

CLIA Waiver by Application
Approval Determination Decision Summary

A. Document Number

CW170003

B. Parent Document Number

K162642

C. Purpose of the Submission

To obtain CLIA waiver for the BinaxNOW Influenza A & B Card 2 Assay with Alere Reader.

D. Measurand (analyte)

Influenza A and influenza B viral nucleoprotein antigens

E. Sample Type

Direct nasal and nasopharyngeal swabs,

F. Type of Test

Qualitative Immunoassay

G. Applicant

Alere Scarborough, Inc.

H. Proprietary and Established Names

Alere BinaxNOW[®] Influenza A & B Card 2
Alere Reader

I. Test System Description

1. Overview

The Alere BinaxNOW Influenza A & B Card 2 is an immunochromatographic membrane assay that detects influenza type A and B nucleoprotein antigens in respiratory specimens. Influenza specific antibodies and a control antibody are immobilized onto a membrane support as three distinct lines on the test strip. The test strip is mounted inside a cardboard, book-shaped hinged test card. The patient nasal or nasopharyngeal swab specimen is placed in a vial containing Elution Solution (supplied with the kit) and swirled vigorously. The liquid sample is then transferred onto the top of the test strip with a fixed volume transfer pipette (100 µL) and the test card is closed. After 15 minutes, the card assay is inserted into the Alere Reader for the interpretation of results.

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

Upon acknowledgement of the test results, the operator is instructed to open the drawer, remove the test card, and close the drawer. The operator is then returned to the Home screen.

The assay kit consists of 22 Test Cards (a cardboard, book-shaped hinged test card containing the test strip), 25 fixed volume (100 µl) transfer pipettes, 22 Elution Solution Vials, 22 nasal swabs, 1 Positive Control swab (inactivated influenza A and B viruses dried onto the swab), and 1 Negative Control swab (inactivated *Streptococcus* Group A dried onto the swab). The kit may be stored at 2-30°C.

A Reader Calibration Check card is provided with the Reader.

2. Results Interpretation

The Reader captures the image on the test strip created by the antigen-antibody complexes coupled with dyed particles and immobilized at the specific test line positions. The Reader analyzes the test and the control lines and results are displayed on the Reader touchscreen as positive or negative for Influenza A and Influenza B along with the procedural control line status.

There are five possible results: (1) Influenza A positive/Influenza B negative; (2) Influenza A negative/Influenza B positive; (3) Influenza A negative/Influenza B negative (4) Influenza A positive/Influenza B positive; and (5) Invalid. If the test is Invalid, another specimen should be collected and tested. Influenza A and B dual positive results should be considered invalid and another specimen should be collected and tested.

For all valid results, “Control: Valid” is displayed on the Reader screen.

J. Demonstrating “Simple”

The Alere BinaxNOW Influenza A & B Card 2 assay with Reader was designed to be simple and easy to use by incorporating the following features:

- The test uses direct nasal and nasopharyngeal specimens.
- The test requires basic, non-technique-dependent specimen and reagent handling to obtain accurate test results.
- The Elution Solution is premeasured and provided in individual vials.
- A fixed volume pipette is provided to ensure proper sample volume.
- The test cards are unitized and contain all the reagents required for analysis.
- The reagents are stable and can be stored at a wide range of temperatures (2-30°C).
- The test does not require any operator intervention during the analysis step. Once the sample is added to the test strip the test card is closed.
- The results are interpreted by the Alere Reader which provides a direct readout of test eliminating subjectivity associated with visual reading of results by the end-user.
- The results are displayed on the screen as positive, negative or invalid and there is no interpretation required.
- The Reader touchscreen is designed for ease of use and features a color display that facilitates easy-to-read messages.
- Calibration check, which is required every 30 days, is easily performed with a provided barcoded Calibration Check Card.
- Error messages are unambiguous and include easy-to-interpret solutions.
- No complex troubleshooting or interpretation of error codes are required to operate the Reader.
- There is no maintenance required other than wiping of the external surface of the Reader.
- There are no serviceable parts and the instrument is to be returned to Alere for repair.
- The test procedure is written at a 7th grade comprehension level.
- An optional printer is available.

K. Demonstrating “Insignificant Risk of an Erroneous Results”- Failure Alerts and Fail-safe Mechanisms

1. Risk Assessment

A comprehensive risk analysis of the Alere BinaxNOW Influenza A & B Card 2 assay with Reader has been conducted in accordance with ISO 14971. The sponsor utilized the Device Hazard Analysis and the Failure Mode Effects Analysis (FMEA) methods to assess the risks of failure that may occur during use or misuse of the device. The FMEA includes identification of potential failure modes and effect of the failure, potential causes, built in design controls and evaluation of severity, frequency of occurrence, and ability to detect the failure. The elements considered included operator errors (human factors), sample and device handling and storage, and environmental factors.

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 that stressed the functional limits of the test system (see below).

