CLIA Waiver by Application Approval

Determination Decision Summary

A. Document Number

CW180008

B. Parent Document Number

K182001

C. Purpose of the Submission

This submission was a Dual 510(k) and CLIA Waiver by Application (Dual Submission) tracked as K182001 and CW180008. CW180008 was submitted to request CLIA waiver for the Acucy Influenza A&B Test performed on the Acucy Reader.

D. Measurand (analyte)

Influenza A and influenza B viral nucleoprotein antigens

E. Sample Type

Direct nasal and nasopharyngeal swab specimens

F. Type of Test

Qualitative Immunoassay with an optoelectronic reader for result interpretation and reporting

G. Applicant

Sekisui Diagnostics, LLC

H. Proprietary and Established Names

Acucy Influenza A&B Test
Acucy Reader

I. Test System Description

1. Overview

The Acucy Influenza A&B Test is a lateral flow immunochromatographic assay in the sandwich immunoassay format. The Acucy Influenza A&B Test consists of a Test

Cassette that detects and differentiates influenza A and influenza B viral antigens from a patient sample. The test sample, a nasal swab or nasopharyngeal swab, is processed to extract nucleoproteins by mixing the swab in Acucy Influenza A&B Extraction Buffer. The mixture is then added to the sample well of the Test Cassette. From there, the sample migrates along the membrane surface. If influenza A or B viral antigens are present, they form a complex with mouse monoclonal antibodies to influenza A and/or B nucleoproteins conjugated to colloidal gold. The complex is then bound by a rat anti-influenza A and/or mouse anti-influenza B antibody coated on the nitrocellulose membrane.

The Acucy Reader is an optoelectronic instrument that uses a reflectance-based measurement method to evaluate the line signal intensities in the results window of the Test Cassette. The Acucy Reader scans the Test Cassette and measures the absorbance intensity by processing the results using method-specific algorithms. The Acucy Reader displays the test results POS (+), NEG (-), or INVALID on the screen. The results can also be automatically printed on the Acucy Printer if this option is selected.

2. Acucy Influenza A&B Test for Use on the Acucy Reader Components

Materials Provided

The Acucy Influenza A&B Test kit contains all the materials needed to run a test, except for the Acucy Reader, which is provided separately. The Acucy Influenza Test Kit contains the following:

- Sterile nasal swabs for specimen collection (25)
- Acucy Influenza A&B Test Cassettes (25)
- Acucy Influenza A&B Extraction Buffer Vials (0.4 mL phosphate buffered salt solution with 0.09% sodium azide as a preservative) (25)
- Extraction Buffer Vial Dropper Tips (25)
- External Quality Control: Influenza A+/B- Positive Control Swab (Formalin inactivated Influenza A containing 0.05% sodium azide. Inactivity confirmed by inability of virus to infect cell culture) (1)
- External Quality Control: Influenza A-/B+ Positive Control Swab (Formalin inactivated Influenza B containing 0.05% sodium azide. Inactivity confirmed by inability of virus to infect cell culture) (1)
- Instructions for Use (IFU) (1)
- Quick Reference Guide (READ NOW and WALK AWAY/NORMAL Modes) (1)
- External Quality Control (QC) Quick Reference Guide (1)
- Workstation (1)

Instrument System

Separately from the Acucy Influenza A&B Test kit, the Acucy Reader is provided with

the following accessories: an Acucy Printer, power cords and adapters, paper roll, Acucy Calibration Device (CAL-Device) with case, USB Memory Drive, and System Manual, as the Acucy System.

Materials Required but Not Provided

- Timer or Watch
- If needed, sterile nasopharyngeal swabs (Copan Catalog # 534CS01)
- If needed, additional external quality controls may be purchased separately (Acucy Influenza A&B Control Kit # 1011).

3. Quality Control

There are three types of Quality Control for the Acucy System and Acucy Influenza A&B Test: Acucy Reader Calibration, Test Cassette Built-In Internal Control, and External Quality Control.

Acucy Reader Calibration

The Acucy Reader calibration is a required function that checks the Acucy Reader optics and calculation systems using a specific CAL-Device. The CAL-Device is supplied in a calibration case with the Acucy System accessories. The Calibration Procedure is performed upon installation to activate the QC TEST and RUN TEST functionality and is required every 30-days. The operator is prompted by the Acucy Reader to conduct calibration with the CAL-Device after the 30-days has elapsed. The Calibration Procedure may also be performed, as directed during troubleshooting or whenever the Acucy Reader date & time has been changed.

Test Cassette Built-In Internal Control

The Acucy Influenza A&B Test Cassette contains a built-in internal control feature. Each time a test is run in the Acucy Reader, the internal control zone is scanned by the reader. A “VALID” test result displayed by the reader indicates that the internal control was present, demonstrates that the test flowed correctly, and that the functional integrity of the Test Cassette and reagents was maintained. An “INVALID” test result displayed by the reader indicates that the internal control was not present, demonstrates that the test did not flow correctly, and that the functional integrity of the Test Cassette and reagents were not maintained. Should this occur, the end user is instructed to review the testing procedure and repeat the test using a new patient sample, Test Cassette and reagents.

External Quality Control (QC)

The Acucy Influenza A&B Test includes one Influenza A+/B- Control Swab (Red Label) and one Influenza A-/B+ Control Swab (Blue Label), each of which contains inactivated virus, for external quality control testing. The Influenza A+/B- Control Swab

acts as an external positive control for influenza A antigen and an external negative control for influenza B antigen, and conversely, Influenza A-/B+ Control Swab serves as an external positive control for influenza B antigen and an external negative control for influenza A antigen.

The External Quality Controls are used to ensure that the assay-specific reagents, Test Cassettes, and Acucy Reader are functioning properly, and to demonstrate proper performance by the operator. External Quality Control requirements should be established in accordance with local, state, and federal regulations or accreditations requirements. Minimally, Sekisui Diagnostics recommends that External Quality Controls be run with each new lot, shipment received and with each new untrained operator.

4. Acucy Reader Modes

The Acucy Reader has two modes of operation, the WALK AWAY/NORMAL and the READ NOW modes. The reader may be set to either one of the two different modes.

WALK AWAY/NORMAL Mode

In the WALK AWAY/NORMAL mode, Test Cassettes are inserted into the reader. Then, after the addition of the sample to the Test Cassette, the development and timing of the test occur within the reader. This allows the user to "walk away" until the test result has been completed.

READ NOW Mode

In the READ NOW Mode, the reader analyzes the results after manually timing the development of the Test Cassette on a flat surface for the full 15 minutes. This mode is helpful if the user is running multiple samples in a batch format.

Running the tests in the wrong modes will produce invalid results. If a user runs a test in the WALK AWAY/NORMAL mode, however, the reader is set incorrectly in the READ NOW mode, the reader screen will display error message "INVALID2." If a user runs a test in the READ NOW mode, however, the reader is set incorrectly in the WALK AWAY/NORMAL mode, the reader screen will display error message "INVALID1."

