510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K182001

B. Purpose for Submission:

New device 510k clearance for the Acucy Influenza A&B Test with the Acucy System

C. Measurand:

Influenza A and influenza B viral nucleoprotein antigens

D. Type of Test:

Qualitative Immunoassay

E. Applicant:

Sekisui Diagnostics, LLC

F. Proprietary and Established Names:

Acucy Influenza A&B Test with the Acucy System

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3328, Influenza virus antigen detection test system

2. Classification:

   Class II

3. Product code:

   PSZ - Devices detecting influenza A, B, and C virus antigens

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   1) The Acucy Influenza A&B Test for the rapid qualitative detection of influenza A and B is composed of a rapid chromatographic immunoassay for the direct and qualitative detection of influenza A and B viral nucleoprotein antigens from nasal and nasopharyngeal swabs of symptomatic patients that is automatically analyzed on the Acucy Reader. The Acucy Influenza A&B Test is a differentiated test, such that influenza A viral antigens can be distinguished from influenza B viral antigens from a single processed sample using a single Test Cassette. The test is intended for use with the Acucy System as an aid in the diagnosis of influenza A and B viral infections. The test is not intended for the detection of influenza C viruses. Negative test results are presumptive and should be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Negative test results do not preclude influenza viral infection and should not be used as the sole basis for treatment or other patient management decisions.

      Performance characteristics for influenza A were established during the 2017-2018 influenza season when influenza A/H3N2 and A/H1N1pdm09 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

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

   2) Acucy Influenza A&B Control Kit is intended for *in vitro* diagnostic use in external quality control testing with the Acucy Influenza A&B Test and Acucy System.

2. Indication(s) for use:

   Same as Intended Use(s)

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Acucy Reader

I. Device Description:

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.

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)

Two extra Test Cassettes, Extraction Buffer Vials, and Extraction Buffer Vial Dropper Tips are included in the kit for External Quality Control (QC) testing.

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.

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)

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 have elapsed. The Calibration Procedure may also be performed, as directed during troubleshooting or whenever the Acucy Reader date and time have 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. A "INVALID" test result displayed by the reader indicates that the internal control was not present, demonstrates that the test did not flow correctly, and/or 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 monitor 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, each shipment received, and with each new untrained operator.

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 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."

Workflow

To perform the Acucy Influenza A&B Test, an operator first collects either a nasal swab (NS) using a nylon sterile flocked nasal swab provided in the test kit or 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.

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 (-), for FLU A and B separately, or INVALID on the screen 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 retested with a new patient sample, reagents, and Test Cassette. A FLU A or FLU B positive result, or a FLU A and 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 and 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 and 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. Substantial Equivalence Information:

1. Predicate device name(s):

   Sofia Influenza A+B FIA (with Sofia or Sofia 2 readers)

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

   K162438

3. Comparison with predicate:

Similarities and Differences		
Item	**Device**	**Predicate**
	Acucy Influenza A&B Test (with the Acucy Reader) (K182001)	**Sofia Influenza A+B FIA (with Sofia or Sofia 2) (K162438)**
Intended Use	The Acucy Influenza A&B Test for the rapid qualitative detection of influenza A and B is composed of a rapid chromatographic immunoassay for the direct and qualitative detection of influenza A and B viral nucleoprotein antigens from nasal and nasopharyngeal swabs of symptomatic patients that is automatically analyzed on the Acucy Reader. The Acucy Influenza A&B Test is a differentiated test, such that influenza A viral antigens can be distinguished from influenza B viral antigens from a single processed sample using a single Test Cassette. The test is intended for use with the Acucy System as an aid in the diagnosis of influenza A and B viral infections. The test is not intended for the detection of influenza C viruses. Negative test results are presumptive, and should be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Negative test results do not preclude influenza viral infection and should not be used as the sole basis for treatment or other patient management decisions. Performance characteristics for influenza A were	The Sofia Influenza A+B FIA employs immunofluorescence to detect influenza A and influenza B viral nucleoprotein antigens in direct nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash specimens and nasopharyngeal swab and nasopharyngeal aspirate/wash specimens in transport media from symptomatic patients. This qualitative test is intended for use as an aid in the rapid differential diagnosis of acute influenza A and influenza B viral infections. The test is not intended to detect influenza C antigens. A negative test is presumptive and it is recommended these results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Negative results do not preclude influenza virus infections and should not be used as the sole basis for treatment or other patient management decisions. This test is intended for professional and laboratory use. The Sofia Influenza A+B FIA may be used with

Similarities and Differences		
Item	**Device**	**Predicate**
	Acucy Influenza A&B Test (with the Acucy Reader) (K182001)	**Sofia Influenza A+B FIA (with Sofia or Sofia 2) (K162438)**
	established during the 2017-2018 influenza season when influenza A/H3N2 and A/H1N1pdm09 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	Sofia or Sofia 2. Performance characteristics for influenza A and B were established during February through March 2011 when influenza viruses A/California/7/2009 (2009 H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008 (Victoria-Like) were the predominant influenza viruses in circulation according to the Morbidity and Mortality Weekly Report from the CDC entitled "Update: Influenza Activity--United States, 2010-2011 Season, and Composition of the 2011-2012 Influenza Vaccine." Performance characteristics may vary against other emerging influenza viruses. If infection with a novel influenza virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, samples should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture samples.
Sample Type	Nasal swab and nasopharyngeal swab	Nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash
Test Results	Qualitative	Same
Test Targets	Influenza A and B viral nucleoprotein antigens	Same
Test Principle	Immunochromatographic device	Immunofluorescence device
Test Format	Lateral flow test cassette	Same
Test Antibodies	Monoclonal antibodies to influenza A and B nucleoproteins	Same
Instrument Detection Method	Absorbance	Fluorescence
Sample Transfer Method	Dropper tip applied to extraction vial to transfer extracted sample	Use a fixed volume pipet to transfer extracted sample
Reporting of Results	Reader displays results on screen, or may be printed	Same
Time to Result	15 minutes	Same

