CLIA Waiver by Application

Approval Determination Decision Summary

A. Document Number:

CW170013

B. Parent Document:

K171792

C. Purpose for Submission:

To request CLIA waiver for the Alere™ i Influenza A & B 2 Assay with the Alere™ i Instrument.

D. Measurand (Analyte):

Influenza A polymerase basic protein 2 (PB2) RNA
Influenza B polymerase acidic protein (PA) RNA

E. Sample Type(s):

Direct Nasal and Nasopharyngeal Swab Specimens

F. Type of Test:

Qualitative isothermal nucleic acid amplification assay for the amplification and detection of specific Influenza A RNA and Influenza B RNA sequences in nasal swab (NS) or nasopharyngeal swab (NPS) specimens.

G. Applicant:

Alere Scarborough, Inc.
10 Southgate Rd, Scarborough, ME 04074

H. Proprietary and Established Names:

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

I. Test System Description:

The Alere i Influenza A & B 2 assay system utilizes an 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
- Alere i Instrument – repeat use reader
- Alere i Influenza A & B 2 positive and negative external controls – for quality control purposes
- Nasal Swabs – sterile swabs for use with the Alere i Influenza A & B 2 Test

The first reaction tube (Tube 1) in the Test Base contains the reagents required for amplification of the influenza A target viral nucleic acid, and the second reaction tube (Tube 2) in the Test Base contains reagents required for amplification of the influenza B target viral nucleic acid and an internal control synthetic RNA sequence. Alere i Influenza A & B 2 utilizes pairs of templates (similar to primers in a PCR reaction) for the specific isothermal amplification of target RNA sequences from influenza A (highly conserved region of the PB2 gene), influenza B (highly conserved region of the PA gene), and the internal control, and fluorescently labeled molecular beacons designed to specifically identify the amplified products. 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.

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 and initiating target amplification. Heating, mixing, and detection by fluorescence are provided by the instrument, with results automatically reported.

Results (positive, negative, or invalid) are displayed by the Alere i Instrument separately for influenza A and influenza B. 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 are also recorded. 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.

J. Demonstrating "Simple":

The Alere i Influenza A & B 2 Assay is a "self-contained" test that uses unprocessed nasal swab specimens that are added directly to the buffer in the Sample Receiver following collection from the patient. The test system requires only basic, non-technique-dependent reagent manipulation; the elution buffer is contained within the Sample Receiver and there is no reagent preparation required of the user.

The test does not require any operator intervention during the analysis step. After adding the sample to the Sample Receiver, the mixture is transferred via a Transfer Cartridge component. Heating, mixing, and detection are performed by the instrument which reports the results automatically.

No technical or specialized operator training is required in order to use the test. The kit is packaged with a Quick Reference Instructions guide that outlines the test process using easy to follow steps with illustrations. Invalid results and error messages are clearly displayed on the instrument screen. Additional information is provided within the instrument user manual.

The test provides a direct readout of test results. No calculations, conversions, or calibrations are required. Results are reported as positive, negative, or invalid for each analyte.

The instrument requires no electronic or mechanical maintenance. There are no serviceable parts and the instrument is to be returned to Alere for repair.

The test procedure in the Quick Reference Instructions guide and in the User Manual are written at a 7^{th} grade comprehension level.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms:

1. <u>Risk Assessment:</u>

   Risk analysis was performed by the firm using the Failure Modes and Effects Analysis (FMEA) Method; the detailed analysis was included in the submission. Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through additional cautions in the labeling. The identified risks which could result in erroneous test results were evaluated in flex studies which stressed the functional limits of the test system.

2. Fail-safe and Failure Alert Mechanisms

a. Lockout features

- The Sample Receiver and Test Base components are color coded and shaped to fit only into the proper location on the instrument and only in the proper orientation.

- The Test Base contains a quick response (QR) code with information such as test type, expiration date, and lot number. The instrument reads the QR code and generates an error message if an expired Test Base is used.

- The instrument uses image analysis to confirm the Transfer Cartridge is present and instructs the user to close the lid before testing can begin. The instrument detects lid closure and automatically begins the testing process when the lid is closed.

- If power is lost during a test run, the test is cancelled and a result is not reported.

- The instrument uses timeout countdowns at every step of the procedure and will invalidate the test automatically if the timer expires.

- Sample Receivers contain the name of the test printed on the foil to help prevent accidental use of an improper test component.