5. Workflow

To perform the Acucy Influenza A&B Test, an operator first either collects a nasal swab (NS) using a nylon sterile flocked nasal swab provided in the test kit or collects a nasopharyngeal swab (NPS) using a nylon sterile flocked nasopharyngeal swab (Copan Catalog # 534CS01). The operator removes the cap off an Extraction Buffer vial and inserts the NS or NPS sample into the Extraction Buffer vial while pressing down on the swab, vigorously mix against the side of the vial 10 times while submerged. The

operator then removes the swab while squeezing the middle of the vial to remove the liquid from the swab, properly discard the swab, adds dropper tip to the Extraction Buffer vial, presses tightly to seal the vial, and labeled the vial with patient identification.

After the swab sample elution/extraction step, the procedure for testing depends on the reader workflow configuration chosen by the operator.

WALK AWAY/NORMAL Mode

In the WALK AWAY/NORMAL mode, the operator first enters the patient identification into the reader by scanning the patient's ID barcode using the reader scanner or by manually entering the patient ID using the keypad on the reader screen. The operator then opens the Test Cassette foil pouch, labels the Test Cassette with the patient ID, opens the reader drawer, and inserts the Test Cassette. The reader automatically scans the Test Cassette.
While the Test Cassette is in the reader drawer, the operator gently mixes the Extraction Buffer vial to agitate sample, and then inverts the Extraction Buffer vial vertically above the Test Cassette to gently squeeze 5 drops of extracted sample into the sample well of the Test Cassette. The operator closes the drawer within 10 seconds to start the test. The Reader automatically times the 15-minute development. After the 15 minutes, the reader automatically displays the test results. The operator subsequently opens the reader drawer, removes the Test Cassette, and disposes it in a proper biohazard container.

READ NOW Mode

In the READ NOW Mode, the operator first opens the Test Cassette foil pouch and labels the Test Cassette with the patient ID. While the Test Cassette is on a flat surface, the operator inverts the Extraction Buffer vial vertically above the Test Cassette and gently squeeze 5 drops of extracted sample into the sample well of the Test Cassette. The operator then starts an external timer for 15 minutes. While the Test Cassette is developing, the operator enters the patient identification into the reader by scanning the patient's ID barcode using the reader scanner or by manually entering the patient ID using the keypad on the reader screen. Once the 15-minute is complete, the operator opens the reader drawer, and inserts the Test Cassette. The reader automatically scans the Test Cassette. The operator then immediately closes the drawer to start the test. The reader automatically displays the test results. The operator subsequently opens the reader drawer, removes the Test Cassette, and disposes it in a proper biohazard container.

6. Results Interpretation

The results are interpreted by the Acucy Reader software from measured absorbance intensity by processing the results using method-specific algorithms. The Acucy Reader displays the test results POS (+), NEG (-), or INVALID on the screen, for FLU A&B separately. There are five possible results: (1) FLU A POS (+)/FLU B NEG (-); (2) FLU A NEG (-)/FLU B POS (+); (3) FLU A NEG (-)/FLU B NEG (-); (4) FLU A POS

(+)/FLU B POS (+); and (5) INVALID. The results can also be automatically printed on the Acucy Printer if this option is selected.

Any INVALID result should be re-tested with a new patient sample, reagents, and Test Cassette. A FLU A or FLU B positive result, or a FLU A&B dual positive result, does not rule out co-infection with other pathogens or identify any specific Influenza A or B virus subtypes. Co-infection with Influenza A and B is rare. It is recommended that a FLU A&B dual positive sample (Influenza A and Influenza B positive) should be re-tested. Repeatable Influenza A and B dual positive results should be confirmed by cell culture or PCR testing before reporting results. A FLU A&B negative result does not exclude influenza viral infection. Negative results should be confirmed by viral culture or an FDA-cleared Influenza A and Influenza B molecular assay.

J. Demonstrating "Simple"

The Acucy Influenza A&B Test with the Acucy 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 Extraction Buffer is premeasured and provided in individual vials.
- Dropper tips are provided to be put on the Extraction Buffer vials contain the eluted/extracted samples. User inverts the vial and applies 5 drops of sample directly to the Sample Well of the Test Cassette using the dropper tip. No pipetting is required.
- The Test Cassettes are unitized and contain all the reagents required for analysis.
- The reagents are stable and can be stored at room temperatures (15 - 30°C).
- The test does not require any operator intervention during the analysis step. In the WALK AWAY/NORMAL mode, once the Test Cassette is inserted into the Acucy Reader, the extracted sample is added to the Test Cassette, and the reader drawer is closed, all subsequent steps are completed automatically by the Reader.
- The results are interpreted by the Acucy 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 prompts and messages in simple English phrases.
- Calibration check, which is required every 30 days, is easily performed with a provided barcoded Acucy Calibration Device (CAL-Device).
- Error messages are unambiguous and include easy-to-interpret solutions.
- No complex troubleshooting or interpretation of error codes are required to operate the Acucy Reader.
- There is no maintenance required other than wiping of the external surface of the Reader.

- There are no user-serviceable parts and the instrument is to be returned to Sekisui Diagnostics for repair.
- The test procedure is written at a 7th grade comprehension level. An Acucy printer is provided as an accessory to the Acucy Reader.

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

1. Risk Analysis

A comprehensive risk analysis of the Acucy Influenza A&B Test with the Acucy 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 FMEAs (Acucy Influenza A&B Design FMEA, Acucy Influenza A&B Process FMEA, and Acucy Influenza A&B User FMEA) include 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 Acucy Reader. The instrument software was reviewed under the parent 510(k) submission (K182001).

2. Fail-safe and Failure Alert Mechanisms

The Acucy Influenza A&B Test with the Acucy Reader was designed to include numerous features and fail-safe mechanisms built into the system to prevent erroneous results.

Design Features

- Each Test Cassette is packaged in a foil pouch with desiccant to maintain the integrity of the reagents.

- Each Test Cassette contains a barcode on the outer cassette housing with critical information, such as the assay type, the lot number and the expiration date. It confirms the identity of the Test Cassette once inserted into the Reader.

- The Reader is designed in a way that it only accepts Test Cassette that are properly inserted/oriented. The Test Cassette design is "keyed" ensuring that the cassette is placed in the Reader in the correct orientation. The Test Cassette clearly displays a directional arrow that visually guide the user to place the Test Cassette in the Reader drawer correctly.

- The Test Cassette clearly displays the text of "5 drops" along with a pictorial illustration of five drops of sample to mark the location of the Sample Well on the Test Cassette, and to visually remind and guide the user to add the correct amount of extracted sample into the Sample Well.

- 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.

Fail-safe Features

- Internal Control: The Acucy Influenza A&B Test Cassette contains a built-in internal control feature. Each time a test is run, the internal control zone is scanned by the Acucy Reader. A valid test result displayed by the Reader indicates that the internal control was present, demonstrates that the test flowed correctly, and that the functional integrity of the cassette and reagents was maintained. The internal control is interpreted by the Acucy Reader after the device has developed for 15 minutes. If the test does not flow correctly, the Acucy Reader will indicate that the result is INVALID, and a patient result will not be reported.