The sponsor provided detailed software validation and verification documentation, including requirements related to assay performance when using the Alere Reader. The instrument software was reviewed under the parent 510(k) submission (K162642).

2. Fail-safe and Failure Alert Mechanisms

The Alere BinaxNOW Influenza A & B Card 2 assay with Reader was designed to include numerous features and fail-safe mechanisms built into the system to prevent erroneous results.

Design Features

- Each test card is packaged in an air-tight aluminum foil pouch to maintain the integrity of the reagents.
- Each test card contains a barcode on the outer foil package with critical information, such as the assay type, the lot number and the expiration date. The outer card housing contains a matching QR code (test ID) that confirms the identity of the test card once inserted into the Reader.
- The test card displays bold directional arrows that visually guide the user to place the sample onto the test strip correctly.
- The Reader touchscreen is designed to facilitate easy operation with clearly labeled “action buttons.”
- Test results are interpreted automatically and a direct readout is provided on the Reader screen.

- The test card design is “keyed” ensuring that the card is placed in the Reader in the correct orientation.

Fail-safe Features

- In-test Strip Control: The built in Control line is intended to detect procedure errors or reagent failure. The Alere Reader recognizes when a control line is not present, either due to a manufacturing error or a procedural error, returning an invalid result.
- External Controls: External Control Swabs, one Positive Control Swab (coated with inactivated influenza A and influenza B viruses) and one Negative Control Swab (coated with inactivated, non-infectious *Streptococcus* Group A), are included in each reagent kit. Each control is processed using a separate test card. Both control swabs are ready to use and are tested using the same procedure used for patient samples. These controls monitor the entire assay and serve to detect product defects or reagent deterioration between the manufacturer’s lot release date and the date of use. The controls also monitor the operator’s use of the test and detect any errors in the procedure. The manufacturer-recommended frequency of running the controls includes: with each new shipment and with each untrained operator.
- A calibration check of the Reader is required at least every 30 days to verify that the camera is functioning properly, that the lens is free from debris and that the Reader is working according to the specifications. Calibration cards are provided with the Reader.
- Each time the Reader is turned on a system self-check is initiated to check the camera functionality, the memory integrity, the calibration status, the drawer state (open/closed), the presence or absence of a test card, and to verify specification settings.
- The Reader has a connectivity capability for interfacing with an in-house “middleware” network allowing automatic uploads of results to the facility’s system. This allows for remote oversight of the results in the CLIA waived areas for facilities that have a central information system (LIS).

Lockout Features and Alert Messages

The Alere Reader has numerous built-in lockout features to minimize the potential for erroneous results, including the following:

- The Reader calibration must be performed every 30 days and the instrument will not proceed until the calibration status is updated, preventing optics drift over time.

- The Reader contains an optional QC lockout feature that prevents a new reagent lot from being used until the external controls have been successfully tested.
- Each test unit has a barcode on the outer foil pouch containing assay information (test type, lot number, and expiration date) that is scanned or entered manually using the Alere Reader touch screen. Additionally, each test card has a barcode that contains assay code (QR code). If a mismatch occurs between the pouched test card barcode and the QR code on the outer card housing an error message will be displayed on the Alere Reader screen and the testing will not proceed.
- The Reader will not proceed when the test card is expired; an error message is generated.
- When the optional QC lockout feature is turned on, a QC test is required when a new lot of reagents is run for the first time.
- The Reader will not proceed when the drawer is open. The Reader contains a sensor and the Read Test sequence does not begin until the drawer is closed.
- The Reader must “see” a valid barcode on the test card, otherwise an error message will alert the user that the barcode could not be read; the testing will not proceed.
- If the drawer of the Reader is opened during the image acquisition, an error will be displayed and the test result will be rendered invalid.

The functionality of the fail-safe mechanisms built into the software of the Alere Reader was demonstrated in studies conducted using the Alere BinaxNOW Influenza A & B Card 2 test cards. Some of the tests performed during the software verification are listed below.

Verification of Software Functionality

Function	User Action	Expected Error Message on the Reader Screen
Calibration Interval	An Alere BinaxNOW Influenza A & B Card 2 test card was placed in the drawer of the Reader and “Read Test” was touched on the screen.	<i>Calibration Check Required. Instrument Calibration Check required. Read Test and Read QC Test are unavailable until a successful Calibration Check is performed.</i>
Barcode Functionality (QC)	“Read QC Test” was tapped on the screen and a BinaxNOW Influenza A & B Card 2 was scanned by the barcode.	<i>Tray Barcode Read Failure. Tray barcode could not be read. Open drawer to check</i>

