2	1.58×10^{4}
A/Texas/50/2012	H3N2	1.06×10^{0}
A/Victoria/3/75	H3N2	1.58×10^{1}
A/Victoria/361/2011	H3N2	2.11×10^{0}
A/Wisconsin/67/2005	H3N2	2.63×10^{1}
B/Bangladesh/3333/2007	Yamagata Lineage	2.11×10^{5}
B/Brisbane/60/2008	Victoria Lineage	3.41×10^{5}
B/Florida/04/2006	Yamagata Lineage	2.97×10^{5}
B/Lee/40	Victoria Lineage	6.81×10^{3}
B/Maryland/1/59	Yamagata Lineage	7.90×10^{3}
B/Montana/05/2012	Victoria Lineage	2.51×10^{6}
B/Ohio/1/2005	Victoria Lineage	3.40×10^{3}
B/Russia/69	Yamagata Lineage	5.93×10^{5}
B/Texas/06/2011	Yamagata Lineage	1.47×10^{6}
B/Victoria/304/2006	Victoria Lineage	1.58×10^{5}
B/Victoria/504/2000	Victoria Lineage	6.81×10^{4}
B/Wisconsin/01/2010	Yamagata Lineage	1.45×10^{4}

h. Analytical Specificity

Cross-Reactivity

To determine the analytical specificity of the Alere BinaxNOW® Influenza A & B Card 2, 58 commensal and pathogenic microorganisms (41 bacteria, 16 viruses and one yeast) that may be present in the nasal cavity or nasopharynx were tested. The bacteria and yeast were re-grown and re-titered by the sponsor; the commercially obtained viruses were tested based on the vendor reported concentrations. For each organism, 10 µL of the stock was added to a vial containing 350 µL of Elution Solution and tested with the Alere BinaxNOW® Influenza A & B Card 2 assay in three replicates according to the test procedure. For each organism, the highest concentration that yielded negative results in all three replicates when tested with the Alere BinaxNOW® Influenza A & B Card 2, is shown below.

Bacteria	**Concentration (in cells/mL, unless indicated otherwise)**
Acinetobacter calcoaceticus	2.2×10^{10}
Bacteroides fragilis	4.4×10^{9}
Bordetella pertussis	9.7×10^{9}
Chlamydia pneumoniae	1.0×10^{9} IFU/mL
Corynebacterium diptheriae	1.2×10^{10}
Enterococcus faecalis	1.0×10^{10}
Escherichia coli	2.2×10^{10}
Gardnerella vaginalis	3.1×10^{9}
Haemophilus influenza	2.9×10^{9}
Haemophilus parainfluenza	9.7×10^{9}
Klebsiella pneumonia	1.7×10^{7}
Lactobacillus casei	6.7×10^{9}
Lactobacillus plantarum	5.5×10^{9}
Legionella pneumophilia	1.3×10^{10}
Listeria monocytogenes	7.2×10^{8}
Moraxella/Branhamella catarrhalis	2.4×10^{10}
Mycobacterium avium	1.3×10^{9}
Mycobacterium intracellulare	1.3×10^{9}
Mycobacterium tuberculosis	1.4×10^{8}
Mycoplasma pneumonia[1]	1.5×10^{3} CFU/mL
Neisseria gonorrhoeae	2.0×10^{9}
Neisseria meningitides	7.7×10^{8}
Neisseria mucosa	9.4×10^{8}
Neisseria sicca	1.0×10^{9}
Neisseria subflava	1.6×10^{9}
Peptostreptococcus anaerobius	9.0×10^{8}
Proteus mirabilis	5.3×10^{8}
Proteus vulgaris	1.5×10^{9}
Pseudomonas aeruginosa	1.5×10^{9}
Serratia marcescens	6.5×10^{7}