- External Controls: Two external control swabs are provided with the Acucy Influenza A&B Test. The Influenza A Positive Control Swab (red label, formalin inactivated Influenza A containing 0.05% sodium azide) also serves as a negative control for influenza B, and the Influenza B Control Swab (blue label, formalin inactivated Influenza B containing 0.05% sodium azide) also serves as a negative control for influenza A. Use of the controls helps to ensure that the reagents, Test Cassette, and Reader are functioning properly, and can demonstrate proper performance by the operator. Each Control Swab is extracted, and the sample is then applied to a Test Cassette, using the same sample extraction and application process used for patient samples. The Influenza A Positive Control Swab is run first and then the Influenza B Positive Control Swab is run next. Each control swab is individually packaged in a foil pouch with a barcode printed on the outer foil package with information such as control swab type and the expiration date. The Reader prompts the user to scan the barcode of each respective control swab and testing will not proceed if the control swabs are tested out of order. Testing will not proceed if an expired control swab is used. These controls are used to verify reagent integrity when a new lot or new shipment of Acucy Influenza A&B kits is received, or to

demonstrate proper performance for each new operator. Controls may also be used to conform with local, state and/or federal regulations, accrediting groups, or the lab's standard quality control procedures.

- Acucy Reader Calibration: A Calibration Device (CAL-Device) is supplied with the Acucy System which is used on a 30-day cycle to check Reader optics, motor, and calculation systems. Calibration is required under the following conditions: when installing a new Acucy Reader; every 30 days; as directed during troubleshooting; and whenever the Reader Date and Time has been changed. The Reader screen has a 30-Day calibration countdown, which reminds the user every day, how many days are left until calibration is due.

- User ID and password required to ensure use by authorized users. Separate authority level for supervisors, who can change Reader settings.

- The Acucy Reader has the potential capability to output data automatically to a computer and/or a central laboratory information system (LIS) from the Mini USB (USB 2.0) cable connection on the rear of the instrument. However, currently this capability is limited to manual data transmission to the Exclusive USB Memory Drive only. USB communication cable transmission is not currently available to the end users, as the Mini USB cable connection is currently covered and will not be used by customers, and the middleware has not been designed for the Acucy Reader to transfer the test results to a LIS.

Lockout Features and Alert Messages

The Acucy 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. After 30 days, the Reader will prevent an External Control and a patient sample test to be run until the Reader is successfully calibrated.

- Calibration will not proceed if an expired calibration device is used.

- If calibration fails two times, the Reader will lock, and the screen will display a message to call customer support.

- Each Test Cassette contains a barcode on the outer cassette housing with critical information (test type, lot number, and expiration date) that is automatically scanned when inserted correctly into the opened drawer of the Reader. The reader will not proceed if inserted cassette does not match the user selected test on the Reader screen.

- The Reader must scan a valid barcode on the Test Cassette, otherwise an error

message will alert the user that a Test Cassette cannot be detected; the testing will not proceed.

- RUN TEST (in both the WALK AWAY/NORMAL and the READ NOW modes) will not proceed when the Test Cassette is expired; an error message is generated.

- RUN TEST (in both the WALK AWAY/NORMAL and the READ NOW modes) will not proceed if a Calibration Device is inserted instead of a Test Cassette.

- RUN TEST (in both the WALK AWAY/NORMAL and the READ NOW modes) will not proceed if the drawer of the Reader is not closed completely.

- If the drawer of the Reader is opened before the measurement is completed, an error message will be displayed and the test result will be rendered INVALID.

- If a user runs a test in the WALK AWAY/NORMAL mode, however, the Reader is set incorrectly in the READ NOW mode, after sample is added to the Test Cassette and the drawer is immediately closed, the Reader scans the Test Cassette and the test will not continue if the Reader does not detect a colored control line. The Reader screen will display the error message of "INVALID2."

- If a user runs a test in the READ NOW mode, however, the Reader is set incorrectly in the WALK AWAY/NORMAL mode, After the developed Test Cassette is inserted into the drawer and the drawer is closed, the Reader scans the test strip and the test will not continue if the Reader detects an intensity in the colored control line above the threshold. The Reader screen will display the error message of "INVALID1".

- An auto-logout function is in place after the Reader has been idle for more than 60 minutes. The Reader will go into Standby mode if it is not used within 90 minutes.

The functionality of the fail-safe and/or lock-out mechanisms built into the software of the Acucy Reader was demonstrated in studies conducted using the Acucy Influenza A&B Test Cassettes. 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 (Description if Applicable)
Calibration	(1) After selecting CALIBRATION from the MAIN MENU, waited for 30 minutes without scanning the CAL-Device barcode.	*DEVICE CAN NOT BE DETECTED*
	(2) Scan the barcode of an expired CAL-Device.	*CAL-DEVICE EXPIRED*
	(3) Scan the barcode other than the CAL-Device barcode.	*WRONG DEVICE DETECTED*
	(4) Open the drawer during the calibration measurement.	*FAILED RETRY CALIBRATION DRAWER OPENED*
	(5) Using a marker, color over Line 1 or Line 2 of the CAL-Device. Use this CAL-Device to perform calibration.	*FAILED RETRY CALIBRATION*
	(6) Using the same CAL-Device as in (5) and perform calibration.	*FAILED CALL CUSTOMER SUPPORT*
QC Test	(1) After selecting QC TEST from the MAIN MENU, waited for 30 minutes without scanning the FLU A QC barcode.	*CONTROL CAN NOT BE DETECTED.*
	(2) Scan an expired FLU A QC barcode.	*CONTROL EXPIRED*
	(3) Scan the barcode other than the FLU A QC barcode.	*WRONG CONTROL DETECTED*
	(4) Scan the barcode of an expired TEST DEVICE.	*CASSETTE EXPIRED*
	(5) After closing the drawer to begin the FLU A QC Test, open the drawer before 15 minutes has passed.	*INVALID DRAWER OPENED*
	(5) Perform the steps to run the QC test properly, however, use a test device that has not had a sample added to it.	*FAILED RETRY QC TEST*

Function	User Action	Expected Error Message on the Reader Screen (Description if Applicable)
RUN Test (READ NOW Mode)	(1) Scan a barcode other than the appropriate test device barcode.	*WRONG CASSETTE DETECTED*
	2) After closing the drawer to begin the RUN TEST, open the drawer before the measurement is complete.	*INVALID DRAWER OPENED*
	(3) On the Patient ID barcode scanning screen, wait for 10 seconds without scanning a barcode.	*ID CAN NOT BE READ*
	(4) On the Test Device barcode scanning screen, wait for 30 minutes without scanning the barcode.	*CASSETTE CAN NOT BE DETECTED*
	(5) Scan the barcode of an expired TEST Device.	*CASSETTE EXPIRED*
	(6) Measure a Test Cassette that has not had any sample added to it, but water instead.	*INVALID2* (the control line was not detected)
	(7) Using a marker pen, draw a small dot on the membrane near the control line location.	*INVALID3* (multiple control lines are detected)
	(8) After adding the sample to the cassette sample well, insert the cassette into the reader before the 15 minutes has elapsed, when the control line has still not fully developed.	*INVALID4* (the development of the control line is weaker than the threshold value)
	(9) Draw two small dots near the Flu A or Flu B line on the membrane, as well as drawing a small dot near the control line.	*INVALID5* (within the scope of the Flu A or Flu B test line, the instrument recognizes more than one line)
RUN Test (WALK AWAY Mode)	(1) Scan a barcode other than the appropriate test device barcode.	*WRONG CASSETTE DETECTED*
	2) After closing the drawer to begin the RUN TEST, open the drawer before the measurement is complete.	*INVALID DRAWER OPENED*