	<p>“External Control Type” was selected on the screen. The test card was inserted into the Reader drawer with a hidden barcode and the drawer was closed.</p>	<p><i>that the device is present in the tray and that the barcode is not obscured. Close drawer and try again.</i></p>
<p>Barcode Functionality (Calibration Check Card QR code)</p>	<p>A Calibration Check Card was inserted with the QR code hidden.</p>	<p><i>Calibration Check Card QR code could not be read. Open drawer, ensure that the Calibration Check Card is present and positioned properly and the QR code is not obstructed. Close the drawer to continue.</i></p>
<p>Barcode Functionality (Reagent Lot Expiration)</p>	<p>An out-of-date expiration date was imbedded in the barcode of an Alere BinaxNOW Influenza A & B Card 2 and the test device was scanned.</p>	<p><i>Test device expired on 07/18/2004. Press OK to try again using an in-date Test Device</i></p>
<p>Barcode Functionality (Calibration Check Card Expiration)</p>	<p>An expired Calibration Check Card was inserted into the Reader drawer</p>	<p><i>Calibration Check Card Expired. Calibration Check Card expired on 07/18/2004. Open drawer, replace with an in-date Calibration Check Card and close drawer to try again.</i></p>
<p>Barcode Functionality (mismatch between the test card and the test selected)</p>	<p>A pouched Alere BinaxNOW Legionella Urinary Antigen Card test was scanned and confirmed that the instrument had identified the test to be run as a Legionella test. Next, a new, un-run Alere BinaxNOW Influenza A & B Card 2 test device was un-pouched and inserted into the Alere Reader and the drawer was closed.</p>	<p><i>Test Device ID mismatch. Foil pouch barcode and test device QR code do not match. Open drawer, insert test device from the scanned foil pouch and close drawer to continue.</i></p>
<p>Barcode Functionality (/valid barcode)</p>	<p>“Read Test” was touched on the screen and a BinaxNOW Influenza A & B Card 2 was scanned by the barcode. Patient ID was entered. The</p>	<p><i>Test Device QR code Failure. Test Device QR code could not be read. Open drawer check that the Test</i></p>

	test device was inserted into the Reader drawer with a QR code hidden and the drawer was closed.	<i>Device and orientation are correct and QR code is not obstructed. Close drawer to continue.</i>
QC Lockout	Read Test tapped and a test card from a new lot was scanned.	<i>QC test required. The instrument's QC policy requires a positive and negative QC tests for test type "Alere BinaxNOW Influenza A&B of lot number 1234567. You cannot proceed until a QC test is completed.</i>
Open Drawer	Place a test card in the drawer but leave the drawer open.	<i>Test device read failure</i>
Missing Control Line (not developed)	A new, unused Influenza A & B Test Card was inserted into the Reader, the tray was closed and the test was started.	Invalid result was returned.
Barcode Functionality (card placement/)	A closed test card was placed in the drawer in the inverted direction, where the Alere logo was closest to the test operator. A second position evaluated was when the test card was placed in the drawer with window facing down.	<i>Test Device QR code Failure. Test Device QR code could not be read. Open drawer check that the Test Device and orientation are correct and QR code is not obstructed. Close drawer to continue.</i>
Integrity of Reader settings after Power Failure	The DC power to the Reader was disconnected without logging out. The power was then reinstated and the instrument was switched on.	The Reader settings (such as date and time) and selections (such as operator IDs, sound preferences, language preferences, etc.) were maintained.
Data Integrity after Power Failure	A new patient record was created by running a mock flu test. The Reader was powered off and then back on.	Patient test results were retained in the history file.

Power down the Reader during the test	The power key was pressed down to attempt to power off the Reader while the Read Test was running.	The Alere Reader does not power down during the execution of the Read Test.
---------------------------------------	----------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------

All tests generated the expected error messages and functionality confirming the effectiveness of the fail-safe mechanisms built into the software.

A full review of the Reader software was conducted under K162642.

3. Flex Studies

The operational limits of the device were evaluated in a series of experiments simulating conditions of use outside of the intended use environment or instances of user errors.

The test samples were prepared using the influenza A and B strains (A/California/07/2009 and B/Nevada/03/2011) diluted to concentrations approximately 2x LoD in negative clinical matrix. Swab samples were coated with 10 µL of diluted virus solutions. Negative samples consisted of unspiked matrix. The three samples prepared, one low positive influenza A, one low positive influenza B and one negative, were each tested in triplicate for each condition being evaluated. Positive swab results were read at 15 minutes and negative swab results were read at both 15 and 30 minutes. The samples were blinded and randomized. For all conditions evaluated, one set of test devices was also used to test the samples under the normal conditions, i.e., without being subjected to the stresses being evaluated, as a control.

The effect of the following conditions on the performance of the assay was evaluated:

Operational Environment

1. Temperature and Humidity

The studies were conducted using environmental chamber. Two simulated environments were evaluated: (a) 50°C and 80% humidity and (b) 2-8°C and ambient humidity. The test cards were removed from the foil pouch and held at each test condition prior to testing for: 30 minutes, 1 hour, 2 hours and 4 hours. Four readers were utilized in this study.