- The instrument displays the name of the test on the user interface panel after reading the corresponding QR code on the Test Base.

b. Built-in Procedural Control

The Alere i Influenza A & B 2 Assay contains a built-in procedural control. The control tests for sample inhibition, amplification, and assay reagent function. The result of the procedural control is displayed on the screen and is automatically stored in the instrument memory with each test result. "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.

c. External Controls

Each Alere i Influenza A & B 2 test kit contains one external positive control swab and one negative control swab. The positive control swab contains inactivated influenza A and inactivated influenza B dried onto the swab head. The negative control contains inactivated Streptococcus Group C dried onto the swab head. Both swabs are tested using the same

procedure used for patient sample swabs and are ready to use. Additional control swabs are available in an accessory kit.

3. Flex Studies:

Operational limits of the device were tested in the following series of experiments:

1. User does not follow instructions on how to mix the sample properly.
2. Sample Receiver is not fully inserted into the instrument.
3. User adds incorrect volume of VTM to the Sample Receiver.
4. Test kit components are not allowed to equilibrate to room temperature prior to starting a test.
5. Sample Receiver foil seal removed prior to warm-up step.
6. Sample Receiver is over-filled or under-filled with buffer.
7. Operator completes each step at the last possible moment before instrument time-out.
8. Sample Receiver removed or replaced during warm up period.
9. Test performed under extremes of temperature and humidity.
10. Test is performed with a different Alere i test Sample Receiver.

Flex Study 1: User does not follow instructions on how to mix the sample properly

The package insert instructs the user to mix the swab or VTM sample for 10 seconds when adding it to the sample receiver. The goal of this study was to determine whether any effect was observed when the user mixes the swab or VTM sample using alternative methods. Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were tested either directly (Swab Direct method) or eluted in VTM (VTM method). All samples were tested in five replicates for each Influenza and specimen type and each test condition. Test conditions were as follows:

Swab Direct Method

- No Mixing – The swab was dipped in and out (no mixing, eluting, or expressing the swab on the side of the Sample Receiver to remove liquid).

- 1 Second of Mixing with No Expressing – The swab was gently mixed in Elution Buffer for 1 second and DID NOT express the swab on the side of the Sample Receiver to remove liquid.

- 2 Seconds of Mixing with No Expressing – The swab was gently mixed in Elution Buffer for 2 seconds and DID NOT express the swab on the side of the Sample Receiver to remove liquid.

- 5 Seconds of Mixing with No Expressing – The swab was gently mixed in Elution Buffer for 5 seconds and DID NOT express the swab on the side of the Sample Receiver to remove liquid.

- 10 Seconds of Mixing with No Expressing – The swab was gently mixed in Elution Buffer for 10 seconds and DID NOT express the swab on the side of the Sample Receiver to remove liquid.

- 10 Seconds of Mixing with Expressing – The swab was gently mixed in Elution Buffer for 10 seconds and expressed swab on the side of the Sample Receiver to remove liquid (SOP condition).

- 10 Seconds of Vigorous Mixing with Expressing – The swab was vigorously mixed in Elution Buffer for 10 seconds covering at least 50% of the surface with bubbles and expressed swab on the side of the Sample Receiver to remove liquid.

VTM Method

- No Mixing – VTM was delivered to the Elution Buffer without swirling the pipette to mix.

- 1 Second of Mixing – VTM was delivered to the Elution Buffer and gently mixed for 1 second by swirling the pipette in the Sample Receiver and DID NOT pipette up and down.

- 2 Seconds of Mixing – VTM was delivered to the Elution Buffer and gently mixed for 2 seconds by swirling the pipette in the Sample Receiver and DID NOT pipette up and down.

- 5 Seconds of Mixing – VTM was delivered to the Elution Buffer and gently mixed for 5 seconds by swirling the pipette in the Sample Receiver and DID NOT pipette up and down.

- 10 Seconds of Mixing – VTM was delivered to the Elution Buffer and gently mixed for 10 seconds by swirling the pipette in the Sample Receiver and DID NOT pipette up and down (SOP condition).

- 2 Seconds of Mixing and Pipette Up and Down – VTM was delivered to the Elution Buffer and gently mixed for 2 seconds by swirling the pipette in the Sample Receiver while pipetting up and down.

- 5 Seconds of Mixing and Pipette Up and Down – VTM was delivered to the Elution Buffer and gently mixed for 5 seconds by swirling the pipette in the Sample Receiver while pipetting up and down.

- 10 Seconds of Mixing and Pipette Up and Down – VTM was delivered to the Elution Buffer and gently mixed for 10 seconds by swirling the pipette in the Sample Receiver while pipetting up and down.