Function	User Action	Expected Error Message on the Reader Screen (Description if Applicable)
	(3) On the Patient ID barcode scanning screen, wait for 10 seconds without scanning a barcode.	*ID CAN NOT BE READ*
	(4) On the Test Device barcode scanning screen, wait for 30 minutes without scanning the barcode.	*CASSETTE CAN NOT BE DETECTED*
	(5) Scan the barcode of an expired TEST Device.	*CASSETTE EXPIRED*
	(6) Measure a used Test Cassette with a control line above threshold.	*INVALID1* (a previously used Test Cassette is used for a test or the operator waits too long to insert the test device into the Reader after applying the sample)
	(7) Using a marker pen, mark the cassette near the Flu A or B test line and run the test without adding a sample to the test device.	*INVALID2* (the control line is not detected)
	(8) Using a marker pen, draw a small dot on the membrane near the control line location, cover the cassette with a piece of paper in the drawer until 2 minutes have elapsed, then remove the paper	*INVALID3* (multiple control lines are detected)
	(9) Using a marker pen, mark the cassette near the Flu A or B test line and run the test without adding a sample to the test device.	*INVALID4* (the development of the control line is weaker than the threshold value)
	(10) Draw a dot near the Flu A line on the membrane, then process a Flu A control swab and run the test normally.	*INVALID5* (within the scope of the Flu A or Flu B test line, the instrument recognizes more than one line)

Function	User Action	Expected Error Message on the Reader Screen (Description if Applicable)
	(11) Measure a Test Cassette that has not had any sample added to it.	*INVALID6* (development of the test device progresses slower than expected)
	(12) Using a marker pen, mark the cassette near the Flu A or B test line and run the test without adding a sample to the test device.	*INVALID7* (while using the WALK AWAY mode with Monitoring ON, unstable development of the test lines is detected)
Integrity of Reader settings after Power Failure	The power to the Acucy 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, and test parameters, etc.) made prior to the power failure were maintained.
Data Integrity after Power Failure	The Acucy Reader was powered off and then back on.	Patient test results from completed runs prior to the power failure were retained.

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 Acucy Reader software was conducted under K182001.

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 influenza A and B strains (A/Hong Kong/4801/14 or A/California/07/09, and B/Phuket/3073/13), diluted to concentrations approximately 2x LoD in Viral Transport Media (VTM). Simulated nasal swab samples were prepared with 50 µL of the diluted virus solutions for positive samples, and with 50 µL of un-spiked VTM for negative samples. The three samples prepared, one low positive influenza A, one low positive influenza B, and one negative, were each tested in three or five replicates per operator for each condition being evaluated. The samples were blinded and randomized. For all conditions evaluated, one set of Test Cassettes 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 test was evaluated:

Operational Environment

1. Temperature and Relative Humidity

1) Flex studies were conducted using an incubation chamber to assess the effect of various environmental conditions. The following simulated environmental conditions were evaluated: (a) Acucy Reader at ambient temperature (15 - 30°C) and Acucy test reagents at 40°C and 70% relative humidity (RH); (b) Acucy Reader and Acucy test reagents all at 40°C and 70% RH; (c) Acucy Reader at ambient temperature (15 - 30°C) and Acucy test reagents at 4°C; (d) Acucy Reader and Acucy test reagents all at 4°C. Test reagents, including un-pouched Acucy Influenza A&B Test Cassettes, Acucy Influenza A&B Extraction Buffer, and Acucy Readers were equilibrated to the specified stress conditions for at least 30 minutes (but no more than 45 minutes) prior to testing.

The results showed that exposure of Acucy test reagents, including un-pouched Test Cassette and Extraction Buffer, and Acucy Readers to 40°C and 70% RH for 30 minutes, or to low temperatures, such as 4°C, for 30 minutes, had no effect on the test performance.

An additional flex study was conducted to further evaluate the effects of temperature and humidity outside of the expected normal conditions of use for the Acucy Reader. Four extreme stress conditions were tested in a controllable temperature/humidity chamber in this study: Acucy Reader operational condition at 4°C with 10% relative humidity (RH); Acucy Reader operational condition at 40°C with 70% RH; Acucy Reader operational condition at 40°C with 95% RH; and Acucy Reader operational condition at 50°C with 95% RH. The control condition was the Reader run at room temperature (15 - 30°C) with ambient RH. Each condition was tested in replicates of 5 on 5 different Readers by one operator. All samples were analyzed using the Walk AWAY/NORMAL mode. All testing produced expected results in this study, except that one false positive Flu B result was observed at 50°C with 95% RH.

The risk of erroneous results under the circumstances of exposing the Reader and the test reagents to extremes of temperature and relative humidity is minimized by clearly indicated operational environmental conditions in terms of temperature and relative humidity (i.e., 15 - 30°C and < 70% RH) in the labeling, including the test Quick Reference Guide (QRG).

2) The effect of increased temperature on Acucy test reagents, including pouched Test Cassettes and Extraction Buffer, was evaluated as a part of the accelerated test kit stability study by storage of three lots of the test kit at 37°C for 50 days, 99 days, 124 days, 132 days, 198 days, and 206 days, at 45°C for 22 days, 44 days, 55 days, 58 days, 88 days, and 91 days, and at 55°C for 8 days, 17 days, 21 days, 22, days, 33 days, and 35 days. All testing of storage at 37°C produced expected results. Testing of storage at 45°C produced expected results, except that initially two false positive results at 44 days using one of the three reagent

lots, one false positive result at 58 days using one of the three reagent lots, and one false positive result at 88 days using one of the three reagent lots, respectively, were generated. All four initially false positive samples produced expected results upon retesting. Testing of storage at 55°C produced expected results, except that initially one false positive result at 8 days using one of the three reagent lots, and one false positive result at 35 days using one of the three reagent lots, respectively, were generated. All two initially false positive samples produced expected results upon retesting.

The risk of erroneous results under the circumstances of exposing the Acucy test reagents, including pouched Test Cassettes and Extraction Buffer, to extremes of operating conditions is minimized by the packaging of the Test Cassettes in individual air-tight foil pouches. Additionally, a caution is included in the test procedure instructing the user not to open the foil pouch of the Test Cassette until the cassette is ready for immediate use.

3) The effect of increased temperature on Acucy test Control Swabs was evaluated as a part of the accelerated test Control Swab stability study by storage of three lots of the Control Swabs at 37°C for 50 days, 99 days, 124 days, 132 days, 198 days, and 206 days, at 45°C for 22 days, 44 days, 55 days, 58 days, 88 days, and 91 days, and at 55°C for 8 days, 17 days, 21 days, 22, days, 33 days, and 35 days. All testing produced expected results.

4) A flex study was carried out to evaluate the effect of ambient temperature on opened Test Cassette, and the effect of ambient and refrigerated temperatures on opened or closed Extraction Buffer by storage of one lot of opened Test Cassette at ambient temperature (15 - 30°C) over 2, 5, 9, 17, and 25 hours, and by storage of one lot of opened or closed Extraction Buffer at ambient temperature (15 - 30°C) and at refrigerated temperature (2 - 8°C) over 2, 5, 9, 17, and 25 hours. All testing produced expected results in this study.

