510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K171792

B. Purpose for Submission:

To establish substantial equivalence to a predicate device and to obtain clearance for a new assay: the Alere i Influenza A & B 2 Test.

C. Measurand:

Influenza A Polymerase gene PB2
Influenza B Polymerase gene PA

D. Type of Test:

Qualitative isothermal nucleic acid amplification assay for the amplification and detection of specific Influenza A and Influenza B RNA sequences.

E. Applicant:

Alere Scarborough, Inc.

F. Proprietary and Established Names:

Alere™ i Influenza A & B 2 Test
Alere™ i Instrument
Alere™ i Influenza A & B Control Swab Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3980, Respiratory Viral Panel Multiplex Nucleic Acid Assay

2. Classification:

   Class II

3. Product code:

   OCC – Respiratory Virus Panel Nucleic Acid Assay System

OZE – Influenza A and Influenza B Multiplex Nucleic Acid Assay
OOI – Real Time Nucleic Acid Amplification System

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For Prescription Use

4. Special instrument requirements:

   To be used only with the Alere i Instrument

I. Device Description:

The Alere i Influenza A & B 2 assay consists of a single-use Sample Receiver containing the elution buffer, a single-use Test Base comprising two sealed reaction tubes, each containing a lyophilized pellet, disposable Transfer Cartridge for transfer of the eluted sample to the Test Base, the Alere i Instrument to process the sample and to record raw data, and embedded software to analyze and interpret the data. The test also includes Alere i Influenza A&B positive and negative external control swabs for quality control purposes. The controls are also available separately as an accessory item. The Alere Universal Printer and Barcode Scanner are also available as accessories.

To perform the assay, the Sample Receiver and the 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. Resuspending the lyophilized pellet contained within the Test Base initiates the target amplification. Heating, mixing, and detection by fluorescence are provided by the instrument, with results automatically reported.

The test performs individual amplification reactions for influenza A and B within the Test Base by targeting regions within the RNA genomes of the two viruses. The amplification target for influenza A is the polymerase basic gene 2 (PB2); the amplification target for influenza B is the polymerase acidic gene (PA). Separate results for influenza A and influenza B are reported by the test. Alere i influenza A & B 2 assay technology is isothermal and does not require a heat denaturation step to generate a single strand target for amplification. Instead a thermostable, strand-displacing DNA polymerase, a thermostable nicking endonuclease, and two oligonucleotide templates (primers) are utilized. Reverse transcription is also performed in the reaction. The products of Alere i influenza A & B 2 amplification are two complementary oligonucleotides 30-40 nucleotides in length. A product of this size is of sufficient length to be highly unique amongst genomes. The exact length of the products depends on the specific placement of the templates relative to one another and relative to their complementary sequences within the target genome.

The Alere i influenza A & B 2 test uses fluorescently-labeled molecular beacon probes for sensitive and specific real-time detection. Results (positive, negative, or invalid) are displayed by the Alere i Instrument. Results 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.

<u>Interpretation of results:</u>

Alere i influenza A & B 2 consists of two fluorescence channels for each tube: ROX for the influenza A or influenza B channels and FAM for the Internal Control (IC) channel. The response curves are analyzed within a decision algorithm to form an assay result. Results are reported to the end user as positive, negative or invalid.

Quality Control

Alere i influenza A & B 2 contains an internal control in the influenza B reaction tube. The control has been designed to monitor functionality of the amplification/detection process and reagents. Detection of the IC occurs via a molecular beacon that specifically detects the amplified product generated from the IC RNA oligonucleotide. In positive samples where target amplification is strong, the internal control is ignored and the target amplification serves as the 'control' to confirm that the clinical sample was not inhibitory and that assay reagent performance was robust. At a low frequency, clinical samples can contain inhibitors that may generate invalid results.

Procedural Control Valid displayed on the instrument screen indicates that the assay reagents maintained their functional integrity and the sample did not significantly inhibit assay performance.

Alere i influenza A & B External Controls are designed for use with Alere i influenza A & B 2 assay. 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 C dried onto a swab. External control swabs are provided with the Alere i influenza A & B 2 kit and are also available separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Alere i Influenza A & B

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

   K163266

3. Comparison with predicate:

Table 1: Alere i Influenza A & B 2 – Comparison With Predicate

	Alere i Influenza A & B 2	Alere i Influenza A & B
510(k) Number	K171792	K163266
Assay Targets	Influenza A and B	Same
Product Code	OCC, OZE, OOI	Same
Assay Targets	Influenza A PB2 segment Influenza B PA segment	Same
Device Technology	Isothermal nucleic acid amplification	Same

Results Interpretation	Automated	Same
Time to Result	<15 minutes	Same
Specimen Types	Nasopharyngeal swab (NPS); NPS eluted in Viral Transport Media (VTM); Nasal swab (NS); NS eluted in VTM	NS; NS or NPS eluted in VTM
Instrument	Alere i Instrument	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.	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 nasal swabs 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 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

	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.	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.

