DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

September 29, 2017

Alere Scarborough, Inc.
Angela Drysdale
VP, Regulatory Affairs – Infectious Disease
10 Southgate Road
Scarborough ME 04074

Re: K171792
Trade/Device Name: Alere i Influenza A & B 2, Alere i Instrument, Alere i Influenza A & B Control Swab Kit
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: II
Product Code: OCC, OZE, OOI
Dated: June 15, 2017
Received: July 3, 2017

Dear Ms. Drysdale:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K171792

Device Name
Alere i Influenza A & B 2

Indications for Use *(Describe)*
The Alere i Influenza A & B 2 assay performed on the Alere i Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in direct nasal or nasopharyngeal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus.

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

Performance characteristics for influenza A were established during the 2016-2017 influenza season when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

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

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K171792

SUBMITTER

Alere Scarborough, Inc.
10 Southgate Road
Scarborough, Maine 04074
Establishment Registration Number: 1221359

CONTACT PERSON

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DATE PREPARED

June 15, 2017

TRADE NAME

Alere™ i Influenza A & B 2

COMMON NAME

Alere™ i Flu, Alere™ i, Alere™ i Flu 2, Alere™ Influenza A & B, Alere™ Influenza A & B 2

CLASSIFICATION NAME

Respiratory Viral Panel Multiplex Nucleic Acid System (per 21 CFR 866.3980)
Instrumentation for Clinical Multiplex Test Systems (per 21 CFR 862.2570)

CLASSIFICATION

Class II

PRODUCT CODE

OCC, OZE, OOI

PANEL

Microbiology (83)

PREDICATE DEVICE

Alere™ i Influenza A & B K163266

DEVICE DESCRIPTION

Alere™ i Influenza A & B 2 is a rapid, instrument-based isothermal test for the qualitative detection and differentiation of influenza A and influenza B from nasal swab or nasopharyngeal swabs tested directly or after elution in viral transport media collected from patients with signs and symptoms of respiratory infection. The Alere™ i Influenza A & B 2 System utilizes isothermal nucleic acid amplification technology and is comprised of:

- Sample Receiver – single use, disposable containing the elution buffer
- Test Base – single use, disposable comprising two sealed reaction tubes, each containing a lyophilized pellet
- Transfer Cartridge – Single use, disposable for transfer of the eluted sample to the Test Base, and
- Alere™ i Instrument – repeat use reader

The reaction tubes in the Test Base contain the reagents required for amplification of the target nucleic acid and an internal control. Alere™ i Influenza A & B 2 utilizes a pair of templates (similar to primers) for the specific amplification of RNA from influenza A and B, which occur in two separate reaction tubes. Each reaction tube contains fluorescently labeled molecular beacon designed to specifically identify the amplified RNA targets. Alere™ i Influenza A & B 2 is performed within the confinement of the Test Base, and no other part of the Alere™ i Instrument has contact with the sample during the amplification process. This minimizes the risk of instrument contamination and sample carry-over between measurements.

To perform the assay, the Sample Receiver and Test Base are inserted into the Alere™ i Instrument and the elution buffer is automatically heated by the instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base, re-suspending the lyophilized pellets contained within the Test Base and initiating target amplification. Heating, mixing and detection by fluorescence are provided by the instrument, with results automatically reported.

Results are displayed by the Alere™ i Instrument and are also stored in an on-board archive and are assigned to a sample ID that has been entered into the Alere™ i Instrument by the operator, and the date/time the test was performed. Data can be retrieved and downloaded by the operator at any time after testing. An external Alere™ Universal Printer can be attached via USB to the Alere™ i Instrument to print test results.

INTENDED USE

The Alere™ i Influenza A & B 2 assay performed on the Alere™ i Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in direct nasal or nasopharyngeal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus.
Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

Performance characteristics for influenza A were established during the 2016-2017 influenza season when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

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

TECHNICAL CHARACTERISTICS

Alere™ i Influenza A & B 2 and the predicate device, Alere™ i Influenza A & B, have the same intended use, indications for use, and utilize similar basic principles of operation. They are both molecular tests for the qualitative detection of influenza A and influenza B viral RNA.

DEVICE COMPARISON

Alere™ i Influenza A & B 2 was compared to the legally marketed predicate device, the Alere™ i Influenza A & B Assay.