The potential risk of erroneous results under the circumstances of exposing the opened Test Cassettes to ambient temperature for a prolonged period is minimized by including caution statements in the test procedure instructing the user not to open the foil pouch exposing the Test Cassette to the ambient environment until ready for immediate use, and once the foil pouch is opened, the Test Cassette must be used within 30 minutes or discarded.

2. Reagent Freeze/Thaw

The study evaluated the effect of repeated freeze/thaw conditions of test kit, such as may occur during shipping. One test kit was 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 one freeze/thaw cycle. A second test kit underwent three freeze/thaw cycles using the same storage scheme. Functional testing was performed using the test kit that underwent one freeze/thaw cycle, and the test kit that underwent three freeze/thaw cycles. Expected results were obtained for all samples at both conditions

tested; no erroneous results were obtained.

3. Vibrations

The study evaluated the effect of vibrations, such as those that may be generated from nearby instrumentation. One Acucy Reader was placed on a lab bench at 21.5 cm, 43.5 cm, 65.5 cm, 88.0 cm, and 112.0 cm away from a centrifuge running at 14,000 rpm. The test samples were loaded on to the Acucy Reader and analyzed using the WALK AWAY/NORMAL mode while the centrifuge was running. Expected results were obtained for all samples tested; no erroneous results were obtained.

4. Geographic Altitude (Barometric Pressure)

The Acucy Reader is specified for use at an altitude of <3000 meters. Studies were conducted to evaluate the effect of operational altitude and barometric pressure on the Acucy Influenza A&B Test results using the Acucy Reader, in order to support the operational altitude claim of <3000 meters.

1) The first study was conducted to examine the effect of >2000 meters altitude on assay performance using the Acucy Reader. In this study, one Acucy Reader was utilized in the testing in San Diego, CA (altitude 134 meters) as the Control Condition. The same Acucy Reader was taken to Big Bear, CA (altitude 2058 meters) to perform further testing. Expected results were obtained for all samples tested.

2) A second study was conducted to examine the effect of a simulated barometric pressure of 400 mmHg, equivalent to air pressure at 5,450 meters altitude, on the assay performance using the Acucy Reader compared to 748 mmHg or 1 atmosphere at 134 meters altitude (Control Condition). In this study, one Acucy Reader was placed in a barometric pressure chamber. A sample Test Cassette was loaded into the Reader drawer and the test started using the WALK-AWAY mode, then the barometric pressure chamber was closed and pressurized. Upon completion of the run the chamber was de-pressurized, and the Test Cassette removed from the Reader drawer and the procedure was repeated for each sample. The same Acucy Reader was run in the laboratory as a control condition in San Diego, CA (altitude 134 meters). Expected results were obtained for all samples tested; no erroneous results were obtained.

5. Testing in Direct Sunlight

The test samples were processed and the Acucy Influenza A&B tests were incubated on a workbench pushcart outdoors in a parking lot (outdoor air temperature 26°C), exposed to direct sunlight. The Reader was kept in the shadow of a car hatchback out of direct sunlight, and then was moved under direct sunlight (with the Reader display facing away from the direct sunlight) for two minutes prior to reading. After the 15-minute incubation under direct sunlight, the test was analyzed using the

READ NOW mode. This direct sunlight condition outdoors caused the Acucy Reader display to fail, and tests were cancelled by the Reader. No results were generated.

6. Testing under Bright Lighting

   The test samples were processed and the Acucy Influenza A&B tests were incubated on a workbench, exposed to bright lighting condition under a desk lamp with 22,800 lumens. After the 15-minute incubation under the desk lamp, the test was analyzed using the READ NOW mode. The Acucy Reader was placed with the front of the instrument directly exposed to the bright lamp light to maximize exposure to the bright light. Expected results were obtained for all samples tested; no erroneous results were obtained.

7. Testing under Low Lighting

   The test samples were processed and the Acucy Influenza A&B tests were incubated on a workbench pushcart, exposed to low lighting condition (below 100 lumens/square meter). After the 15-minute incubation under low lighting, the test was analyzed using the READ NOW mode. The Acucy Reader was placed with the front of the instrument directly under the low lighting condition. Expected results were obtained for all samples tested; no erroneous results were obtained.

8. Testing under “Draft” Conditions

   The test samples were processed and the Acucy Influenza A&B tests were incubated inside a chemical fume hood to simulate an air draft. After the 15-minute incubation in the chemical fume hood, the test was analyzed using the READ NOW mode. The Acucy Reader was operated inside the chemical fume hood throughout the testing. Expected results were obtained for all samples tested; no erroneous results were obtained.

9. Testing in an Upright Position

   This study evaluated the test performance when the Test Cassette is placed in a vertical of 90° angle during sample incubation for the READ NOW mode. The test samples were processed on a bench top and the Test Cassettes were propped upright at 90° angle immediately after sample addition. The tests were allowed to incubate for 15 minutes in the upright position. Expected results were obtained for all samples tested; no erroneous results were obtained.

10. Testing when “Bumped”

    This study evaluated the assay performance when the Test Cassettes were intentionally bumped on a bench top during incubation for the READ NOW mode. Expected results were obtained for all samples tested; no erroneous results were obtained.

Operator Errors/Human Factors

1. Contamination of Test Cassette during Handling

This study examined the effect of inadvertent touching of the Acucy Influenza A&B Test Cassette read window by the user with a gloved hand (nitrile powder-free exam gloves), a bare hand, a hand treated with a hand cream, and a hand treated with a sanitizer gel immediately prior to testing. No interference was observed from the substances tested; expected results were obtained for all samples tested.

2. Dropped Test Cassettes

This study examined the effect of inadvertent dropping of a Test Cassette before and after sample addition to the cassette. The Test Cassettes were removed from their foil pouch and dropped to the ground from a standard laboratory bench height (approximately 36 inches) before and after sample addition to the cassettes. False positive results were observed for two samples tested in the "before" condition, and for one sample tested in the "after" condition. However, it appears that the false positive results were not caused by the impact of the Test Cassette with the floor, but were most likely caused by particulate matter from the floor that sticks to the membrane in the Read Window of the Test Cassette.

To minimize the risk of false positive results caused by particulate matter sticking to the membrane in the Read Window of the Test Cassette, a precaution has been added to the Instructions for Use and the test Quick Reference Guide (QRG), stating that all dropped Test Cassettes (regardless of whether samples were added to the Test Cassettes) should be discarded.

3. Sample Preparation/Test Procedure

This study was designed to evaluate the effect of different swab extraction times on test results. The test procedure instructs the user to rotate the swab in the Extraction Buffer against the side of the vial 10 times. Contrived sample swabs were extracted into the Extraction Buffer vials by rotating against the side of the vial for 2, 5, 10 (control), 15, 20, 50, and 100 times. All samples were tested using the READ NOW mode. All samples generated expected results, except that one false positive result was observed initially at 50 rotations. Expected negative result was obtained upon retesting.

4. Sample Addition/ Test Procedure

These studies evaluated the effect of errors that may occur during the addition of the sample to the Test Cassette. The following describes the conditions evaluated and the observed results:

1) Volume of the sample added

Varying volumes (drops) of extracted sample were added to the Sample Well on the Test Cassette to simulate user error during this step. Each of the following volumes was evaluated: 3 drops, 4 drops, 5 drops (the volume specified in the test procedure), 6 drops, 7 drops, 8 drops, and all drops.