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

Not applicable.

L. Test Principle:

Isothermal nucleic acid amplification

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility Study

A reproducibility study of Alere i Influenza A & B 2 was conducted by operators at three sites using panels of blind coded specimens containing negative, low positive , and moderate positive influenza A and influenza B samples. Nine operators from three sites (3 operators per site) participated in the study. The study was conducted over five days each for the VTM and the Swab Direct panels. On each day of testing each operator tested a blinded panel of 10 swabs comprised of the following sample types: true negative (clinical matrix diluent), moderate positive (3x LOD), and low positive (2x LOD) of each influenza type A and B. Swab samples were prepared using the influenza A and B strains diluted to the appropriate concentration in clinical matrix diluent.

For VTM samples, the percent agreement with expected results for the influenza A moderate positive and low positive samples was 100% (90/90) and 98.9% (89/90), respectively. The percent agreement with expected result for the influenza B moderate positive and low positive samples was 100% (90/90). All of the true negative samples (90) generated negative test results. For Direct Swab samples, the percent agreement with

expected results for the influenza A moderate positive and low positive samples were 100% (90/90). The percent agreement with expected result for the influenza B moderate positive and low positive samples were 100% (89/89) and 98.9% (89/90), respectively. 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 runs (five different days), between sites (three sites), or between operators (nine operators).

The Reproducibility Study site-to-site qualitative results (agreements with expected results) are presented in Tables 2 and 3 below.

Table 2: Alere i Influenza A & B 2 - Overall Reproducibility for VTM Samples

Sample Type		Site 1: Detection % and (count)	Site 2: Detection % and (count)	Site 3: Detection % and (count)	Overall Detection
Influenza A	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
	Low Positive	100% (30/30)	96.7% (29/30)	100% (30/30)	98.9% (89/90)
Influenza B	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)
True Negative[1]		100% (30/30)	96.7% (29/30)	90.0% (27/30)	95.6% (86/90)

[1] Percent agreement correlates to the percent of negative results

Table 3: Alere i Influenza A & B 2 - Overall Reproducibility for Swab Direct Samples

Sample Type		Site 1: Detection % and (count)	Site 2: Detection % and (count)	Site 3: Detection % and (count)	Overall Detection
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% (29/29)	100% (89/89)
Influenza B	Moderate Positive	100% (30/30)	100% (30/30)	100% (29/29)	100% (89/89)
	Low Positive	100% (30/30)	96.7% (29/30)	100% (30/30)	98.9% (89/90)
True Negative[1]		100% (30/30)	100% (29/29)	100% (30/30)	100% (89/89)

[1] Percent agreement correlates to the percent of negative results

b. Linearity/assay reportable range:

Not applicable; this is a qualitative assay.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Specimen Stability

To provide data supporting the specimen storage recommendations stated in the product package insert, an analytical study was carried out to evaluate specimen stability.

Contrived positive nasal swab samples were prepared using the Influenza A/Texas/250/2012 and Influenza B/Brisbane/60/2008 dilutions in pooled negative clinical matrix with targeted concentrations near the respective limit of detection (LOD) levels. Mock negative nasal swab samples were prepared using pooled clinical matrix. Swabs were prepared by coating 10 µL of the virus dilution or clinical matrix onto the swab. Swab coating was done by lightly scratching the swab surface with the pipette tip as the dilution was pipetted to ensure liquid was absorbed into the swab tip. All prepared swabs were tested at N=10 at each of the following three time points/conditions: For Direct Swabs, Time 0, 3 hours at 30 °C, 22 hours at 2-8 °C. Swabs tested after 22 hours at 2-8 °C were obtained from the group of swabs stored for 3 hours at 30 °C for a total of 25 hours elapsed before testing. For swabs eluted in VTM, 200 µL was tested according to the VTM assay procedure at the following time points: 0, 9 hours at 30 °C, 45 hours at 2-8 °C, and 64 hours at 2-8 °C. Positive samples were considered stable as long as they tested positive in Alere i Influenza A & B 2. Negative samples were considered stable as long as they generated negative results in the test. Results of the Direct Swab and VTM stability studies are shown in Tables 4 and 5 below.

Table 4: Alere i Influenza A & B 2 Swab Direct Sample Storage

Sample Type	Time 0		3 Hours at 30 °C		22 Hours at 2-8 °C	
	Flu A[1]	Flu B[1]	Flu A[1]	Flu B[1]	Flu A[1]	Flu B[1]
Negative Matrix	-	-9 / +(1)[2]	-	-	-	-9 / +(1)[2]
Influenza A	+	-	+	-	+	-
Influenza B	-	+9 / -(1)[2]	-	+	-	+

[1] Unless otherwise indicated, + or – represents 10/10 concurrence with the expected result.
[2] Retest from the same sample receiver generated the expected result.