The results showed that exposure of un-pouched test cards to 50°C and 80% humidity or to low temperatures, such as 2-8°C for 30 minutes had no effect on the assay performance. However, exposure for 1 hour or longer to either of the conditions may result in incorrect results. The effect of increased temperature on pouched test cards was evaluated by storage at 45°C for two weeks and all testing produced expected results. The risk of erroneous results under the circumstances of exposing the test cards to extremes of operating conditions is minimized by the packaging of the units

in individual air-tight foil pouches. Additionally, a caution is included in the test procedure instructing the user to leave the test card sealed in the pouch until just prior to testing.

2. Reagent Freeze/Thaw

The study evaluated the effect of repeated freeze/thaw conditions of reagents, such as may occur during shipping. The test devices were placed at -20°C for 24 hours and then removed and placed at 30°C (maximum storage temperature) for a minimum of 24 hours for 1 freeze/thaw cycle. Functional testing was performed on test devices that underwent 1 freeze/thaw cycle and 3 freeze/thaw cycles (the freeze/thaw process was repeated for a total of 3 freeze/thaw cycles). One Reader was utilized in this study. Expected results were obtained for all samples at both conditions tested.

3. Vibrations

The study evaluated the effect of vibrations, such as those that may be generated from nearby instrumentation. Three Readers were placed on a lab bench within 3 feet of a centrifuge running at 14,000 rpm. The test samples were processed and read by the Reader while the centrifuge was running (3 Readers were used to read the results in this study). Expected results were obtained for all samples tested.

4. Geographic Altitude (Barometric Pressure)

The Alere Reader is specified for use at an altitude of 0-2000 meters. This study examined the effect of > 2000 meters altitude on the assay performance. Initially, three Alere Readers underwent a full-suite of Production End of Line tests (PTR), a scanner test and a printer test at the Scarborough, ME facility to ensure that the Readers were operating as expected. Then the Readers were taken to Idaho Springs, CO (altitude 2289 meters) where the full-suite of PTR tests, a scanner tests and a printer test were repeated. Upon completion of the testing, the Readers were taken to Saco, ME (altitude 6 meters) and the same series of tests was repeated. The results from all tests passed all the specifications at all altitude conditions tested.

5. Testing in Direct Sunlight

The test samples were processed and the tests were allowed to incubate on a windowsill under direct sunlight (a calibrated light meter was used to record the amount of Lux in the testing area). One Reader was utilized in this study. Expected results were obtained for all samples tested; no erroneous results were obtained.

6. Testing under Low Lighting

The test samples were processed and the test devices were allowed to incubate under poor lighting conditions (a calibrated light meter was used to record the amount of Lux in the testing area). One Reader was utilized in this study. Expected results were obtained for all samples tested; no erroneous results were obtained.

7. Testing under “Draft” Conditions

The test samples were processed and the test devices were allowed to incubate in a chemical fume hood. The hood was turned on to create air flow. One Reader was utilized in this study. Expected results were obtained for all samples tested; no erroneous results were obtained.

8. Testing in an Upright Position

The test samples were processed on a bench top and the test cards were propped upright at 90° angle immediately after sample addition. The tests were allowed to incubate for 15 minutes in the upright position. One Reader was utilized in this study. Expected results were obtained for all samples tested; no erroneous results were obtained.

Operator Errors/Human Factors

1. Contamination of Test Pad during Handling

The study examined the effect of inadvertent touching of the sample pad by the user with a gloved hand (nitrile powder-free exam gloves), bare hand, hand treated with a hand cream, hand treated with a sanitizer gel and hand treated with baby powder immediately prior to testing. Three readers were utilized in this study. No interference was observed from the substances tested; expected results were obtained for all samples tested.

2. Dropped Test Cards

The study examined the effect of inadvertent dropping of the test cards, simulating dropping from a storage shelf prior to use. The test devices were removed from their foil pouch and dropped to the ground from the height of 6 feet. The test cards were evaluated for any visible damage. No visible damage was observed on any of the test devices and they were used to test the samples. One Reader was utilized in this study. Expected results were obtained for all samples tested.

3. Sample Preparation/Test Procedure

The study was designed to evaluate the effect of improper specimen preparation (not following the test procedure) which includes rotation of the swab in the Elution Solution three times while pushing against the bottom of the vial. The following deviations from the procedure were evaluated:

- a. Swab was dipped in and out of the elution solution; no rotating or expressing the swab on the side of the vial to remove liquid was performed.
- b. Swab was dipped in the elution solution, rotated once and WAS NOT expressed on the side of the vial to remove liquid.
- c. Swab was dipped in the elution solution, rotated three times and WAS NOT expressed on the side of the vial to remove liquid.

- d. Swab was dipped in the elution solution, rotated six times and expressed swab on the side of the vial to remove liquid.

Two Readers were utilized in this study. All samples generated expected results.