Staphylococcus aureus	2.6×10^9
Staphylococcus epidermidis	5.2×10^9
Streptococcus mutans	9.9×10^8
Streptococcus pneumoniae	1.6×10^8
Streptococcus salivarius	6.0×10^8
Streptococcus sanguinis	6.0×10^8
Streptococcus Group A	3.0×10^8
Streptococcus sp. Gp. B	2.0×10^9
Streptococcus sp. Gp. C	1.2×10^9
Streptococcus sp. Gp. F	3.0×10^8
Streptococcus sp. Gp. G	1.7×10^9
Yeast	
Candida albicans	3.8×10^8
Viruses	**Concentration (in $TCID_{50}$/mL, unless indicated otherwise)**
Adenovirus type 1	1.6×10^7
Adenovirus type 7	2.8×10^6
Cytomegalovirus[2]	8.9×10^4
Human Coronavirus OC43	2.8×10^5
Human Coronavirus 229E[2]	2.8×10^4
Enterovirus/Coxsackievirus B4	1.6×10^7
Human Cytomegalovirus strain AD-169[2]	8.8×10^4
Human metapneumovirus	1.4×10^5
Rhinovirus type 1A	1.6×10^8
Measles virus, strain Edmonston	1.6×10^7
Mumps virus, strain Enders	1.6×10^5
Parainfluenza virus 1	1.6×10^8 $CEID_{50/mL}$
Parainfluenza virus 2	1.7×10^7
Parainfluenza virus 3	1.6×10^7
Respiratory Syncytial virus, type B, strain 18537	8.9×10^5
Epstein Barr virus, strain P-3	Not available

[1]*Mycoplasma pneumonia* 10^3 was the maximum cfu/mL that could be achieved for growth
[2]Virus tested at the highest concentration available from the vendor.

Interference

The effect of substances naturally present in respiratory specimens, or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated with the Alere BinaxNOW® Influenza A & B Card 2 assay. One strain of influenza A and influenza B was diluted in pooled pre-screened negative nasal clinical matrix diluent. The targeted concentrations of the viruses used in the study were at approximately 2 times the pre-determined LoD and are shown below.

Influenza Strain	**$TCID_{50}$/mL**
A/California/7/2009	1.19×10^4

B/Nevada/03/2011	1.94×10^4

Swabs were prepared by coating 10 µL of a virus dilution or clinical matrix diluent onto the swab. Each potential interfering substance was added to 315 µL of the Elution Solution at 35 µL volume. One of the Flu A or Flu B inoculated swabs from above (or a clinical matrix diluent representing a negative sample) was placed into each tube. Each substance was tested in triplicate. The following substances, at the concentrations shown, had no effect on the detection of influenza A or influenza B.

Substance	Concentration
Mucin	2% (w/v)
Whole Blood	1% (v/v)
Sinus Buster Nasal Spray	20% (v/v)
NeoSynephrine Cold & Sinus Extra Strength Spray	20% (v/v)
Zicam Extreme Congestion Relief	20% (v/v)
4-acetamidophenol	203 µg/mL
Acetylsalicylic acid (aspirin)	652 µg/mL
Albuterol	399 ng/mL
Chlorpheniramine	142 ng/mL
Dexamethasone	0.8 mg/mL
Dextromethorphan	1 µg/mL
Diphenhydramine	5 µg/mL
Doxylamine Succinate	232 ng/mL
Ephedrine	276 ng/mL
Flunisolide	6.8 ng/mL
Guaiacol glycerol ether (pseudoephedrine)	3.58 ng/mL
Mupirocin	12 mg/mL
Oxymetazoline	0.6 mg/mL
Phenylephrine	12 mg/mL
Relenza	284 ng/mL
Rebetol	4.4 µg/mL
Rimantadine	0.28 ng/mL
Tamiflu	1.102 µg/mL
Tobramycin	2.4 mg/mL
Triamcinolone	40 µg/mL

i. Inhibition by Other Common Respiratory Pathogens

A study was conducted to evaluate the Alere BinaxNOW® Influenza A & B Card 2 test performance in the presence of non-influenza respiratory pathogens. Vendor provided stocks of one strain of influenza A and one strain of influenza B were diluted in clinical matrix to approximately 2 times the limit of detection. Contrived influenza A and B positive swab specimens were prepared by coating 10 µL of the virus dilutions onto each swab. The co-infection (non-influenza) viruses were added onto the same swabs in 10 µL

volumes using the original stock concentrations obtained from a commercial vendor. The co-infection viruses were also applied onto clean (not coated with influenza viruses) swabs as controls. Each swab was tested in triplicate according to the Alere BinaxNOW® Influenza A & B Card 2 test procedure.

The following panel of non-influenza viruses was tested at the concentrations provided in the table below.