Parameter	Alere™ i Influenza A & B 2	Alere™ i Influenza A & B (K163266)
FDA Product Code	OCC, OZE, OOI	Same
Assay Target	Influenza A, Influenza B	Same
Intended Use	The Alere™ i Influenza A & B 2 assay performed on the Alere™ i Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in direct nasal or nasopharyngeal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus. Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Performance characteristics for influenza A were established during the 2016-2017 influenza season when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	The Alere™ i Influenza A & B assay performed on the Alere™ i Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in direct nasal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus. Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Performance characteristics for influenza A were established during the 2012-2013 and the 2014-2015 influenza seasons when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
2Intended Environment for Use	Professional use, in a medical laboratory or point-of-care	Same
Instrumentation	Alere™ i Instrument	Same
Self-Contained System	Integrated PC, Software and Touch Screen Display	Same
Automated Assay	Yes. Sample preparation, amplification, detection, and result interpretation.	Same
Assay Information		
Sample Type	Nasopharyngeal Swab, Nasopharyngeal Swab	Nasal Swab, Nasal Swab or Nasopharyngeal

Parameter	Alere™ i Influenza A & B 2	Alere™ i Influenza A & B (K163266)
	eluted in Viral Transport Media, Nasal Swab, Nasal Swab eluted in Viral Transport Media	Swab eluted in Viral Transport Media
Influenza A Target	PB2 Segment	Same
Influenza B Target	PA Segment	Same
Technology	Isothermal nucleic acid amplification for detecting the presence/absence of viral RNA in clinical specimens.	Same
Internal Control	Yes	Same
Results Interpretation	Automated	Same
Assay Result	Qualitative	Same
Time to Result	<15 minutes	Same

PERFORMANCE SUMMARY

CLINICAL STUDY

The clinical performance of Alere™ i Influenza A & B 2 was established in a multi-center, prospective clinical study conducted at ten US trial sites during the 2016-2017 respiratory season.

A total of 1110 nasal or nasopharyngeal swab specimens were enrolled in this study. Of those, 36 nasal or nasopharyngeal swab specimens did not meet eligibility criteria. A total of 1074 specimens were tested with Alere™ i Influenza A & B 2. 56% of the population tested was female and 44% was male.

In this study, two nasopharyngeal swabs were collected from each Subject. One swab was tested directly with Alere™ i Influenza A & B 2, according to product instructions for testing direct swabs. The other swab was eluted in VTM, and a sample of the VTM eluate was tested with Alere™ i Influenza A & B 2. An FDA-cleared real-time Polymerase Chain Reaction (RT-PCR) test was utilized as the comparator method for this study.

Of the 1074 specimens, Alere™ i Influenza A & B 2 generated invalid results for 4 direct swab specimens after repeat testing per the product instructions, resulting in a total of 1070 specimens for direct swab performance analysis. Alere™ i Influenza A & B 2 generated invalid results for 11 viral transport media specimens after repeat testing per the product instructions and an additional 6 specimens did not meet eligibility criteria, resulting in a total of 1057 specimens for viral transport media performance analysis.

Compared to the comparator method, the performance of Alere™ i Influenza A & B 2 is presented in the tables below.

Direct Nasal or Nasopharyngeal Swab – Performance Obtained for Influenza A with Alere™ i Influenza A & B 2 against the Comparator Method

Alere™ i Influenza A & B 2 - Flu A	Comparator Method		
	Positive	Negative	Total
Positive	260	21[a]	281
Negative	10[b]	779	789
Total	270	800	1070
Sensitivity: 260/270	96.3% (95%CI: 93.3%-98.2%)		
Specificity: 779/800	97.4% (95%CI: 96.0%-98.4%)		

[a] *Flu A nucleic acid was detected in 6/21False positive specimens using a second FDA-cleared molecular test*

[b] *Flu A nucleic acid was not detected in 4/10 False negative specimens using a second FDA-cleared molecular test*

Direct Nasal or Nasopharyngeal Swab – Performance Obtained for Influenza B with Alere™ i Influenza A & B 2 against the Comparator Method

Alere™ i Influenza A & B 2 - Flu B	Comparator Method		
	Positive	Negative	Total
Positive	97	28[a]	125
Negative	0	945	945
Total	97	973	1070
Sensitivity: 97/97	100% (95%CI: 96.3%-100%)		
Specificity: 945/973	97.1% (95%CI: 95.9%-98.1%)		

[a] *Flu B nucleic acid was detected in 21/28 False positive specimens using a second FDA-cleared molecular test*

Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media – Performance Obtained for Influenza A with Alere™ i Influenza A & B 2 against the Comparator Method

Alere™ i Influenza A & B 2 - Flu A	Comparator Method		
	Positive	Negative	Total
Positive	246	12[a]	258
Negative	19[b]	780	799
Total	265	792	1057
Sensitivity: 246/265	92.8% (95%CI: 89.0%-95.6%)		
Specificity: 780/792	98.5% (95%CI: 97.4%-99.2%)		