Table 5: Alere i Influenza A & B 2 Swab Eluted in VTM Sample Storage

Sample Type	Time 0		9 Hours at 30 °C		45 Hours at 2-8 °C		64 Hours at 2-8 °C	
	Flu A[1]	Flu B[1]	Flu A[1]	Flu B[1]	Flu A[1]	Flu B[1]	Flu A[1]	Flu B[1]
Negative Matrix	-	-9 / +(1)[2]	-	-	-	-	-	-
Influenza A	+	-	+	-	+	-	+	-
Influenza B	-	+	-	+	-	+	-	+

[1] Unless otherwise indicated, + or – represents 10/10 concurrence with the expected result.
[2] Retest from the same sample receiver generated the expected result.

Direct swab method samples generated expected results for each sample type at each storage time point except at Time 0 when one Flu B false negative result was generated; repeat testing using the same Sample Receiver generated expected results and all later testing generated expected results. One presumed negative swab generated a Flu B false positive result; repeat testing using the same Sample Receiver generated expected results. After 22 hours of storage at 2-8°C, one presumed negative swab generated a Flu B false positive result; repeat testing using the same Sample Receiver generated expected results. VTM method samples generated expected results for each sample type at each storage time point except at Time 0 for the clinical matrix diluent which generated one Flu B false positive result. Repeat testing using the same Sample Receiver generated expected results and all later testing generated expected results.

The package insert states that nasal or nasopharyngeal swab samples can be stored at room temperature for up to two hours. If the swab will be held longer than two hours, it must be refrigerated at 2-8°C and tested within 24 hours from the time of sample collection.

The package insert also states that VTM samples can be held at room temperature for up to eight hours. If the eluted swab sample will be held longer than eight hours, it must be refrigerated at 2-8°C and tested within 72 hours from the time of sample collection.

Shelf Life

Alere performed a product stability study to generate stability data to support the expiry assigned to Alere i influenza A & B 2 test kits and control swabs. The test components were tested at specified time points using the influenza A & B Positive QC LOD Controls, and influenza Positive Control Swabs as the positive samples. The QC LOD Controls are approximately 3X the LOD when 50µl of a control is added to the sample receiver. Elution Buffer, UTM, Negative Controls Swabs, and presumed negative nasal swabs were tested as negative samples. Table 6 details the sample type and number of replicates tested at each time point for each condition.

Table 6: Alere i Influenza A & B 2 Kit Stability Test Conditions

Condition / Time Point	Sample Type	Replicates	Total Tests
Time 0 All conditions	Flu A LOD Flu B LOD Flu B/WI LOD Elution Buffer VTM Negative Swab Pos. Control Swabs Neg. Control Swabs	105 105 105 130 40 50 5 5	545
28-32°C: Months 1 and 3	Flu A LOD Flu B LOD Flu B/WI LOD Elution Buffer	10 10 10 10	40
45°C: Months 1,2,3 and 4	Flu A LOD Flu B LOD Flu B/WI LOD Elution Buffer Pos. Control Swabs Neg. Control Swabs	10 10 10 10 5 5	50

To perform testing of influenza A and B QC LOD controls, 50µL of the control material was added to the Sample Receiver and mixed by pipetting up and down and swirling the pipette tip for 10 seconds. To perform testing of the Elution Buffer, no samples was added to the Sample Receiver. The buffer was transferred to the Test Base using the Transfer Cartridge when prompted by the instrument. For testing VTM samples, 200µL of VTM was added to the Sample Receiver and mixed by pipetting up and down and swirling the pipette tip for 10 seconds. Tests were considered stable as long as they continued to produce positive results for positive samples and negative results for negative samples.

Data generated so far supports the following conditions for Alere i Influenza A & B 2 kit storage conditions:

Table 7: Alere i Influenza A & B 2 Kit Stability Results

Lot	Months of Stability at Various Temperatures	
	28-32°C	45°C
Transfer Lot 1	4 Months	4 Months
Transfer Lot 2	4 Months	4 Months
Transfer Lot 3	4 Months	4 Months
Transfer Lot 4	2 Months	2 Months

Swab Type Compatibility Study

Alere performed a swab type validation study to establish which swabs are acceptable for use with the Alere i Influenza A & B 2 assay. Swab samples were prepared using influenza A and influenza B strains diluted to approximately 3 times the limit of detection. Swabs were prepared by coating 10µL of the influenza A virus dilution, influenza B virus dilution or clinical matrix diluent on the swab. Ten samples of each swab type were prepared for each sample type (influenza A, influenza B, or clinical matrix). Swab coating was done by lightly scratching the swab surface with the pipette tip as the solution was pipetted to ensure liquid was absorbed into the swab tip. All swabs were tested within 10 minutes of coating.