4. Sample Addition/ Test Procedure

The study evaluated the effect of errors that may occur during the addition of the sample to the test pad. Two Readers were utilized in the three studies below. The following describes the conditions evaluated and the observed results:

- a. Volume of the sample added

Varying volumes of sample were added to simulate user error during this step. Each of the following volumes was evaluated: 50 μL , 75 μL , 100 μL (the optimal volume specified in the test procedure), 150 μL , and 200 μL

Expected results were generated for all samples tested at volumes of 75 μL , 100 μL and 200 μL . A volume of 50 μL generated invalid results because not enough liquid was added to allow for sample flow. A volume of 150 μL generated two incorrect results (2/3 Flu B LoD samples gave false positive Flu A results).

The risk of an erroneous result is mitigated by including a fixed volume pipette in the test kit. Additionally, a caution is included in the test procedure emphasizing that the provided fixed volume pipette must be used to add the sample to the test pad. The test procedure includes explicit instructions on the use of the pipette.

- b. Speed of Adding the Sample

The study evaluated the effect of adding the sample to the test by rapid squeezing of the transfer pipette, rather than adding the sample in a dropwise fashion.

- 100 μL of sample was added drop wise (as specified in the procedure) to the sample pad,
- 100 μL of sample was added in one quick burst to the sample pad

The speed of the sample addition did not appear to affect the test results. Expected results were generated for all samples tested.

c. Sample placement on the test strip (using 100 μ L sample volume)

This study was designed to examine the effect of placing the sample outside of the designated area on the test strip. In this study, the specified volume of sample (100 μ L) was added to the following locations on the test strip:

- The top of the sample receiving pad (test procedure)
- The center of the sample receiving pad
- The bottom of the sample receiving pad, just above the conjugate pad
- The overlapped blank H&V bridge pad
- The conjugate pad
- The top of the absorbent pad

Expected results were obtained for all samples that were applied to the sample receiver pad, whether it was the top half, the center, or the bottom half of the pad. Erroneous results were observed when the sample was applied to the overlapped blank H&V bridge pad or to the conjugate pad area. All samples applied to the absorbent pad area gave invalid results. The data showed that the sample application position is critical in obtaining the correct results.

Mitigation measures have been implemented through the labeling, although this error is not entirely preventable. To minimize the risk of applying the sample incorrectly, the graphics on the test card include bold arrows directing the user to the area of the sample receiving pad. Additionally, the test procedure includes a note cautioning the user not to add sample to the pink/red colored pad (the conjugate pad).

9. Sample Elution/Test Procedure

This study evaluated the effect of incorrect Elution Solution volume due to either overfilled vials due to manufacturing errors or low volume due to inadvertent spillage by the user. The Elution Solution is provided in pre-filled vials of 350 μ L. The test performance was evaluated with the following Elution Solution volumes: 75 μ L, 125 μ L, 175 μ L, 225 μ L, 350 μ L, 425 μ L, and 500 μ L.

One Reader was utilized in this study. Expected results were obtained for all samples with Elution Solution volume ranging from 225 μ L to 500 μ L. When the Elution Solution volume was < 225 μ L, the results were invalid for all samples. No erroneous results were obtained.

10. Read Time

This study evaluated the effect of reading the test results outside of the specified 15 minutes. The following read times (incubation intervals) were evaluated: 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes, and 60 minutes. Two Readers were utilized in this study. Expected results were generated for all samples evaluated at 10, 15, 20, 30 and 45 minutes. Erroneous results were obtained at the

5 minute read time and at 60 minute read time. The results of this study demonstrate that the device has flexibility in the timing of the incubation of samples prior to reading. To minimize the potential for reading of the results outside of the specified 15 minute interval, the product labeling cautions the operator in bold not to read the test results before or after 15 minutes.

11. Using Cold Reagents

The objective of this study was to evaluate the assay performance when the kit components are stored refrigerated and are not sufficiently equilibrated to room temperature (20-25°C) before use. One test kit was evaluated after storing in a refrigerator (2°- 8°C) for at least 24 hours and then using the test components to test the samples immediately after removal from the refrigerator. One Reader was utilized in this study. Expected results were obtained for all samples tested; no erroneous results were obtained.

12. Using Expired Elution Solution

The performance of the assay was evaluated when testing was performed with expired elution solution and non-expired test cards. The elution solution and the test cards used in this study were manufactured approximately one year apart. One Reader was utilized in this study. Expected results were obtained for all samples tested; no erroneous results were obtained.

13. Reliability of the QC Material

Four different lots of Positive Control swabs and Negative Control swabs were used with five different lots of the reagents (Test Cards) between February and November 2016. A total of 498 Positive Control swabs and 495 Negative Control swabs were tested. There were 3 invalid results and 9 failed results (all passed upon repeat testing). The failure rate (including the failed and the invalid results) was 1.2% (12/993), with 95% CI: (0.7%-2.1%).

Specimen Integrity and Handling

1. Effect of Testing Temperature on Eluted Sample

This study evaluated the effect of temperature on the samples eluted in the Elution Solution and either leaving the samples at increased ambient temperature or placing them in a refrigerator prior to testing, without bringing them to room temperature (15-30°C), as directed in the test procedure. Two Readers were utilized in this study.