[a] *Flu A nucleic acid was detected in 5/12 False positive specimens using a second FDA-cleared molecular test*

[b] *Flu A nucleic acid was not detected 6/19 in False negative specimens using a second FDA-cleared molecular test*

Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media – Performance Obtained for Influenza B with Alere™ i Influenza A & B 2 against the Comparator Method

Alere™ i Influenza A & B 2 - Flu B	Comparator Method		
	Positive	Negative	Total
Positive	97	22[a]	119
Negative	0	938	938
Total	97	960	1057
Sensitivity: 97/97	100% (95%CI: 96.3%-100%)		
Specificity: 938/960	97.7% (95%CI: 96.6%- 98.6%)		

[a] *Flu B nucleic acid was detected in 18/22 False positive specimens using a second FDA-cleared molecular test*

During the prospective clinical study, the initial invalid rate for direct nasal or nasopharyngeal swab samples (before repeat testing per the product instructions) was 0.8% (9/1074) (95% CI: 0.4% to 1.6%). After repeat testing per the product instructions, the invalid rate was 0.4% (4/1074) (95% CI: 0.1% to 1.0%).

The initial invalid rate for swabs eluted in viral transport media was 2.2% (24/1074) (95% CI: 1.5% to 3.2%). After repeat testing per the product instructions, the invalid rate was 1.0% (11/1074) (95% CI: 0.6% to 1.8%).

ANALYTICAL STUDIES

ANALYTICAL SENSITIVITY

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

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

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	LoD ($TCID_{50}$/mL)	LoD ($TCID_{50}$/Swab)*	LoD (Genome Equivalents/mL)	LoD (Genome Equivalents/Swab)*
A/Texas/50/2012	A/H3N2	1.00×10^{-1}	1.00×10^{-3}	1.06×10^{4}	1.06×10^{2}
A/California/7/2009	A/2009 H1N1 pdm	2.00×10^{0}	2.00×10^{-2}	1.60×10^{4}	1.60×10^{2}
B/Brisbane/60/2008	Victoria lineage	5.20×10^{1}	5.20×10^{-1}	6.60×10^{3}	6.60×10^{1}
B/Wisconsin/1/2010	B Yamagata lineage	5.01×10^{2}	5.01×10^{0}	1.11×10^{4}	1.11×10^{2}

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

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

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	LoD ($TCID_{50}$/mL)	LoD ($TCID_{50}$/Swab)*	LoD (Genome Equivalents/mL)	LoD (Genome Equivalents/Swab)*
A/Texas/50/2012	A/H3N2	1.00×10^{0}	1.00×10^{-2}	2.10×10^{5}	2.10×10^{3}
A/California/7/2009	A/2009 H1N1 pdm	5.00×10^{1}	5.00×10^{-1}	3.83×10^{5}	3.83×10^{3}
B/Brisbane/60/2008	Victoria lineage	1.20×10^{3}	1.20×10^{1}	1.51×10^{5}	1.51×10^{3}
B/Wisconsin/1/2010	B Yamagata lineage	9.66×10^{3}	9.66×10^{1}	2.14×10^{5}	2.14×10^{3}

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

ANALYTICAL REACTIVITY (INCLUSIVITY)

The influenza A and B strains listed below were tested and generated positive Alere™ i Influenza A & B 2 test results at the concentrations indicated in the table.