Swabs tested in this study include: Puritan Small Foam Tipped Swab; Puritan Regular Foam Swab; Puritan Regular Rayon Swab; Puritan Mini Rayon Swab; Puritan PurFlock Standard Swab; Puritan PurFlock Mini Swab; Puritan HydraFlock Flocked Mini Swab; Puritan HydraFlock Standard Swab; Copan Mini-tip Nylon Flocked Swab; Copan Standard Flocked Swab; and Copan Standard Rayon Swab.

All of the swabs, except the Puritan PurFlock Standard Flocked Swab, Puritan PurFlock Mini-tip swab, and the Copan Standard Rayon Swab generated the expected results for all ten replicates for each sample type. A precaution has been added to the package insert warning the Puritan PurFlock Standard Flocked Swab, Puritan PurFlock Mini-tip swab, and the Copan Standard Rayon Swabs are not acceptable to be used with this assay. All other commonly used flocked swabs appear to be acceptable.

Viral Transport Media Validation Study

Sixteen common transport media were tested in a VTM validation study to determine if the particular media type is acceptable for use with VTM specimens in the Alere i Influenza A & B 2 assay. Presumed negative nasal swabs were eluted in 3 mL of each VTM type by swirling and mixing the swab for 10 seconds. For each VTM type, n=10 clinical matrix diluent (negative sample), influenza A, and influenza B samples were tested at a volume of 200 µL on the Alere i Influenza A & B 2 test according to the VTM assay procedure. Dilutions of influenza A and influenza B were prepared at a concentration approximately 3 times the VTM limit of detection level in each VTM type clinical matrix diluent. A VTM was considered acceptable if positive influenza samples generated positive influenza result and negative samples (clinical matrix diluent) generated negative results. A summary of the testing is shown in the table 8 below.

Table 8: Alere i Influenza A & B 2 VTM Validation Study Results

VTM Type	Sample Type	Replicates	Influenza A Results*	Influenza B Results*
Liquid Amies Transport Media	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
UTM	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
HBSS	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
M4	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
M4-RT	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
M5	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
M6	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
DMEM	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+

Starplex Multitrans Media	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
Liquid Stuart's Media	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
Tryptose Phosphate Broth	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+5 / -5
Brain Heart Infusion Broth	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+9 / Invalid 1	-8 / Invalid 2
	Influenza B Dilution	N=10	-	+
0.9% Saline Solution	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
Phosphate Buffered Saline	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+	-
	Influenza B Dilution	N=10	-	+
2.5% Veal Infusion Broth	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+8 / -2	-
	Influenza B Dilution	N=10	-	+6 / -4
Wako E-MEM	Matrix Diluent	N=10	-	-
	Influenza A Dilution	N=10	+3 / -7	-
	Influenza B Dilution	N=10	-	+

* Unless otherwise indicated, + or – represents 10/10 concurrence with the expected result.

The results of this study indicate Tryptose Phosphate Broth, Brain Heart Infusion Broth, Veal Infusion Broth, and Wako's E-MEM are not compatible with the Alere i Influenza A & B 2 assay. A note has been added to the package insert stating that these four media are not appropriate for use in this assay. All other media tested produced the expected

results and are acceptable for use in the assay.

d. Detection limit:

The objective of the Analytical Sensitivity Study was to identify the limit of detection (LOD) of the Alere i Influenza A & B 2 using characterized strains of influenza A and influenza B. For the purposes of the study, the LOD level was defined as concentration of influenza A and influenza B that produced positive Alere i Influenza A & B 2 test results approximately 95% of the time when tested in multiple replicates.

The Swab Direct Method samples were tested on the Alere i Influenza A & B 2 test according to the swab direct assay procedure. VTM samples were each eluted into a separate vial containing 3 mL of UTM. Four swab eluates were combined and mixed thoroughly by gentle vortex. The four eluates were combined in order to prepare homogenous sample volume sufficient to test 40 replicates. Each swab eluate was tested on the Alere i Influenza A & B 2 test according to the VTM assay procedure.

For both methods, Probit analysis was first performed to determine the LOD of the sample. The LOD was then verified by testing 20 replicates. If 95% or greater detection was obtained with the concentration then that concentration was considered the LOD for that lot. If less than 95% positive results were obtained, a 2-fold higher concentration of virus was tested at n=20 replicates. If the 2x more concentrated panel resulted in 100% detection, a 0.5x dilution was tested to ensure the LOD is not lower than the level determined via Probit analysis.

A minimum of 0.3 mL of each dilution was retained at -80°C for determination of the concentration in targets/mL via qPCR. The empirically determined LOD for each specimen type and each influenza strain is shown in the table below along with the corresponding genome equivalents/mL.