- 10 Seconds of Vigorous Mixing– VTM was delivered to the Elution Buffer and vigorously mixed for 10 seconds covering at least 50% of the surface with bubbles.

Results: There was one Influenza A sample from the Swab Direct method which produced an incorrect dual-positive (i.e.; positive for Influenza A and Influenza B) result in the test group '10 seconds vigorous mixing with expressing'. Quantitative PCR analysis of the remaining eluate from this sample identified a low level of Influenza B which may account for the unexpected result. All other tests produced the correct results. This study demonstrates the test is not sensitive to a user utilizing some mixing methods that do not precisely match the instructions.

Flex Study 2: Sample Receiver is not fully inserted into the instrument

The Alere i Instrument contains a sensor to detect when the Sample Receiver has been inserted into the instrument. The objective of this study was to evaluate the effect on test performance when the Sample Receiver was not fully seated in the Sample Receiver. Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were tested either directly (Swab Direct method) or eluted in VTM (VTM method). All samples were tested in five replicates for each Influenza and specimen type and each test condition. Steps of the Alere i Influenza A & B 2 procedure were followed until insertion of the Sample Receiver. The Sample Receiver was slowly inserted into the Alere i Instrument until it was detected by the instrument, triggering the countdown for the warming cycle, but was not fully seated in the instrument.

Results: All test samples produced the expected results. This study demonstrates the test is not sensitive to the situation whereby the operator inserts the Sample Receiver to the minimum depth needed to allow the instrument to perform the test, even though it is not fully seated in the instrument.

Flex Study 3: User adds incorrect volume of VTM to the Sample Receiver

The product insert instructs the user to add 200 µL of VTM sample to the Sample Receiver. The objective of this study was to evaluate the effects on test

performance when different volumes of VTM are delivered to the Sample Receiver. Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were then eluted in VTM. Eight coated swabs were eluted into one vial containing 3 mL of UTM by swirling the swab for 10 seconds and expressing the swab head on the side of the vial. The eight eluates were pooled into one vial and mixed by gentle vortex to combine eluates in order to have a sufficient volume for the testing procedure.

The following VTM volumes were tested:

- 100 µL (-50%)
- 150 µL (-25%)
- 175 µL (-12.5%)
- 200 µL (SOP Delivery Volume)
- 225 µL (+12.5%)
- 250 µL (+25%)
- 300 µL (+50%)
- 400 µL (+100%)

Results: All test samples produced the expected results. This study demonstrates the test is not sensitive to the situation whereby the operator uses between 100 and 400 microliters of VTM sample in the Sample Receiver when performing a test.

Flex Study 4: Test kit components are not allowed to equilibrate to room temperature prior to starting a test

The Alere i Influenza A & B 2 Package Insert instructs the user to allow all test pieces to reach room temperature prior to use. The objective of this study was to evaluate the effects on test performance when test components are not allowed to reach room temperature before performing Alere i Influenza A & B 2. Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were tested either directly (Swab Direct method) or eluted in VTM (VTM method). All samples were tested in five replicates for each Influenza and specimen type and each test condition. Test conditions for this study were as follows: 1) Pouched Sample Receivers, Test Bases, and Transfer Cartridges were stored at 2-8°C, removed and tested immediately; or 2) Pouched Sample Receivers, Test Bases, and Transfer Cartridges were stored at 2-8°C, removed and allowed to equilibrate to room temperature prior to beginning the test (SOP).

Results: All test samples produced the expected results. This study demonstrates the test is not sensitive to the situation whereby the operator does not follow the instructions and uses one or more kit components before they have reached room temperature.

Flex Study 5: Sample Receiver foil seal removed prior to warm-up step

The Alere i Instrument User Manual and instructions on the screen instruct the user to remove the foil seal after the Sample Receiver has warmed up. The objective of this study was to evaluate the effect on test performance when the foil seal is removed from the Sample Receiver before the Sample Receiver is warmed up. Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were tested either directly (Swab Direct method) or eluted in VTM (VTM method). All samples were tested in five replicates for each Influenza and specimen type and each test condition. Test conditions for this study were as follows: 1) Foil seal removed immediately after inserting the Sample Receiver; 2) Foil seal removed after 1 minute; 3) Foil seal removed after 2 minutes; 4) Foil seal removed when warm-up has completed and the operator has been instructed by the instrument (SOP).

Results: All test samples produced the expected results. This study demonstrates the test is not sensitive to the situation whereby the operator does not follow the instructions and removes the foil seal prior to being instructed to do so.