Expected results were generated for all samples tested at volumes of 3 drops, 4 drops, 5 drops, and 6 drops. A volume of 7 drops generated one false positive Flu A result (1 of 9 Flu B samples), and a volume of 8 drops generated four incorrect results (1 of 9 Flu A samples gave a false negative result, 1 of 9 Flu B samples gave a false positive Flu A result and a false negative Flu B result, and 1 of 9 negative samples gave a false positive Flu A result). When all drops of the extracted sample were added to the Sample Well, expected results were obtained for 35% of the samples tested. Invalid or incorrect results were observed for 65% of the samples tested (31 invalids and 4 false positive or false negative results).

The risk of an erroneous result is mitigated by the design of the Test Cassette where the text "5 drops" along with a pictorial illustration of 5 drops of same are clearly and prominently displayed. Additionally, the test procedure includes explicit instructions on the use of the inverted Extraction Buffer vial to gently squeeze 5 drops of the extracted sample into the Sample Well of the Test Cassette. Furthermore, a warning statement is included in the test procedure to alert the user that adding more than 5 drops of sample to the Test Cassette may generate invalid or false results.

2) Speed of adding sample

This study attempted to evaluate the effect of "speed" when adding the sample to the Test Cassette. Each of the following conditions were tested initially: adding 5 drops of sample in succession (the control condition); adding 5 drops of sample slowly by pausing for 2 seconds between the 5 drops; and adding 5 drops of sample as fast as possible (in one quick squeeze, not in a dropwise fashion). Expected results were obtained for all samples tested, except that one of the three operators generated three invalid results and two false negative results for all five replicates of the Flu A positive sample tested.

Since it is difficult to discern whether the invalid and incorrect results occurred was due to speed of sample addition or overshoot of the volume, which is known to cause failures in lateral flow tests, to tease out the cause of the observed unexpected results, addition of the entire volume of 5 drops of sample in one quick squeeze of an exact volume pipette was tested as an additional condition. There were no unexpected results when testing with this condition across all three operators for all samples. The results of this additional testing seem to suggest that the cause of the initially observed unexpected results was most likely overshoot volume related issues, not speed of sample addition.

3) Sample placement on the Test Cassette

This study was designed to examine the effect of placing the sample into the Read Window Well, instead of the Sample Well in the Test Cassette. Invalid or incorrect results were observed for 93% of the samples tested (26 invalids and 2 false positive or false negative results). The data showed that the sample addition to the Sample Well is critical in obtaining the correct results.

To minimize the risk of applying the sample incorrectly, the Test Cassette clearly displays the text of "5 drops" along with a pictorial illustration of five drops of sample to mark the location of the Sample Well on the Test Cassette, and to visually remind and guide the user to add the correct amount of extracted sample into the Sample Well. Additionally, the test procedure includes a note cautioning the user not to add sample to the Read Well.

4) Sample dropper angle while dispensing sample

This study evaluated the effect of varying the dropper (inverted Extraction Buffer vial) angle while dispensing sample into the Sample Well in the Test Cassette. Five drops of extracted sample were dispensed into the Sample Well while holding the vial either vertically 90° (control) or at an angle of approximately 45°. Expected results were obtained for all samples tested.

5. Sample Elution/Test Procedure

This study evaluated the effect of incorrect Extraction Buffer volume due to either overfilled vials due to manufacturing errors or low volume due to inadvertent spillage by the user. The Extraction Buffer is provided in pre-filled vials of 400 µL. The test performance was evaluated with the following Extraction Buffer volumes: 200 µL (-50%); 240 µL (-40%); 300 µL (-25%); 360 µL (-10%); and 440 µL (+10%). Expected results were obtained for all samples with Extraction Buffer volume ranging from 240 µL to 440 µL. When the Extraction Buffer volume was 200 µL, the results were invalid for 6 of the 45 samples tested; 39 of the 45 samples tested generated expected results. No erroneous results were obtained.

6. Read Time

This study evaluated the effect of reading the test results outside of the specified 15 minutes using the READ NOW mode. The following read times (incubation intervals) were evaluated: 5, 10, 15 (control condition), 20, 30, 45, and 60 minutes. Expected results were generated for all samples evaluated at 5, 10, 15, 20, 30 and 45 minutes. Only one erroneous result was obtained at the 60-minute read time (false positive Flu B for 1 of the 9 negative samples tested). 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 in the READ NOW mode, the product labeling, including the test Quick Reference Guide (QRG), contains a warning that it is

important to allow the Test Cassette to develop for the full 15 minutes before placing it into the Reader drawer, and allowing the Test Cassette to develop for more than 45 minutes may generate erroneous results.

7. Using Cold Test Reagents

   Conducted as a part of the Temperature and Relative Humidity flex studies, the objective of this study was to evaluate the assay performance when the kit components are inadvertently stored refrigerated and are not sufficiently equilibrated to room temperature (15 - 30°C) before use. One test reagent kit was evaluated after storing in a refrigerator (2 - 8°C) for 30 minutes and then using the test components to test the samples immediately after removal from the refrigerator. Expected results were obtained for all samples tested; no erroneous results were obtained.

8. Using Expired Extraction Buffer and Test Cassette (Technically Expired, Expiration Date Not Barcoded)

   This study evaluated the effect of using expired Extraction Buffer or Test Cassette. One Acucy Influenza A&B Test Cassette lot and one Acucy Extraction Buffer lot were technically expired (barcode still indicates unexpired) by 6 months based on current real-time stability data of 16 months. The following stress conditions were evaluated: expired Test Cassette, unexpired extraction buffer; unexpired Test Cassette, expired extraction buffer; expired Test Cassette and extraction buffer. The control condition for the study was using unexpired Test Cassette and extraction buffer. Expected results were obtained for all samples tested; no erroneous results were obtained.

9. Using Barcoded Expired Test Cassette, Quality Controls, and Calibration Devices

   The study was to confirm the design feature of the Acucy Reader to identify expired (barcoded) Test Cassettes, quality control materials, and calibration devices, to avoid erroneous results. Expired (barcoded) Acucy Influenza A+/B- controls, Acucy Influenza A-/B+ controls, Acucy Influenza A&B Test Cassettes, and Acucy Influenza A&B Cal-Devices were tested with the Acucy Reader. One operator attempted to test 3 replicates of each expired component using both the READ NOW and the WALK Away modes. The Acucy Reader did not proceed, and the appropriate error message was generated when expired material was tested in all attempts for all testing conditions in this study.

10. Improper Positioning of Test Cassette in the Reader

    This study evaluated the effect of improper positioning of the Acucy Influenza A&B Test Cassettes within the Acucy Reader drawer. The following improper positions were tested: Test Cassette placed backwards (arrow pointing to the test operator); Test Cassette placed upside down (bottom of the Test Cassette facing up); and Test Cassette placed sideways (arrow pointing to the left or right of the test operator). One operator made 3 attempts to load the Test Cassette into the

Acucy Reader drawer for each of the improper test positions, using both the READ NOW and the WALK AWAY modes. The Acucy Reader did not proceed, and the appropriate error message was generated when the Test Cassette was incorrectly positioned in the Acucy Reader drawer in all attempts for all testing conditions in this study.