Table 9: Alere i Influenza A & B 2 Limits of Detection

Specimen Type	Strain	$TCID_{50}$/mL	$TCID_{50}$/Swab*	Copies/mL	Copies/Swab*
Swab Direct	A/Texas/50/2012	1.00×10^{-1}	1.00×10^{-3}	1.06×10^{4}	1.06×10^{2}
	A/California/7/2009	2.00×10^{0}	2.00×10^{-2}	1.60×10^{4}	1.60×10^{2}
	B/Brisbane/60/2008	5.20×10^{1}	5.20×10^{-1}	6.60×10^{3}	6.60×10^{1}
	B/Wisconsin/1/2010	5.01×10^{2}	5.01×10^{0}	1.11×10^{4}	1.11×10^{2}
VTM	A/Texas/50/2012	1.00×10^{0}	1.00×10^{-2}	2.10×10^{5}	2.10×10^{3}
	A/California/7/2009	5.00×10^{1}	5.00×10^{-1}	3.83×10^{5}	3.83×10^{3}
	B/Brisbane/60/2008	1.20×10^{3}	1.20×10^{1}	1.51×10^{5}	1.51×10^{3}
	B/Wisconsin/1/2010	9.66×10^{3}	9.66×10^{1}	2.14×10^{5}	2.14×10^{3}

e. Analytical reactivity:

Various influenza A and influenza B strains were tested to examine the ability of the Alere i Influenza A & B 2 to detect a wide variety of virus strains in a clinical setting. Samples were prepared as swab specimens and tested using the swab direct procedure.

Dilutions of each virus were prepared in clinical matrix diluent and 10µL of each dilution was dispensed on to sterile foam swabs. All viral dilutions were run in triplicate. Swab direct samples were prepared using the influenza A and B strains diluted in clinical matrix to approximately three times the swab direct limit of detection levels as defined in the Analytical Sensitivity Study. If initial testing generated at least one negative result at the initial concentration, a higher concentration was tested and then diluted 2-fold until negative results were obtained. The lowest level of each strain that generated positive results on all three replicates was identified as the lowest level detected by Alere i Influenza A & B 2. A dilution level was considered positive if all three replicates generated a positive result. At least one out of three negative results was considered a negative result in the study.

The lowest detectable dilutions were stored frozen at -80 °C until conclusion of testing each day. This dilution was used to determine concentration in targets/mL via validated qPCR. A summary of the results of the reactivity testing is presented in the table 10 below.

Table 10: Alere i Influenza A & B 2 Analytical Reactivity Results

Strain	Subtype or Lineage	Concentration (copies/µL)	Alere i Influenza A & B 2 Result[1]	
			Flu A	Flu B
A/New Caledonia/20/1999	A/H1N1	3.00×10^1	+	-
A/New Jersey/8/76	A/H1N1	3.00×10^1	+	-
A/Brisbane/59/2007	A/H1N1	3.00×10^1	+	-
A/WSN/33	A/H1N1	3.00×10^1	+	-
A/California/4/2009	A/H1N1	3.00×10^1	+	-
A/Maryland/04/2011	A/H1N1	3.00×10^1	+	-
A/New York/18/2009	A/H1N1	3.00×10^1	+	-
A/South Carolina/2/2010	A/H1N1	3.00×10^1	+	-
A/Port Chalmers/1/73	A/H3N2	3.00×10^1	+	-
A/Hong Kong/8/68	A/H3N2	3.00×10^1	+	-
A/Aichi/2/68	A/H3N2	3.00×10^1	+	-
A/Perth/16/2009	A/H3N2	3.00×10^1	+	-
A/Victoria/3/75	A/H3N2	3.00×10^1	+	-
A/Wisconsin/67/2005	A/H3N2	3.00×10^1	+	-
A/Brisbane/10/2007	A/H3N2	3.00×10^1	+	-
A/Victoria/361/2011	A/H3N2	3.00×10^1	+	-
A/Indiana/10/2011	A/H3N2v	3.00×10^1	+	-
A/Sichuan/26221/2014*	A/H5N6	3.00×10^1	+	-
A/Anhui/1/2013*	A/H7N9	6.70×10^1	+	-
A/Anhui/1/2013* (repeat)	A/H7N9	6.70×10^1	+	-
A/Anhui/1/2013*	A/H7N9	5.00×10^1	+2 / -1	-
B/Lee/40	Victoria	2.25×10^1	-	+
B/Victoria/504/2000	Victoria	2.25×10^1	-	+
B/Nevada/03/2011	Victoria	2.25×10^1	-	+
B/Montana/05/2012	Victoria	2.25×10^1	-	+
B/Maryland/1/59	Yamagata	2.25×10^1	-	+
B/Russia/69	Yamagata	7.23×10^2	-	+
B/Russia/69	Yamagata	5.78×10^2	-	+2 / -1
B/Russia/69	Yamagata	3.61×10^2	-	+2 / -1
B/Bangladesh/3333/2007	Yamagata	2.33×10^1	-	+
B/Massachusetts/2/2012	Yamagata	2.25×10^1	-	+
B/Malaysia/2506/2004	Yamagata	2.25×10^1	-	+
B/Texas/06/2011	Yamagata	2.25×10^1	-	+

[1] Unless otherwise indicated, + or – represents 3/3 concurrence with the expected result.
* Inactivated virus

f. Analytical specificity:

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 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). Cross-reactivity was observed for *E. coli* at concentrations greater than 2.20 x10^9, *Moraxella catarrhalis* at concentrations greater than 2.40 x10^8, and *Proteus vulgaris* at concentrations greater than 1.50 x 10^8 and has been noted in the package insert.