Flex Study 6: Sample Receiver is over-filled or under-filled with buffer

The objective of this study was to evaluate the effects on test performance when using Sample Receivers with low volume of Elution Buffer (due to test operator spilling) or high volume of Elution Buffer (due to overfilling during manufacture). Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were tested either directly (Swab Direct method) or eluted in VTM (VTM method). All samples were tested in five replicates for each Influenza and specimen type and each test condition. Seven volumes of Elution Buffer were tested (in milliliters): 0, 0.25, 0.5, 1, 2, 2.5 (SOP condition) and 3 ml. All testing was performed according to the Alere i Influenza A & B 2 assay procedure.

Results: For the swab direct method, invalid results were generated when tested with 0, 0.25 and 0.5 ml of Elution Buffer. One Flu A swab generated a Flu A/Flu B positive result when tested with 1 ml of Elution Buffer. Results were as expected for Elution Buffer volumes of 2, 2.5 and 3 ml.

For the VTM method, invalid results were generated when tested with 0 and 0.25 ml of Elution Buffer. An Influenza A and Influenza B false negative result was observed for the 0.5 ml condition. Results were as expected for Elution Buffer volumes of 1, 2, 2.5 and 3 ml.

This study demonstrates that invalid or false results may occur if the elution buffer is below 2 ml from the 2.5 ml manufacturing specification. The package insert contains multiple precautions warning the user not to open the sample receiver prior to inserting it into the instrument, not to use damaged kit components, and to replace a sample receiver if any buffer is spilled prior to testing.

Flex Study 7: Operator completes each step at the last possible moment before instrument time-out

The objective of this study was to evaluate the effects on test performance when each step of the Alere i Influenza A & B 2 assay procedure was delayed to the end of the time out period, but executed before the Instrument timed out. Additionally, the effect on performance of reusing a Test Base and Sample Receiver after a timeout was determined. Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were tested either directly (Swab Direct method) or eluted in VTM (VTM method). All samples were tested in five replicates for each Influenza and specimen type and each test condition. The conditions tested were: 1) Samples were tested by allowing the instrument to reach the final seconds prior to each of the four Timeout lockouts; 2) samples were tested using a Test Base which had been the subject of a Timeout lockout; 3) samples were tested using a Test Base and Sample Receiver which had been the subject of a Timeout lockout.

The four Timeout lockouts are: 1) The user must insert a Sample Receiver within 10 minutes of the instrument detecting a Test Base; 2) the user must choose ‘Ok’ to indicate the sample has been added within 10 minutes of the Sample Receiver being detected by the instrument; 3) there is a 10 minute timer after the user pushes ‘Ok’ when the sample must be detected in the Test Base (i.e.; transferred via the transfer cartridge); 4) there is a 30 second time limit after transfer when the lid must be closed.

Results: All test samples produced the expected results. This study demonstrates the test is not sensitive to the situation in which the operator waits the maximum amount of allowable time before performing a test step, or if the operator re-uses a previously rejected Test Base or Sample Receiver.

Flex Study 8: Sample Receiver removed or replaced during warm up period

The objective of this study was to evaluate the effect on test performance when the Sample Receiver (SR) is removed from the Instrument after the warm up period and a new SR is inserted in the instrument without allowing the SR to warm up. Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were tested either directly (Swab Direct method) or eluted in VTM (VTM method). All samples were tested in five replicates for each Influenza and specimen type and each test condition. Two test conditions were examined: 1) The SR was removed after warm-up and replaced with another SR already at room temperature; 2) The SR was removed after warm-up and replaced with another SR which had not been allowed to equilibrate to room temperature (2°C -8°C).

Results: False positive results were observed for the test condition whereby the SR was replaced with a new one that had not been allowed to equilibrate to room temperature. There were no unexpected results for the condition tested whereby the SR was replaced with a room temperature SR. This study demonstrates there is risk for erroneous result if the SR is removed after warm-up and replaced with a new SR. The precautions section of the package insert specifically warns the operator not to replace the SR after warm-up has started.

Flex Study 9: Test performed under extremes of temperature and humidity

The objective of this study was to evaluate the effects on test performance if the Alere i Influenza A & B 2 test is performed outside of the temperature and humidity ranges specified in the User Manual. Swab samples were prepared using Influenza A or Influenza B in UTM to concentrations near their respective LoD. Ten microliters of sample was added to the swab and the swab samples were tested either directly (Swab Direct method) or eluted in VTM (VTM method). All samples were tested in five replicates for each Influenza and specimen type and each test condition. Test components were allowed to equilibrate (60 minutes) and testing was performed in conditions with 1) >80% Relative Humidity (RH); 2) <10% RH; 3) >30°C; and 4) <15°C.