Virus Panel	Concentration ($TCID_{50}$/ml)
Adenovirus Type 1	1.58×10^7
Rhinovirus Type 1A	1.58×10^8
Respiratory Syncytial Virus, Type B, Strain 18537	8.89×10^5

All tests gave expected results, i.e., all swabs coated with influenza A virus tested positive for Flu A, all swabs coated with influenza B tested positive for Flu B, and all swabs coated only with the co-infection pathogens tested negative for Flu A and for Flu B.

j. Co-infection with Influenza Viruses

A study was conducted to determine if the Alere BinaxNOW® Influenza A & B Card 2 test can detect weakly positive influenza A samples in the presence of concentrated influenza B virus and weakly positive influenza B samples in the presence of concentrated influenza A virus.

The weakly reactive (reference) swab samples were prepared using the influenza A and influenza B strains diluted to approximately 2 times the pre-determined LoD with pooled pre-screened clinical nasal matrix diluent. Swabs were prepared by coating 10 µL of the virus dilution onto the swab.

The same influenza A and influenza B strains were also diluted in clinical matrix diluent to 20 times (20X) the pre-determined LoD with pooled pre-screened clinical nasal matrix diluent. To prepare the co-infection swabs, 10 µL of the 20X diluted influenza A strain was added to the reference influenza B swabs prepared above. Similarly, 10 µL of the 20X diluted influenza B strain was added to the reference influenza A swabs prepared above using the same method. The concentration of each influenza strain tested is presented below.

Swab Type	Strain	Concentration ($TCID_{50}$/mL)
Reference Swab	A/California/7/2009	1.19×10^4
	B/Nevada/03/2011	1.94×10^4
Co- Infection Swab	A/California/7/2009	1.19×10^5
	B/Nevada/03/2011	1.94×10^5

The reference swabs and co-infection swabs were each tested in triplicate with the Alere BinaxNOW® Influenza A & B Card 2 test. All swabs gave expected results for all replicates tested. The summary of the results is presented below.

Sample	# Tested	BinaxNOW® Influenza A & B Card 2 Result	
		Flu A	Flu B
Flu A Reference Swab	3	+	-
Flu B Reference Swab	3	-	+
Flu A (2x LoD) Co-Infection w/ Flu B (20X LoD)	3	+	+
Flu B (2x LoD) Co-Infection w/ Flu A (20X LoD)	3	+	+

The results of this study showed that the ability of the Alere BinaxNOW® Influenza A&B Card 2 test to detect influenza A in weakly positive samples in the presence of high titers of influenza B virus is not affected. Similarly, the ability of the device to detect influenza B in weakly positive samples in the presence of high titers of influenza A virus is not affected.

k. Controls

External Controls

One swab each, a low positive control for influenza A/B and a negative control, are provided with each kit. The Alere BinaxNOW® Influenza A & B External Controls are designed for use with Alere BinaxNOW® Influenza A & B Card 2. The Positive Control swab is coated with inactivated Influenza A and B viruses dried onto a swab. The Negative Control swab is coated with inactivated *Streptococcus* Group A dried onto a swab. Additional controls (10 Positive and 10 Negative Control Swabs) are available as an accessory to the kit.

Process Control (internal, on every test strip)

A built in assay control is present on every test strip (visible as a blue line on each unused cartridge) and consists of purified rabbit anti-mouse antibodies. It serves to control for the proper flow of reagents across the test strip.

2. Clinical study:

Clinical performance characteristics of the Alere BinaxNOW® Influenza A & B Card 2 were evaluated in a multi-site prospective study during the 2015-2016 flu season (December 2015 to April 2016) in the U.S. A total of 12 investigational sites throughout the U.S. participated in the study. To be enrolled in the study, patients had to be presenting at the participating study centers with flu-like symptoms. Either two nasopharyngeal swabs or two nasal swabs were collected from one nostril from each patient with flu-like symptoms using standard collection methods. In each case, one of the collected swabs was eluted in the Elution Solution and tested using the Alere BinaxNOW® Influenza A & B Card 2 at the study sites according to product instructions.

The other swab was eluted in 1 mL of Viral Transport Media (VTM) and shipped to a central laboratory for testing with a FDA cleared influenza real-time Polymerase Chain Reaction (RT-PCR) assay used as the comparator for this study.