Table 11: Alere i Influenza A & B 2 Analytical Specificity

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

In addition, *in silico* analysis was performed to determine whether there is any significant overlap between Alere i Influenza A & B 2 target nucleic acid sequences and the genomes 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.

Table 12: Alere i Influenza A & B 2 Specificity (*in silico*)

Viruses	Bacteria
Adenovirus 2	*Bordetella bronchiseptica*
Adenovirus 3	*Chlamydia pneumoniae*
Adenovirus 4	*Chlamydia trachomatis*
Adenovirus 5	*Neisseria mucosa*
Adenovirus 11	*Neisseria gonorrhoeae*
Adenovirus 14	*Proteus mirabilis*
Adenovirus 31	
Coronavirus NL63	
Coxsackievirus B35	
Echovirus 6	
Echovirus 9	
Echovirus 11	
Enterovirus 71	

g. Potentially Interfering Substances:

An analytical study was performed to assess the potential interference effects of 17 substances naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity/nasopharynx.

Table 13: Alere i Influenza A & B 2 Interfering Substances Tested

Substance	Concentration
Mucin	0.5% (w/v)
Whole Blood	1% (v/v)
NeoSynephrine Cold and Sinus Extra Strength Spray	20% (v/v)
Afrin PumpMist Original	20% (v/v)
Ocean Saline	20% (v/v)
Chloroseptic Max	20% (w/v)
Zicam Allergy Relief	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
Tobramycin	1.44 mg/mL
Triamcinolone	0.04 mg/mL
Zanamivir (Relenza)	0.284 mg/mL

Swab samples were prepared using influenza A and influenza B strains diluted to approximately three times the swab direct limit of detection in clinical matrix diluent. Swabs were prepared by coating 10µL of the virus dilution onto the swab. Swab coating was done by lightly scratching the swab surface with the pipette tip as the solution was pipetted to ensure liquid was absorbed into the swab tip.

A volume of 50µL of each interfering substance was dispensed into sterile tubes. Blank swabs or influenza A or influenza B inoculated swabs were placed into each tube in triplicate. Swabs were stirred to absorb all of the liquid into the swab head. Blank swabs containing only the potentially interfering substance were tested to determine if the substance causes false positive or invalid results in the assay. None of the substances were found to affect test performance at the concentrations tested. False negative results were observed when mucin was present at a concentration of 1% w/v or greater and has been noted in the package insert Limitations section.

h. Microbial Interference:

Alere i Influenza A & B 2 testing was performed in the presence of non-influenza respiratory pathogens. Vendor provided stocks of influenza A and B strains were diluted in clinical matrix to approximately three times the swab direct limit of detection. Contrived swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. Fifty microliters of each of the three potentially interfering viruses were dispensed into sterile tubes.

One of the influenza A or influenza B inoculated swabs was placed into each tube (n=3 influenza A and n=3 influenza B swabs total for each non-influenza virus). Swabs were stirred to absorb all of the liquid into the swab head. Swabs were tested immediately as a swab direct sample according to the Alere i Influenza A & B 2 test procedure. The potentially interfering viruses, the concentrations tested, and the test results are shown in the table 14 below.

Table 14: List of Potentially Interfering Organisms and Concentrations Tested

Co-infection Test	Co-infection Concentration	Alere i Flu A Test Result[1]	Alere i Flu B Test Result[1]
Influenza A + Adenovirus Type 1	5.89×10^7 ($TCID_{50}$/mL)	+	-
Influenza A + Rhinovirus Type 1A	1.58×10^8 ($TCID_{50}$/mL)	+	-
Influenza A + RSV	4.80×10^4 (PFU/mL)	+2 / -1	-
	2.40×10^4 (PFU/mL)	+	-
Influenza B + Adenovirus Type 1	5.89×10^7 ($TCID_{50}$/mL)	-	+2 / -1
	2.95×10^7 ($TCID_{50}$/mL)	-	+
Influenza B + Rhinovirus Type 1A	1.58×10^8 ($TCID_{50}$/mL)	-	+
Influenza B + RSV	4.80×10^4 (PFU/mL)	-	+2 / -1
	2.40×10^4 (PFU/mL)	-	+2 / -1
	1.20×10^4 (PFU/mL)	-	+2 / -1
	6.00×10^3 (PFU/mL)	-	+2 / -1
	3.00×10^3 (PFU/mL)	-	+

[1] Unless otherwise indicated, + or – represents 3/3 concurrence with the expected result.