Results: One negative control swab produced a false positive result. The test was repeated and produced the expected result. All other samples produced the expected result. This study demonstrates the test is not sensitive to the situation in which the operator performs the test under conditions of extreme temperature or humidity.

Flex Study 10: Test is performed with a different Alere i test Sample Receiver

The objective of this study was to evaluate the effect on test performance when an Alere i Influenza A & B or Alere i Strep A Sample Receiver is used with an Alere i Influenza A & B 2 Test Base. Swab samples were prepared using Influenza A or Influenza B near their respective LoD. Testing was performed using the Swab Direct method. All samples were tested in five replicates for each Influenza type and each test condition. Test condition variables included the following: 1) SR is the Alere i Influenza A & B (previous generation test); 2) Alere i Influenza A & B 2 SR (proper kit component); 3) Alere i Strep A SR; 4) Alere i Strep A 2 SR (generation 2 Strep test). The Alere i RSV SR was not tested because the buffer concentrations are identical.

Results: Two false negative Influenza B results were obtained when the Alere i Strep A or the Alere i Strep A 2 Sample Receivers were used in the test. All other test conditions produced the expected results. This study demonstrates there is chance of false results if an Alere i Strep A SR is used in place of the Influenza A & B 2 SR when performing the Alere i Influenza A & B 2 Test. To further mitigate this risk, the foil seal of the SR is printed with the test name. Upon placement into the Alere i Instrument and before removing the foil seal the test name will be visually available to the test operator.

L. Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy:

1. Study Design:

The objective of the study was to evaluate the performance of the Alere i Influenza A & B 2 Assay in the hands of the intended users when performed in a CLIA waived setting.

a. Study Sites and Duration:

Clinical performance characteristics of the Alere i Influenza A & B 2 Assay were evaluated in a multi-site prospective study during the 2016-2017 influenza season in the U.S. Ten sites throughout the U.S. participated in the method comparison portion of the clinical study. The sites consisted of primary and urgent care clinics, pediatric offices, and family practice offices. All the sites qualified as representative of CLIA waived intended use sites for this device.

b. Operators:

A total of 35 operators representative of intended CLIA waived users across the ten clinical testing sites participated in the study. The operators consisted of administrative personnel, medical assistants, nurses, research/study coordinators, administrative managers, and other patient care providers. The operators who participated in the study were untrained

in the use of the Alere i Influenza A & B 2 Assay and none were trained laboratory technicians. Upon completion of the study, the operators at each site were asked to complete an Operator Questionnaire that asked them to rate the ease of use of the test procedure.

c. Instructions for Use:

The operators were given the product instructions and the Quick Reference Guide. No other materials or instructions were provided and the operators received no training in the use of the test.

d. Subjects (Patients):

To be enrolled in the study, subjects had to be patients presenting at the participating study sites with symptoms of respiratory disease. Patient ages ranged from < 1 year old to ≥ 60 years old. All patients had to have completed informed consent forms prior to sample collection.

e. Samples:

Two nasal or nasopharyngeal swabs were collected from each subject using standard collection methods. One swab was tested directly using the Alere i Influenza A & B 2 Assay. The second swab was eluted in VTM for 10 seconds according to the assay procedure and an aliquot tested on the Alere i Influenza A & B 2 Assay. The remainder of the eluted sample was sent to the reference lab for comparator testing according to the shipping instructions for the comparator test.

f. Comparative Method:

An FDA-cleared molecular influenza assay was used as the comparator method for demonstrating the performance accuracy in support of the CLIA waiver.

g. Exclusions:

A total of 1110 nasopharyngeal swab specimens were enrolled in the study. Of those, 31 specimens did not meet eligibility criteria. A total of 1079 nasopharyngeal swab specimens were considered evaluable. Of the 1079 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 (i.e.; sample handling) leaving a total of 1075 direct swab specimens and 1062 VTM specimens evaluable for performance accuracy estimates.

2. Test Performance:

a. Method Comparison:

Samples with invalid results by the Alere i Influenza A & B 2 Assay were retested per the product instructions. During the clinical study, the initial invalid rate for direct nasopharyngeal swab samples (before repeat testing per the product instructions) was 0.8% (9/1079) (95% CI: 0.4% to 1.6%). After repeat testing per the product instructions, the invalid rate was 0.4% (4/1079) (95% CI: 0.1% to 0.9%).