Specimen Integrity and Handling

1. Stability of Collected Samples

An analytical study was carried out to evaluate the effect of storage temperature and time on the stability of undiluted nasopharyngeal swab (NPS) and nasal swab (NS) samples. The following storage conditions were evaluated in this study: undiluted NPS and NS samples stored at 15 - 30°C for 0, 1, 2, 3, 8, and 9 hours; and undiluted NPS and NS samples stored at 2 - 8°C for 0, 1, 4, 8, 16, and 25 hours. All testing produced expected results in this study.

The study results support the conclusion that the collected undiluted NPS and NS samples are stable for up to 8 hours at room temperature (15 - 30°C), and for up to 24 hours at refrigerated temperature (2 - 8°C), before testing with the Acucy Influenza A&B Test run on the Acucy Reader.

2. Effect of Elevated Ambient Temperature on Eluted Sample

This study evaluated the effect of increased ambient temperature on the samples eluted in the Extraction Buffer by leaving the samples at increased ambient temperature prior to testing, without bringing them to room temperature (15 - 30°C), as directed in the test procedure. The test samples were eluted in the Extraction Buffer and placed in an incubator set to 37°C for 1, 2, and 4 hours prior to testing. The samples were then removed from the incubator and tested immediately, without bringing to room temperature. The study results showed that exposure of eluted samples to 37°C for 1 hour prior to testing had no effect on the assay performance. However, exposure of eluted samples to 37°C for 2 hours or longer may result in incorrect results.

3. Stability of Eluted Samples

In an event that immediate testing of eluted samples is not possible due to workload or staffing limitations, collected specimens could possibly be eluted in the Extraction Buffer and stored for testing at a later time. The storage stability conditions for a sample eluted in the Extraction Buffer were evaluated by testing the samples after storage at room temperature (15 - 30°C) for 0, 1, 2, 4, 8, 9, 12, 13, 16, and 25 hours; after storage at refrigerated temperature (2 - 8°C) for 0, 1, 2, 4, 8, 16, and 25 hours; and after storage at -5°C to -25°C for 0, 1, 7, 14, 21, and 31 days.

The study results support the conclusion that the eluted samples in Extraction

Buffer vials are stable for up to 12 hours at room temperature (15 - 30°C), for up to 24 hours at refrigerated temperature (2 - 8°C), and for up to 30 days at -5°C to -25°C, before testing with the Acucy Influenza A&B Test run on the Acucy Reader.

Hardware Robustness

1. Non-level Work Surface

This study evaluated the test performance when Acucy Reader was placed on a bench top in 4 positions, tilted to produce a 15° incline in the front, back, and at each side. Test samples were either loaded onto the Acucy Reader and analyzed using the WALK AWAY/NORMAL mode or inserted into the Reader after the 15-minute incubation on the bench top and analyzed using the READ NOW mode. Expected results were obtained for all tested positions for both reading modes.

2. Mishandling of the Acucy Reader Drawer

This evaluation was attempted to assess the robustness of the Reader drawer design. The Reader drawer was pulled out all the way, the operator then attempted to pick up the Reader by the Reader drawer. Although the Reader drawer has a drawer stop, the stop does not have a locking mechanism. In attempting to pick up the Reader by the drawer in order to lift the Reader completely off the work bench, the drawer detached from the Reader.

3. Movement during the Test

This study evaluated the effect of inadvertent "bumping" of the Acucy Reader during its operation by the user. To simulate the "bumping" conditions, the Reader was bumped during Test Cassette incubation and during test line interrogation, hard enough to make it move. Bumping during incubation of the test was evaluated with samples tested using the WALK AWAY mode. Bumping during test line interrogation of the test was evaluated with samples tested using the READ NOW mode. Expected results were obtained for all tested conditions in this study.

Based on assessment of the design features, robustness of the test 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 Acucy Influenza A&B Test used with the Acucy Reader when performed by untrained operators was evaluated in the CLIA waiver clinical study conducted during the 2017-2018 influenza season testing patients of all ages who presented with signs and symptoms of influenza. The patients were prospectively enrolled in the study at 16 point of care (POC) clinical sites located across the United States. Consented subjects were randomized to have either two nasal swabs or two nasopharyngeal swabs

collected per standard collection methods; both swabs were collected from the same nostril. One swab specimen was processed with the Extraction Buffer and tested with the Acucy Influenza A&B Test using the Acucy Reader on-site immediately after specimen collection. The other swab specimen was eluted in viral transport media (VTM) and sent to a clinical laboratory for reference testing with cell culture, and two FDA-cleared Influenza molecular assays. The swabs were collected in no set order for testing (i.e., one for Acucy Influenza A&B Test and the other for reference methods testing).

1. Testing Sites and Operators

The 16 clinical sites participating in the study represented CLIA waived testing locations and included all POC sites, such as physician's offices and outpatient clinics. A total of 32 operators performed the testing with the Acucy Influenza A&B Test with the Acucy Reader.

The operators were selected from among the staff of the healthcare providers enrolled in the study. The participating operators had no formal training or experience in laboratory testing and included nurses, phlebotomists, medical assistants, and administrator/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.

2. Study Samples

Test samples were collected from 1053 subjects enrolled in the clinical study. There were 41 swab samples excluded from the performance analyses due to patient eligibility and sample handling issues, and inconclusive reference testing results, leaving a total of 1012 prospectively collected swab samples to be included in the evaluation of the assay performance (please see section below for details concerning the excluded samples).

In total, the Acucy Influenza A&B Test with the Acucy Reader was evaluated with 1012 swab specimens and the results were compared to a composite reference, which was calculated from the results of three comparator methods: two FDA-cleared molecular tests and cell culture.

The initial invalid rate observed during the study was 1.8% (18/1012), 95% CI: (1.1% - 2.8%). Nine of the 18 samples with an initially invalid result generated valid results upon retest, while 9 of the 18 samples with an initially invalid result were not retested resulting in 1003 samples with valid results.

3. Performance of Quality Control Material

A total of 960 Influenza A+/B- Control Swabs and 957 Influenza A-/B+ Control Swabs were tested during the clinical evaluation of the Acucy Influenza A&B Test with the Acucy Reader by operators representative of those in CLIA waived settings

who were not selected to participate in the clinical evaluation of the Acucy Influenza A&B Test with the Acucy Reader. Three different lots of Influenza A+/B- Control Swab and Influenza A-/B+ Control Swab were used with one lot of the test reagents (Test Cassette and Extraction Buffer) between December 2017 and May 2018. Upon initial testing, there were 14 invalid results (for both the Influenza A+/B- Control Swab and the Influenza A-/B+ Control Swab), 8 failed results for the Influenza A+/B- Control Swab, and 11 failed results for the Influenza A-/B+ Control Swab. All controls with initial invalid or failed results generated expected results upon repeat testing. The failure rate upon initial testing (including the failed and the invalid results) of the external quality control materials was 1.7% (33/1917), with 95% CI: (1.2% - 2.4%).

4. Test Performance

The performance of the Acucy Influenza A&B Test with the Acucy Reader when used by CLIA waiver intended operators representative of those in CLIA waived settings was evaluated against a compostite reference, which was calculated from the results of three reference methods: two FDA-cleared molecular methods and cell culture. Results obtained from the 1003 specimens were used in data analysis. The performance of the assay, when in the hands of untrained operators, is presented below as sensitivity and specificity against the composite reference method[1].