This study demonstrated that RSV may potentially interfere with the ability of the test to positively identify influenza A or influenza B if present as a co-infection. A limitation has been added to the package insert to inform the user of such a possible outcome.

The Sponsor also tested influenza A and influenza B near the LOD when a high titer co-infection with the other influenza type was present (i.e., influenza A + 30x LOD co-infection with influenza B or vice versa). All tests produced the expected results for all replicates (n=3).

i. Carry-over:

To test for the possibility of cross-contamination between runs, samples containing both influenza A and influenza B were prepared in clinical matrix diluent at approximately 30 times each strain's limit of detection level. Swab direct samples were prepared by coating 10µL of the virus dilution onto the swab. Swab coating was done by lightly scratching the swab surface with the pipette tip as the solution was pipetted to ensure liquid was absorbed into the swab tip. VTM method samples were tested on the Alere i Influenza A & B 2 test according to the VTM assay procedure.

For each testing method (Swab Direct and VTM), testing alternated between positive and negative samples for a total of 15 rounds (15 positives and 15 negatives) on one Alere i instrument. All positive and negative daily controls generated the expected

results on each day of testing. A summary of the results of the Direct Swab and VTM testing is shown in the table below.

Table 15: Alere i Influenza A & B 2 Carryover Testing Results

Sample Type	Number Tested	Alere i Flu Result (Positive Samples)		Alere i Flu Result (Negative Samples)	
		Flu A Result	Flu B Result	Flu A Result	Flu B Result
Swab Direct	15	+	+	-	-
VTM	15	+	+	-	-

All positive samples generated influenza A positive and influenza B positive signals. All negative samples generated influenza A negative and influenza B negative signals.

j. Assay cut-off:

Alere i Influenza A & B 2 consists of three fluorescence channels, ROX for influenza A, FAM for influenza B, and ROX for the Internal Control (IC). Their fluorescent processes physically occur in two separate tubes: ROX for influenza A in Tube 1 while ROX for influenza B and FAM for the Internal Control in Tube 2. The response curves are analyzed within a decision algorithm to form an assay result. For each response curve, the gradient values within the threshold window are compared to the gradient threshold and the results of this comparison determine the channel result, "Asserted", "Not Asserted", or "Indeterminate". The gradient threshold (cut-off) in the algorithm was derived through the data collected to support feasibility and from verification studies. These thresholds were subsequently verified during the clinical study performed during the 2016-2017 respiratory season.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Performance of Alere i Influenza A & B 2 was evaluated against the comparator method in a prospective clinical study.

b. Matrix comparison:
Not applicable.

3. Clinical Studies:

A multi-center study was conducted at ten study sites located throughout the U.S. during the 2016-2017 respiratory season. Alere i Influenza A & B 2 was used to evaluate fresh, prospectively collected nasopharyngeal swab specimens from children and adults of all ages presenting with flu-like symptoms and meeting inclusion/exclusion criteria. Samples were tested with Alere i Influenza A & B 2 within two hours of sample collection. At all

sites, one swab specimen was tested directly on Alere i Influenza A & B 2 according to product instructions for testing swabs. The other swab was swirled in VTM for 10 seconds and an aliquot tested using the VTM method. The remaining VTM containing the residual swab was stored refrigerated and shipped to the central laboratory on cold packs for reference testing.

A total of 1110 nasopharyngeal swab specimens were enrolled in the study. Of those, 36 specimens did not meet eligibility criteria. A total of 1074 nasopharyngeal swab specimens were considered evaluable. Of the 1074 Subjects that met inclusion/exclusion criteria, four direct swab samples and eleven VTM samples produced invalid results. Additionally, there were six VTM samples that were handled outside of protocol instructions leaving a total of 1070 direct swab specimens and 1057 VTM specimens evaluable for the purpose of data analysis. Patient age and gender distribution for the evaluable specimens is presented in table 16 below.

Table 16: Clinical Study Participant Demographics

Age Group (Years)	Female	Male
<1	30	30
1-5	80	104
6-10	57	61
11-15	39	48
16-21	48	39
>21-60	299	161
≥60	44	34
Total	597	477

During the prospective clinical study, the initial invalid rate for direct 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 nasopharyngeal 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%).

Compared to an FDA-cleared molecular assay, the performance of the Alere i Influenza A & B 2 for direct NPS and NPS swabs eluted in VTM is presented below, respectively.