- a. The test samples were eluted in the Elution Solution and placed in an incubator set to 37°C for two hours prior to testing. The samples were then removed from the incubator and tested immediately, without bringing to room temperature. Expected results were obtained for all samples tested.

- b. The test samples were eluted in the Elution Solution and placed in a refrigerator (2-8°C) for two hours prior to testing. The samples were then removed from the refrigerator and tested immediately, without bringing to room temperature. Expected results were obtained for all samples tested.

2. Stability of Eluted Samples

In an event of workload or staffing limitations, specimens could possibly be eluted in the Elution Solution and placed in a refrigerator for testing at a later time. Specimen storage stability period of a sample eluted in the Elution Solution and not immediately tested, as instructed, was evaluated by testing the samples with the BinaxNOW Influenza A & B Card 2 assay after 1 hour, 17 hours, 25 hours and 37 hours after storage in a refrigerator (2-6°C). Expected results were obtained for all samples tested; no erroneous results were observed.

Hardware Robustness

1. Non-level Work Surface

The study evaluated the assay performance when the Reader was placed on a bench top and tilted to produce a 15° incline. This was evaluated in 4 positions, front, back and at each side. This study utilized one positive test card to be read on each of the three Readers used in this study for each position evaluated. Expected results were obtained for all test positions.

2. Mishandling of the Drawer

The study evaluated the robustness of the Reader drawer design. The Reader drawer was pulled out all the way and the Reader was picked up by the test drawer, lifting it completely off the work bench surface and across the bench by a foot or so, before placing it back down prior to each test being run. The testing was performed with three prefabricated test strips:

- Strip 1: low positive Flu A and Flu B lines with a developed Control line,
- Strip 2: negative Flu A and Flu B lines with a developed Control line
- Strip 3: high positive Flu A and Flu B lines with a developed Control line

Each strip was evaluated in replicates of three; one Reader was utilized. Expected results were obtained for each test.

3. Movement during the Test

This study evaluated the effect of inadvertent “bumping” of the Reader, while reading of the image is taking place. Each one of the three test samples was evaluated in 6 replicates; one Reader was utilized. As soon as the screen displayed the message “Reading Patient Test,” the Reader was bumped once, hard enough to make it move. Of the 18 samples evaluated, 6 produced invalid results, while the remaining 12 generated expected results.

4. Positioning of the Test Card in the Drawer

During the normal operation the user inserts the closed test card into the drawer with the barcode and result window facing up and closes the drawer. The barcode on the test card must be in a specific location in order to read the test correctly.

This study was designed to evaluate the effect of improper positioning of the test card within the Reader drawer that may be due to a user error or due to bent test card. The study was performed using the Flu A/B Quality Controls as positive samples and the Elution Solution was used as a negative sample. All samples were tested in 6 replicates for each condition; one Reader was utilized. The following placement conditions were evaluated:

- No manipulation
- Test card bent upward in the center lengthwise
- Test card bent upward in the center widthwise
- Test card bent downward in the center lengthwise
- Test card bent downward in the center widthwise
- Test card left open
- Test card placed upside down (Alere logo placed closest to the test operator)
- Test card placed with window facing down

Positive and negative results were read at 15 minutes. If the Reader was unable to read the card with the first test the remaining samples were not read (this occurred with open card, upside down card and window facing down card conditions). The obtained test results included 62 valid expected results, 15 invalid results and 13 barcode failures. One false positive result was obtained due to a bent card that caused a break in the cellulose pad on the Flu A line.

Based on assessment of the design features, robustness of the assay system and device labeling, all the hazards and sources of potentials errors have been mitigated to reasonably acceptable levels.

L. Demonstrating “Insignificant Risk of an Erroneous Result” - Accuracy

Clinical Performance

The sensitivity and specificity of the Alere BinaxNOW Influenza A & B Card 2 assay used with the Alere Reader when performed by untrained operators was evaluated in the CLIA waiver clinical study conducted during the 2015-2016 flu season testing patients of all ages who presented with flu-like symptoms. The patients were prospectively enrolled in the study at clinical sites located across the United States. Two nasal swabs or two nasopharyngeal swabs were collected from each subject; both swabs were collected from the same nostril. One swab specimen was eluted with the elution solution and tested (either immediately or within two hours) with the Alere BinaxNOW Influenza Card 2 assay. The other swab specimen was eluted in a viral transport medium (VTM) and sent to a clinical laboratory for testing with a comparator method, an FDA cleared PCR assay.

a. Testing Sites and Operators

The 12 clinical sites participating in the study represented CLIA waived testing locations and included Emergency Rooms, physician's offices, and outpatient clinics. A total of 36 operators performed the testing with the Alere BinaxNOW Influenza Card 2 assay with Reader.