A total of 645 Subjects were enrolled in the study. Of those, 60 swab samples did not meet eligibility criteria due to protocol deviations (sample handling or storage errors or subject withdrawal). A total of 585 nasal or nasopharyngeal swab specimens were considered evaluable. Patient age and gender distribution for all the evaluable specimens are shown below.

Age Group	Female	Male
<1 year	18	21
1 to 5 years	57	62
6 to 10 years	45	46
11 to 15 years	21	18
16 to 21 years	18	14
>21 to 60 years	143	85
>60 years	25	12
Total	327	258

Among the 585 samples tested, 20 samples gave invalid results (these samples were not re-tested), leaving 565 samples to be included in the calculations of assay performance. The invalid rate observed was 3.4% (20/585) with the 95% confidence interval 2.2% - 5.2% (the invalid rate includes 19 results that were positive for both influenza A and B). The clinical performance of the Alere BinaxNOW® Influenza A & B Card 2 assay, as compared to an FDA cleared molecular method for detection of influenza A and B, is shown below.

Clinical Performance of the Alere BinaxNOW® Influenza A & B Card 2

Influenza A

		Molecular Comparator		
		Positive	**Negative**	**Total**
Alere BinaxNOW® Influenza A & B Card 2	**Positive**	113	23	136
	Negative	21	408	429
	Total	134	431	565
	Sensitivity: 84.3% (113/134)	(95%CI: 77.2%-89.5%)		
	Specificity: 94.7% (408/431)	(95%CI: 92.1%-96.4%)		

Influenza B

		Molecular Comparator		
		Positive	**Negative**	**Total**
Alere BinaxNOW® Influenza A & B Card 2	**Positive**	51	3	54
	Negative	6	505	511
	Total	57	508	565
	Sensitivity: 89.5% (51/57)	(95%CI: 78.9%-95.1%)		

	Specificity: 99.4% (505/508) (95%CI: 98.3%-99.8%)

b. Other clinical supportive data:

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected Values

The number and percentage of influenza A and influenza B positive cases per specified age group, as determined by the Alere BinaxNOW® Influenza A & B Card 2 assay during the clinical study conducted in the 2015-2016 influenza season, are presented in the two tables below:

Influenza A

	Prospective Clinical Study During the 2015/2016 Influenza Season		
Age Group	**No. of Nasal or NP Swab Specimens**	**No. of Influenza A Positives**	**Influenza A Positivity Rate**
<1 year	39	3	7.7%
1 to 5 years	119	23	19.3%
6 to 10 years	91	17	18.7%
11 to 15 years	39	10	25.6%
16 to 21 years	32	8	25.0%
>21 to 60 years	228	65	28.5%
>60 years	37	10	27.0%
Total	585	136	23.2%

Influenza B

	Prospective Clinical Study During the 2015/2016 Influenza Season		
Age Group	**No. of Nasal or NP Swab Specimens**	**No. of Influenza B Positives**	**Influenza B Positivity Rate**
<1 year	39	1	2.6%
1 to 5 years	119	7	5.9%
6 to 10 years	91	15	16.5%
11 to 15 years	39	4	10.25%
16 to 21 years	32	7	21.8%
>21 to 60 years	228	15	6.6%
>60 years	37	5	13.5%

Total	585	54	9.2%

N. Instrument Name:

Alere™ Reader

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes___X_____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X___ or No _____

3. Specimen Identification:

   Specimens are identified by scanning a barcode or by manual entry using touch screen.

4. Specimen Sampling and Handling:

   The samples are collected and processed outside of the Reader. The instrument does not perform any sample handling and is used only to read the final signal generated on the test strip.

5. Calibration:

   The Alere™ Reader is supplied with a Calibration Check Card to check the assay reading performance. A calibration check verifies that the internal digital camera is functioning correctly, the lens is free from debris, and the Alere™ Reader is working to specification. The calibration check is required to be performed at least every 30 days. If a calibration check has not been performed in the last 30 days, an error is registered and no tests are

processed until the calibration check is performed.

The calibration check consists of performing a multi-point reading over the surface of the test strip. If the result of any read point is outside a specified range, an error is returned, and no further assay reading can be performed until a successful calibration check is run.

P. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

Not applicable.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.