Table 17: Alere i Influenza A & B 2 Direct Nasopharyngeal Swab Performance Compared to FDA-cleared Molecular Comparator – Influenza A

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]Influenza A nucleic acid was detected in 6/21 false positive specimens using an alternative FDA-cleared molecular test

[b]Influenza A nucleic acid was not detected in 4/10 false negative specimens using an alternative FDA-cleared molecular test

Table 18: Alere i Influenza A & B 2 Direct Nasopharyngeal Swab Performance Compared to FDA-cleared Molecular Comparator – Influenza B

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]Influenza B nucleic acid was detected in 21/28 false positive specimens using an alternative FDA-cleared molecular test

Table 19: Alere i Influenza A & B 2 Nasopharyngeal Swab Eluted in VTM Performance Compared to FDA-cleared Molecular Comparator – Influenza A

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]Influenza A nucleic acid was detected in 5/12 false positive specimens using an alternative FDA-cleared molecular test
[b]Influenza A nucleic acid was not detected in 6/19 false negative specimens using an alternative FDA-cleared molecular test

Table 20: Alere i Influenza A & B 2 Nasopharyngeal Swab Eluted in VTM Performance Compared to FDA-cleared Molecular Comparator – Influenza B

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%)			

[b]Influenza A nucleic acid was not detected in 18/22 false positive specimens using an FDA-cleared molecular test

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   In the Alere i Influenza A & B 2 prospective clinical study (described in the "Clinical Studies" section above), a total of 1070 direct NPS specimens and 1057 VTM specimens were evaluable by the Alere i Influenza A & B 2 assay. The number and percentage of influenza positive cases per specified age group, as determined by the Alere i Influenza A & B 2 assay, are presented in the table below:

Table 21: Alere i Influenza A & B 2 Expected Values for Influenza A – Swab Direct

Age Group (Years)	Number of Subjects	Positive by Alere i	% Positivity
<1	60	6	10.0%
1-5	184	47	25.5%
6-10	115	41	35.7%
11-15	87	30	34.5%
16-21	86	30	34.9%
>21-60	460	108	23.5%
≥60	78	18	24.4%
Total	1070	281	26.3%

Table 22: Alere i Influenza A & B 2 Expected Values for Influenza B – Swab Direct

Age Group (Years)	Number of Subjects	Positive by Alere i	% Positivity
<1	60	4	6.7%
1-5	184	28	15.2%
6-10	115	27	23.5%
11-15	87	20	23.0%
16-21	86	10	11.6%
>21-60	460	29	6.3%
≥60	78	7	9.0%
Total	1070	125	11.7%

Table 23: Alere i Influenza A & B 2 Expected Values for Influenza A – VTM

Age Group (Years)	Number of Subjects	Positive by Alere i	% Positivity
<1	59	7	11.9%
1-5	177	45	25.4%
6-10	116	40	34.5%
11-15	86	28	32.6%
16-21	84	28	33.3%
>21-60	457	91	19.9%
≥60	78	19	24.4%
Total	1057	258	24.4%

Table 24: Alere i Influenza A & B 2 Expected Values for Influenza B – VTM

Age Group (Years)	Number of Subjects	Positive by Alere i	% Positivity
<1	59	4	6.8%
1-5	177	25	14.1%
6-10	116	28	24.1%
11-15	86	18	20.9%
16-21	84	11	13.1%
>21-60	457	26	5.7%
≥60	78	7	9.0%
Total	1057	119	11.3%

N. Instrument Name:

Alere i Instrument

O. System Descriptions:

1. Modes of Operation:

The Alere i Instrument is a portable bench-top unit designed to perform in laboratory and point-of care environments. Alere i Influenza A & B 2 components are color-coded to match the corresponding holder on the instrument and designed to only fit in the corresponding holder in one direction. Once properly placed into the instrument, the Alere i Instrument uses image analysis to automatically detect the presence of the Test

Base and to check the test type and expiry date on the barcode present on the Test Base. This triggers the test sequence. Heating, mixing, and detection by fluorescence are provided by the instrument. The Alere i Instrument is equipped with two optical fluorescence modules, FAM and ROX. Each optical module consists of a LED excitation source and photodiode receiver configured to the fluorescent channel. Fluorescence signals are optically filtered by a confocal lens and mirror arrangement and processed by software running on an on-board computer. The results are calculated automatically and presented on the instrument graphical user interface and available for export in JSON format or printing.

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:

   Specimen ID is entered using on screen keyboard or barcode scanner.

4. Specimen Sampling and Handling:

   Not applicable. The specimens are manually inserted in the Sample Receiver in the instrument.

5. Calibration:

   The Alere i Instrument is factory calibrated and does not require any further calibration and verification at user site. However, if the instrument was transported or moved, a performance check using Alere i Positive and Negative Controls is recommended to ensure proper functionality by the manufacture.

6. Quality Control:

   Quality control is addressed for each specific FDA-cleared assay to be run on the

instrument (separately cleared).

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.