Performance of Acucy Influenza A&B Test with Clinical Specimens: Performance against a Composite Reference Method in the Hands of Untrained Users

	Influenza A		
Acucy Influenza A&B Test	Composite Reference Method		
	Positive	Negative	Total
Positive	216	31	247
Negative	8	748	756
Total	224	779	1003
Sensitivity: 96.4% (216/224), 95%CI: (93.1% - 98.2%)			
Specificity: 96.0% (748/779), 95%CI: (98.5% - 99.8%)			

[1] Composite Reference Method result for each sample tested by all 3 reference methods is based on the following algorithm:

- At least 2 out of 3 reference methods positive = positive result for either Flu A or B
- 1 out of 3 reference methods positive = negative result for either Flu A or B
- At least 2 out of 3 reference methods negative = negative for either Flu A or B

	Influenza B		
Acucy Influenza A&B Test	Comparator Method		
	Positive	Negative	Total
Positive	130	16	146
Negative	28	829	857
Total	158	845	1003
Sensitivity: 82.3% (130/158), 95%CI: (75.6% - 87.4%)			
Specificity: 98.1% (829/845), 95%CI: (96.9% - 98.8%)			

The 41 samples excluded from the calculations of performance (as mentioned above) included 4 samples where the testing deviated from the Quick Reference Guide (QRG), specifically, the samples were tested outside of the acceptable time window (i.e., the time from start of test to the time of result was ≥ 45 minutes); 16 samples that were deviated from the clinical study protocol; 5 samples that did not satisfy the clinical study inclusion/exclusion criteria; and 16 samples where the composite reference results were inconclusive (i.e., only two of the three reference methods generated valid results and the two valid results did not agree).

Eight out of these 41 samples had a conclusive composite reference method result, including 7 samples that were influenza A and B negative, and 1 sample that was influenza B positive, as determined by the composite reference method. Acucy Influenza A&B Test with the Acucy Reader generated 7 true negative results for influenza A and B, 1 true positive result for influenza B, and 1 false positive result for influenza A. After inclusion of these samples in the data analyses, performance estimates of the assay were similar to the estimates shown in the table above.

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 at the Limit of Detection (LoD) was conducted.

Three CLIA-waived sites that participated in the prospective clinical study participated in the this study. The testing was performed by three untrained intended operators at each of the sites. The test panel consisted of four contrived samples in negative clinical nasal matrix applied to nasal swabs: a Low Positive influenza A sample at 1x LoD (C_{95}), a High Negative influenza A sample at 0.1x LoD (C_5), a Low Positive influenza B sample at 1x LoD (C_{95}), and a High Negative influenza B sample at 0.25x LoD (C_5). Low Positive influenza A samples are expected to give a positive result for influenza A and a negative result for influenza B; Low Positive influenza B samples are expected to give a positive result for influenza B and a negative result for influenza A, and High Negative samples are expected to give a negative result for both influenza A and influenza B.

Samples were masked as subject samples and were presented to the intended use

operators for testing throughout the course of a normal work day. Testing took place over the course of two weeks on non-consecutive days. Each operator tested seven samples each testing day. Each site ultimately tested a panel of 84 samples.

The results from this study are shown below.

Performance of Acucy Influenza A&B Test 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})	100% (21/21)	100% (21/21)	100% (21/21)	100% (63/63)	94.3% - 100%
Flu B Low Positive (C_{95})	95.2% (20/21)	95.2% (20/21)	100% (21/21)	96.8% (61/63)	89.1% - 99.1%
Flu A High Negative (C_5)	100% (21/21)	100% (21/21)	100% (21/21)	100% (63/63)	94.3% - 100%
Flu B High Negative (C_5)	95.2% (20/21)	100% (21/21)	100% (21/21)	98.4% (62/63)	91.5% - 99.7%

There were no significant differences in the observed reactivity of the device with weakly reactive samples between sites or between operators. 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 LoD.

Quick Reference Guide (QRG)

The QRGs for the use of the Acucy Influenza A&B Test with the Acucy Reader are written in simple language (at or below 7th grade reading level based on an evaluation using the Flesch-Kincaid Reading Level tool) and contain pictorial descriptions of the individual steps. In addition to the Acucy Influenza A&B Test QRG (a two-sided card) contains test procedure for patient samples using either the WALK AWAY/NORMAL mode or the READ NOW mode, the Acucy Influenza A&B External Quality Control (QC) QRG contains instructions for performing QC testing with external controls, the Acucy System Calibration Procedure QRG contains instructions for performing calibration, and the Acucy System Start Up QRG contains instructions for unpacking and setting up the Acucy Reader and the Acucy Printer.

Operator Questionnaire Results:

Upon completion of the prospective clinical study, the operators at each site were asked to complete a questionnaire to help assess whether the participants understood how to use the Acucy Influenza A&B Test with the Acucy 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. A total of 53 operators, including 31 operators who participated in the prospective clinical study and 22 operators who performed QC

testing during the clinical study, completed the questionnaire (one of the two operators who did not return the questionnaires is no longer employed at the clinical site). The participants generally found the test to be easy to use and the instructions easy to understand

M. Labeling for Waived Devices

The labeling consists of:

1. Acucy Influenza A&B Test Instructions for Use (Package Insert)
2. Acucy Influenza A&B Test Quick Reference Guide
3. Acucy Influenza A&B Control Kit Instructions for Use
4. Acucy Influenza A&B External Quality Control (QC) Quick Reference Guide
5. Acucy System Calibration Procedure Quick Reference Guide
6. Acucy System Start Up Quick Reference Guide
7. Acucy System Manual

The following elements are appropriately present:

- The Quick Reference Guides 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 Acucy Influenza A&B Test QRG 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 Acucy Influenza A&B Test QRG 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 provided in the Acucy Influenza A&B Control Kit Instructions for Use, as well as in the Acucy Influenza A&B External Quality Control (QC) QRG, and are also integrated with procedural instructions for performing the test in the package insert.
- Instructions for Calibration are provided in the Acucy System Calibration Procedure QRG, and are also integrated with procedural instructions for performing the test in the package insert.
- Appropriate cautions have been added to the Package Insert and Quick Reference Guides to ensure safe use of the product.

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

N. Conclusion

Sekisui Diagnostics conducted an appropriate clinical study evaluating the clinical performance of the Acucy Influenza A&B Test with the Acucy Reader when used by untrained operators in CLIA waived healthcare settings. The test results were compared to an acceptable comparator method; a composite reference method consists of two

FDA-cleared molecular influenza A and B assays and cell culture. The sponsor also conducted appropriate flex studies to demonstrate that the test system is robust, and included design and labeling mitigations to minimize erroneous results.

FDA has evaluated the benefits and risks of using the Acucy Influenza A&B Test with the Acucy 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 Acucy Influenza A&B Test with the Acucy 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 performance of the Acucy Influenza A&B Test with the Acucy Reader for influenza A and influenza B is at least as good as or similar to that of other recently waived influenza tests of this type.
- 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 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 Acucy Influenza A&B Test with the Acucy 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.