The initial invalid rate for nasopharyngeal swabs eluted in viral transport media was 2.2% (24/1079) (95% CI: 1.5% to 3.3%). After repeat testing per the product instructions, the invalid rate was 1.0% (11/1079) (95% CI: 0.6% to 1.8%). There were no valid double positive Alere i Influenza A & B 2 Assay results in the study.

Sensitivity and Specificity for the Alere i Influenza A & B 2 Assay are shown below:

Table 1 – 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	261	21[a]	282
Negative	11[b]	782	793
Total	272	803	1075
Sensitivity: 96.0% (261/272) (95%CI: 92.9-98.0%)			
Specificity: 97.4% (782/803) (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 4/11 false negative specimens using an alternative FDA-cleared molecular test.

Table 2 - 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	950	950
Total	97	978	1075
Sensitivity: 100% (97/97) (95%CI: 96.3-100%)			
Specificity: 97.1% (950/978) (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 3 – 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	248	12[a]	260
Negative	19[b]	783	802
Total	267	795	1062
Sensitivity: 92.9% (248/267) (95%CI: 89.1-95.7%)			
Specificity: 98.5% (783/795) (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 4 - 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	943	943
Total	97	965	1062
Sensitivity: 100% (97/97) (95%CI: 96.3-100%)			
Specificity: 97.7% (943/965) (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.

The study results demonstrated that users untrained in the test procedure of the Alere i Influenza A & B 2 assay were able to perform the test correctly and obtain the results with high accuracy. Performance was comparable across study sites and operators.

b. Performance with Analyte Concentrations Near the Cutoff:

A study was conducted to evaluate the performance of Alere i Influenza A & B 2 assay with weakly reactive samples when testing was performed by untrained users. Randomized blind-coded panels, containing negative, low positive (C_{95}), or moderate positive (3x LoD) Influenza A or Influenza B specimens were tested with the Alere i Influenza A & B 2 assay at three sites representative of CLIA waived sites (60 tests in total per site). Six untrained users (2 operators per site) at the CLIA waived sites participated in the study. The panel testing was conducted over 14 days at each site, and the testing was integrated into the users' daily work flow. The testing results of the Alere i Influenza A & B 2 assay with samples near the assay cutoff are summarized in the tables below. Moderate positive samples (i.e.; samples not close to the cutoff) are not shown.

Table 5 - Detection of Influenza A and Influenza B with Samples Near the Cutoff – Direct Swab Method

Sample Type	Site 1 Detection	Site 2 Detection	Site 3 Detection	Overall Detection	Overall 95% CI
Flu A Low Positive	100.0% (20/20)	95.0% (19/20)	100.0% (20/20)	98.3% (59/60)	91.1-99.7%
Flu B Low Positive	100.0% (20/20)	100.0% (18/18)	95.0% (19/20)	98.3% (57/58)	90.9-99.7%
True Negative[1]	100.0% (20/20)	100.0% (20/20)	100.0% (20/20)	100% (60/60)	94.0-100%

[1] "Detection" count and percent correlates to a negative Flu A/Flu B result.

Table 6 - Detection of Influenza A and Influenza B with Samples Near the Cutoff – VTM Method

Sample Type	Site 1 Detection	Site 2 Detection	Site 3 Detection	Overall Detection	Overall 95% CI
Flu A Low Positive	100.0% (20/20)	100.0% (20/20)	100.0% (20/20)	100% (60/60)	94.0-100%
Flu B Low Positive	100.0% (20/20)	100.0% (20/20)	100.0% (20/20)	100% (60/60)	94.0-100%
True Negative[1]	100.0% (20/20)	100.0% (20/20)	100.0% (20/20)	100% (60/60)	94.0-100%

[1] "Detection" count and percent correlates to a negative Flu A/Flu B result.

The study results demonstrated that users untrained in the test procedure of the Alere i Influenza A & B 2 assay were able to perform the test correctly and the test provided the expected result for samples near the cut-off.

c. Operator Questionnaire Results:

Thirty-three operators completed the Operator Questionnaire and the results do not raise any concerns about the ability of untrained users to perform the test at intended use sites.

M. Proposed Labeling:

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

The labeling includes an assay package insert, Instrument manual, and a Quick Reference Card which is written in simple language and contains graphics to facilitate comprehension of the instructions.

N. Conclusion:

The submitted information in this CLIA waiver application supports a CLIA Waiver approval decision.