The operators were healthcare professionals with no formal training or experience in laboratory testing and included nurses, phlebotomists, medical assistants, study coordinators, and clerical office staff. Information on the operators' current job title, education, laboratory experience and the number of years of relevant work experience was provided. The education of the operators ranged from high school graduates to postgraduate degrees. As additional information, the sponsor provided similar information for other individuals employed at the testing sites that were not selected to participate in the study.

b. Study Samples

Test samples were collected from 642 subjects enrolled in the clinical study. There were 33 swab samples excluded due to patient eligibility or sample handling issues, leaving a total of 609 prospectively collected samples to be included in the evaluation of the assay performance (please see section below for the details concerning the excluded samples).

Due to a low prevalence of influenza observed during the study, the study samples were supplemented with 105 swab samples prepared from archived respiratory specimens that were obtained from patients with influenza-like symptoms and were confirmed positive or negative by an FDA cleared molecular assay for influenza A and influenza B. These samples (20 swabs positive for influenza A, 65 swabs positive for influenza B and 20 swabs negative for both influenza A and influenza B) were distributed (blinded and randomized) among 3 of the testing sites and the testing was incorporated into the daily workflow at each site.

In total, the Alere BinaxNOW Influenza A & B Card 2 with Reader assay was evaluated with 714 swab specimens and the results were compared to an FDA cleared molecular-based comparator method. There were 21 invalid results obtained with the Alere BinaxNOW Influenza A & B Card 2 with Reader, resulting in a total of 693 samples with valid results. The invalid rate observed during the study was 2.9% (21/714), 95% CI: (1.9% - 4.5%) and included 19 dual positive results (positive for both influenza A and influenza B).

c. Assay Performance

The performance of the Alere BinaxNOW Influenza A & B Card 2 assay with Reader when used by operators representative of those in CLIA waived settings was evaluated against an FDA cleared molecular comparator method. Results obtained from the 693 specimens (588 prospectively collected and 105 contrived) were used in data analysis. The performance of the assay, when in the hands of untrained

operators, is presented below as positive percent agreement (PPA) and negative percent agreement (NPA) with the comparator method.

**Performance of Alere BinaxNOW Influenza A & B Card 2 Assay with Clinical Specimens: Percent Agreement with a Molecular Comparator Method
(in the hands of untrained users)**

		Influenza A		
Alere BinaxNOW [®] Influenza A & B Card		Comparator Method		
		Positive	Negative	Total
Positive		141	23	164
Negative		21	508	529
Total		162	531	693
PPA: 87.0% (141/162), 95%CI: (81.0% - 91.4%)				
NPA: 95.7% (508/531), 95%CI: (93.6% - 97.1%)				
		Influenza B		
Alere BinaxNOW [®] Influenza A & B Card		Comparator Method		
		Positive	Negative	Total
Positive		116	3	119
Negative		6	568	574
Total		122	571	693
PPA: 95.1% (116/122), 95%CI: (89.7% - 97.7%)				
NPA: 99.5% (568/571), 95%CI: (98.5% - 99.8%)				

The 33 samples excluded from the calculations of performance (as mentioned above) included 17 samples where the testing deviated from the QRI. Specifically, 4 samples negative by the comparator were tested with the BinaxNOW Card 2 assay later than 2 hours after collection (a deviation from the QRI); all these samples also tested negative with the BinaxNOW Card 2 assay. Additionally, there were 13 samples that were tested later than 30 minutes after sample addition to the test card (a deviation from the QRI):

- a) Of the 13 samples, 5 tested negative with the comparator method and 4 tested negative with the BinaxNOW Card 2 assay, while one sample generated an invalid result.
- b) Of the 13 samples, 6 tested positive for influenza A with the comparator method and 4 tested positive for influenza A with the BinaxNOW Card 2 assay, while 2 samples tested negative with the BinaxNOW Card 2 assay.
- c) Of the 13 samples, 2 samples tested positive for influenza B with the comparator method and both also tested positive for influenza B with the BinaxNOW Card 2 assay.

After inclusion of these samples in the data analyses, performance estimates of the assay were similar to the estimates shown in the table above.

This information is presented to reflect the potential performance of the BinaxNOW Card 2 assay when used in clinical practice by untrained operators in CLIA waived environments.

Performance with Analyte Concentrations near the Assay Cutoff:

A study designed to evaluate the ability of the intended untrained users to perform the testing and obtain accurate results with samples at virus concentrations near the assay cutoff was conducted. The test samples were contrived by spiking inactivated strains of influenza A and influenza B into VTM to create low positive samples (at the limit of detection, C₉₅) and high negative samples (below the limit of detection, C₅). A negative sample consisting of unspiked VTM was also included in the test panel. The samples were blinded and distributed in randomized panels among three CLIA waived clinical testing sites (an urgent care clinic and two physician offices). A total of six operators, across the three sites, performed testing with the Alere BinaxNOW Influenza A & B Card 2 assay with Reader. All operators were untrained in laboratory procedures and qualified as intended operators encountered at CLIA waived sites. On the day of testing, each test operator tested blinded swab samples at random from their designated sample swab panel over a period of 16 days. The number of sample swabs tested each day was at the discretion of the test operator allowing the testing to be integrated into their normal work day. This practice simulated how testing may actually happen in the intended use environment. The results from this study are shown below.