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	Test Concentration (in $TCID_{50}$ or Genome Equivalents)			
		$TCID_{50}$/mL	$TCID_{50}$/Swab*	Genome Equivalents/mL	Genome Equivalents/Swab*
A/New Caledonia/20/1999	A/H1N1	2.74×10^{4}	2.74×10^{2}	3.00×10^{4}	3.00×10^{2}
A/New Jersey/8/76	A/H1N1	8.62×10^{-1}	8.62×10^{-3}	3.00×10^{4}	3.00×10^{2}
A/Brisbane/59/2007	A/H1N1	2.44×10^{0}	2.44×10^{-2}	3.00×10^{4}	3.00×10^{2}
A/WSN/33	A/H1N1	2.78×10^{2}	2.78×10^{0}	3.00×10^{4}	3.00×10^{2}
A/California/4/2009	A/H1N1	1.26×10^{1}	1.26×10^{-1}	3.00×10^{4}	3.00×10^{2}
A/Maryland/04/2011	A/H1N1	1.55×10^{3}	1.55×10^{1}	3.00×10^{4}	3.00×10^{2}
A/New York/18/2009	A/H1N1	9.08×10^{0}	9.08×10^{-2}	3.00×10^{4}	3.00×10^{2}
A/South Carolina/2/2010	A/H1N1	3.47×10^{1}	3.47×10^{-1}	3.00×10^{4}	3.00×10^{2}
A/Port Chalmers/1/73	A/H3N2	2.02×10^{3}	2.02×10^{1}	3.00×10^{4}	3.00×10^{2}
A/Hong Kong/8/68	A/H3N2	2.16×10^{1}	2.16×10^{-1}	3.00×10^{4}	3.00×10^{2}
A/Aichi/2/68	A/H3N2	3.58×10^{0}	3.58×10^{-2}	3.00×10^{4}	3.00×10^{2}
A/Perth/16/2009	A/H3N2	6.12×10^{2}	6.12×10^{0}	3.00×10^{4}	3.00×10^{2}
A/Victoria/3/75	A/H3N2	9.61×10^{-1}	9.61×10^{-3}	3.00×10^{4}	3.00×10^{2}
A/Wisconsin/67/2005	A/H3N2	1.60×10^{3}	1.60×10^{1}	3.00×10^{4}	3.00×10^{2}
A/Brisbane/10/2007	A/H3N2	5.48×10^{1}	5.48×10^{-1}	3.00×10^{4}	3.00×10^{2}
A/Victoria/361/2011	A/H3N2	6.41×10^{0}	6.41×10^{-2}	3.00×10^{4}	3.00×10^{2}
A/Indiana/10/2011	A/H3N2v	7.02×10^{3}	7.02×10^{1}	3.00×10^{4}	3.00×10^{2}
A/Sichuan/26221/2014 (inactivated)	A/H5N6	N/A	N/A	3.00×10^{4}	3.00×10^{2}
A/Anhui/1/2013 (inactivated)	A/H7N9	N/A	N/A	6.70×10^{4}	6.70×10^{2}
B/Lee/40	Victoria Lineage	1.60×10^{0}	1.60×10^{-2}	2.25×10^{4}	2.25×10^{2}
B/Victoria/504/2000	Victoria Lineage	1.45×10^{2}	1.45×10^{0}	2.25×10^{4}	2.25×10^{2}
B/Nevada/03/2011	Victoria Lineage	3.66×10^{2}	3.66×10^{0}	2.25×10^{4}	2.25×10^{2}
B/Montana/05/2012	Victoria Lineage	2.21×10^{2}	2.21×10^{0}	2.25×10^{4}	2.25×10^{2}
B/Maryland/1/59	Yamagata Lineage	8.42×10^{2}	8.42×10^{0}	2.25×10^{4}	2.25×10^{2}
B/Russia/69	Yamagata Lineage	9.38×10^{2}	9.38×10^{0}	7.23×10^{5}	7.23×10^{3}

B/Bangladesh/3333/2007	Yamagata Lineage	4.64×10^2	4.64×10^0	2.33×10^4	2.33×10^2
B/Massachusetts/2/2012	Yamagata Lineage	4.30×10^2	4.30×10^0	2.25×10^4	2.25×10^2
B/Malaysia/2506/2004	Yamagata Lineage	3.25×10^3	3.25×10^1	2.25×10^4	2.25×10^2
B/Texas/06/2011	Yamagata Lineage	5.33×10^2	5.33×10^0	2.25×10^4	2.25×10^2

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

ANALYTICAL SPECIFICITY (CROSS-REACTIVITY)

To determine the analytical specificity of Alere™ i Influenza A & B 2, 36 commensal and pathogenic microorganisms (18 bacteria, 17 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 10^3 to 10^{10} cells/mL or CFU/mL (bacteria), 10^4 to 10^8 $TCID_{50}$/mL (viruses), and 10^8 cells/mL (yeast).

Bacteria	**Viruses**	**Yeast**
Bordetella pertussis	Adenovirus type 1	*Candida albicans*
Corynebacterium diphtheriae	Adenovirus type 7	
*Escherichia coli**	Human Coronavirus OC43	
Haemophilius influenzae	Echovirus 7	
Klebsiella pneumoniae	Human Coronavirus 229E	
Lactobacillus plantarum	Enterovirus 70	
Legionella pneumophila	Coxsackievirus B4	
*Moraxella/Branhamella catarrhalis**	Human Cytomegalovirus (CMV) (Herpes V)	
Mycobacterium tuberculosis	Human metapneumovirus	
Mycoplasma pneumoniae	Rhinovirus 1A	
Neisseria meningitidis	Measles (Edmonston)	
*Proteus vulgaris**	Mumps (Enders)	
Pseudomonas aeruginosa	Parainfluenza 1	
Staphylococcus aureus	Parainfluenza 2	
Staphylococcus epidermidis	Parainfluenza 3	
Streptococcus pneumoniae	Respiratory Syncytial virus, type B	
Streptococcus salivarius	Epstein Barr Virus	
Streptococcus pyogenes		

* Some cross-reactivity was observed for *E. coli* concentrations greater than 2.20×10^9, *Moraxella catarrhalis* at concentrations greater than 2.40×10^8, and *Proteus vulgaris* at concentrations greater than 1.50×10^8 .