Performance of Alere BinaxNOW Influenza A & B Card 2 Assay with Samples near the Assay Cutoff: Percent Agreement with Expected Results

Sample Type	Site 1	Site 2	Site 3	Overall	95% CI
Flu A Low Positive (C ₉₅)	95.0% (19/20)	100% (20/20)	95.0% (19/20)	96.7% (58/60)	88.6%, 99.1%
Flu B Low Positive (C ₉₅)	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	94.0%, 100%
Flu A High Negative (C ₅)	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	94.0%, 100%
Flu B High Negative (C ₅)	100% (20/20)	95.0% (19/20)	95.0% (19/20)	96.7% (58/60)	88.6%, 99.1%
True Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	94.0%, 100%

There were no significant differences in the observed reactivity of the device with weakly reactive samples between sites or between operators. All negative samples yielded negative results. The study results demonstrated that untrained users were able to perform the test correctly and the test provided the expected results for samples with virus concentrations near the assay cutoff.

Quick Reference Instructions (QRI)

The QRI (also referred to as “Procedure Card”) for the use of the Alere BinaxNOW Influenza A & B Card 2 Assay with the Alere Reader is written in simple language (at 7th grade reading level) and contains pictorial descriptions of the individual steps. In addition to the test procedure for patient samples, the QRI includes a section on performing QC testing with external controls. A section on specimen collection and handling is also included in the QRI.

Operator Questionnaire Results:

Upon completion of the prospective clinical study and the contrived sample testing study, the operators at each site were asked to complete a questionnaire to help assess whether the participants understood how to use the Alere BinaxNOW Influenza A&B Card 2 assay with Alere Reader correctly. The questionnaire consisted of a series of questions pertaining to the ease of use of the test with answers rated on a scale from 1-5. Forty five operators completed the questionnaire (the test operators who did not return questionnaires are no longer employed at the clinical site). The participants found the test to be easy to use and the instructions easy to understand.

M. Labeling for Waived Devices:

The labeling consists of:

- a. Package insert,
- b. Quick Reference Instructions (QRI),
- c. Alere Reader Quick Start Guide, and
- d. Alere Reader User Manual

The following elements are appropriately present:

- The Quick Reference Instructions and the Alere Reader Quick Start Guide are written at no higher than a 7th grade reading level and, where appropriate, contain graphic representation of system components and procedure steps.
- The package insert and the QRI identify the test as CLIA waived, and contain a statement that a Certificate of Waiver is required to perform the test in a waived setting; information on how users can obtain a certificate is also provided.
- The package insert and the QRI contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. 42 CFR 493.15(e)(1).
- Instructions for quality control (QC) are integrated with procedural instructions for performing the test in both the package insert and the QRI.
- Appropriate cautions have been added to the Package Insert and Quick Reference Instructions to ensure safe use of the product.
- The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

N. Conclusion:

Alere conducted an appropriate clinical study evaluating the clinical performance of the assay when used by untrained operators in CLIA waived healthcare settings. The test results were compared to an acceptable comparator method, FDA-cleared molecular influenza A and B Assay. The sponsor also conducted appropriate flex studies to demonstrate that the test system is robust, including design and labeling mitigations to minimize erroneous results.

FDA has evaluated the benefits and risks of using the Alere BinaxNOW Influenza A&B Card 2 assay with Alere Reader in CLIA waived settings and concluded that the medical benefit/risk profile favors the decision to grant CLIA waiver for this test. As a general consideration, the benefits of availability of rapid antigen-based influenza detection tests include the following:

- Simplicity, allowing healthcare professionals not skilled in laboratory testing to perform the test with ease;
- Short time to results, leading to early diagnosis and treatment; and
- Widespread use of the tests allowing for prompt detection of outbreaks and better infection control.

The specific benefits of the Alere BinaxNOW Influenza A&B Card 2 assay with Alere Reader in CLIA waived settings include the following:

- The test system includes a digital reader for result interpretation eliminating the subjectivity associated with the visual interpretation of results inherent in older rapid influenza detection tests;
- Based on the literature review, the demonstrated sensitivity of the Alere BinaxNOW Influenza A&B Card 2 with Reader for influenza B is at least as good as the sensitivity of other recently waived flu tests of this type (for tests CLIA waived since 2011, the demonstrated sensitivity for influenza B ranges from 74.3% to 89.7%).
- The intended use of the test clearly states that negative test results should be confirmed by culture or an FDA-cleared molecular method, mitigating the risk of false negative results.
- The product labeling incorporates bold cautions in the written test procedure to safeguard against procedural errors.
- The test includes a built-in procedural control to further safeguard against procedural errors or reagent malfunction.

In summary, the Alere BinaxNOW Influenza A&B Card 2 assay with Alere Reader presents low risk of erroneous results and is suitable for use in CLIA waived environments.

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