In addition, *in silico* bioinformatics analyses were performed to assess the level of sequence similarity between the influenza A and B target nucleic acid sequence and the genomic sequences of the following upper respiratory tract microorganism. None of the organisms maintained genomic sequence that was significantly similar to the Alere™ i Influenza A & B 2 target sequences.

Bacteria	**Viruses**
Bordetella bronchiseptica	Adenovirus 2
Chlamydia pneumoniae	Adenovirus 3
Chlamydia trachomatis	Adenovirus 4
Neisseria gonorrhoae	Adenovirus 5
Neisseria mucosa	Adenovirus 11
Proteus mirabilis	Adenovirus 14
	Adenovirus 31
	Coronavirus NL63
	Coxsackievirus B35
	Echovirus 6
	Echovirus 9
	Echovirus 11

Enterovirus 71

INTERFERING SUBSTANCES

The following substances, naturally present or artificially introduced into the nasal cavity or nasopharynx were evaluated with Alere™ i Influenza A & B 2 at the concentrations listed below and were found not to affect test performance.

Substance	Concentration
Mucin	0.5% w/v
Whole Blood	1% v/v
NeoSynephrine Nasal Spray	20% v/v
Afrin Original Nasal Spray	20% v/v
Ocean Saline Nasal Spray	20% v/v
Chloroseptic Max	20% w/v
Zicam	20% v/v
Beclomethasone	0.068 mg/mL
Budesonide	0.051 mg/mL
Dexamethasone	0.48 mg/mL
Flunisolide	0.04 mg/mL
Fluticasone	0.04 mg/mL
Mometasone	0.04 mg/mL
Mupirocin	4.3 mg/mL
Tobryamycin	1.44 mg/mL
Triamcinolone	0.04 mg/mL
Zanamivir (Relenza)	0.284 mg/mL

Inhibition by other Microorganisms

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

Virus Panel	Concentration
Adenovirus Type 1	2.95×10^7 $TCID_{50}$/mL
Rhinovirus Type 1A	1.58×10^8 $TCID_{50}$/mL
Respiratory Syncytial Virus, Type B, Strain 18537	3.00×10^3 (PFU/mL)

Inhibition by High Levels of Influenza A and B

Alere™ i Influenza A & B test performance in the presence of high levels of influenza A and B was evaluated. Vendor provided stocks of influenza A and B strains were diluted in UTM to approximately 3 times the limit of detection. Contrived influenza A and B positive swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. To create the co-infection swabs, diluted influenza A (at a concentration approximately 30 times the LoD) was added to the near LoD Flu B swab. Likewise, diluted influenza B (at a concentration approximately 30 times the LoD) was added to the near LoD Flu A swab. No impact on test performance was observed.

Carry-Over Contamination

An analytical carry-over study was performed to demonstrate that when recommended laboratory practices are followed, there is little risk of false positive results caused by carryover or cross-contamination in the Alere™ i Influenza A & B 2 test. Vendor provided stocks of influenza A and B strains were diluted in UTM to approximately 30 times the limit of detection. Contrived influenza A and B positive swab specimens were prepared by coating 10

microliters of virus dilution onto each swab. Testing of the contrived positive swabs was alternated with testing of a negative swab sample for a total of 15 rounds. There were no false positive results obtained.

An additional analytical carry-over study was performed testing contrived positive VTM samples alternated with negative VTM samples following the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media for a total of 15 rounds. There were no false positive results obtained.

REPRODUCIBILITY

A reproducibility study of Alere™ i Influenza A & B 2 was conducted by operators from 3 sites using panels of blind coded specimens containing negative, low positive (at the limit of detection), and moderate positive (above the limit of detection) influenza A and B samples. Participants tested each sample multiple times on 5 different days. The percent agreement with expected results for the influenza A moderate positive and low positive samples was 100% (90/90). The percent agreement with expected result for the influenza B moderate positive and low positive samples were 100% (89/89), 98.9% (89/90), respectfully. All of the true negative samples (89) generated negative test results. There were no significant differences observed within run (replicates tested by one operator), between run (five different days), between sites (three sites), or between operators (nine operators).