Similarities and Differences		
Item	**Device**	**Predicate**
	Acucy Influenza A&B Test (with the Acucy Reader) (K182001)	**Sofia Influenza A+B FIA (with Sofia or Sofia 2) (K162438)**
Reader Modes	Read Now or Walk Away/Normal	Same
Intended Use Locations	Clinical laboratories and Point of Care (POC) sites	Same
Calibrator	Yes – QC verification cassette provided	Yes – Calibration cassette and QC card provided
Storage Temperature	Room temperature	Same
Test Internal Control	Internal procedure control	Same
Reader Quality Control Features	• Scanning procedural control zone for adequate flow • Reader prevents use of used cassettes • Reader prevents use of expired cassettes • Prevents improper cassette insertion	Same
External Controls	• Influenza A Positive/B Negative Control • Influenza B Positive/A Negative Control	• Positive Influenza A/Influenza B Control • Negative Control
User	• CLIA moderate complexity laboratory technologist. • Users in CLIA waived settings, such as nurses and medical assistants at emergency rooms, urgent care clinics, and outpatient clinics, including physician's offices.	Same

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

- Food and Drug Administration, 21 CFR 866.8328: Microbiology Devices; Reclassification of Influenza Virus Antigen Detection Test Systems Intended for Use Directly with Clinical Specimens, Final Order, January 12, 2017
- Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses, July 15, 2011
- Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices, January 30, 2008
- Select Updates for Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices, Draft Guidance, November 29, 2017
- Recommendations for Dual 510(k) and CLIA Waiver by Application Studies, Draft Guidance, November 29, 2017
- Administrative Procedures for CLIA Categorization, Guidance for Industry and Food and Drug Administration Staff, October 2, 2017
- FDA Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, May 11, 2005

- General Principles of Software Validation, Final Guidance for Industry and FDA Staff, January 11, 2002
- Content of Premarket Submissions for Management of Cybersecurity in Medical Devices, Guidance for Industry and FDA Staff, October 2, 2014

L. Test Principle:

The Acucy Influenza A&B Test is a lateral flow immunochromatographic assay in the sandwich immunoassay format. In the sandwich immunoassay format, the antibody-conjugate is mixed with the antigen of a sample to form a complex in the glass fiber pad before the test line, which is then transported by diffusion along a flow path to the test site. At the test site, the antigen bound with the conjugate reacts with the immobilized first binding protein to form a "sandwich" of the first protein, antigen, second protein, and colored particles. The sandwich complex is progressively produced at the test site as sample continuously passes by, filling the reservoir. As more and more antigen-conjugate is immobilized, the colored particles aggregate at the test site and become visible through the window, indicating the presence of the antigen in the sample.

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, which then migrates along the membrane surface. If influenza A and/or B viral antigens are present in the sample, 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 presence of colored line at the Flu A position of the results window indicates the presence of influenza A, and a colored line at the Flu B position indicates the presence of influenza B. The intensity of the lines is related to the amount of antigen in the sample.

The Acucy Reader scans the test strip, measures the absorbance of the colored Flu A and Flu B lines, and uses analyte-specific algorithms to determine whether the sample is positive for influenza A and/or influenza B. Depending on the user's choice, the Test Cassette may be either placed inside the Acucy Reader for automatically timed test development (WALK AWAY/NORMAL mode), or placed on the bench top for manually timed development and then placed into the Acucy Reader to be scanned (READ NOW mode).

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Four independent studies: within-laboratory repeatability, instrument-to-instrument precision, reagent lot-to-lot precision, and external multi-site user-to-user

reproducibility, were carried out to evaluate the precision and reproducibility of the Acucy Influenza A&B Test with the Acucy Reader.

A seven-member panel was used for all four studies. The panel was made with two viral strains: Influenza A/Hong Kong/4801/14 (H3N2) and Influenza B/Phuket/3073/13. Each strain was diluted into Viral Transport Medium (VTM) at three different target levels: Moderate Positive (MP) (~2xLoD), Low Positive (LP) (~1xLoD), and High Negative (HN) (0.1xLoD for Influenza A and 0.25xLoD for Influenza B). Panel members were formulated with only one target present (Flu A or Flu B). A Negative sample (VTM only) was also used. Simulated nasal swabs were prepared by pipetting 50 µL of sample (virus dilution) onto the head of the swab. Each swab was allowed to air dry and then sealed in a desiccated pouch prior to testing.

Detection rates and percent agreement scores were calculated for each study. Percent agreement was calculated as the number of expected results divided by the total number of replicates tested. For Moderate and Low Positive samples, detection of target is the expected result. For High Negative and Negative samples, the absence of target detection is the expected result.

Within-Laboratory Repeatability Study

The seven-member panel was tested twice per day with a minimum of two replicates per testing period, for 20 non-consecutive days. Testing at a manufacturer's internal site was conducted by one operator using one lot of the Acucy Influenza A&B Test and one Acucy Reader. Table 1 below shows the results of the within-laboratory repeatability study.

Table 1: Within-Laboratory Repeatability Study Results

Sample	Count	Agreement	95% CI	Mean mAbs*	%CV
Flu A MP	80/80	100%	95.4 - 100%	40.9	7.6
Flu A LP	80/80	100%	95.4 - 100%	19.1	9.2
Flu A HN	80/80	100%	95.4 - 100%	0.0	N/A
Flu B MP	80/80	100%	95.4 - 100%	22.9	8.5
Flu B LP	80/80	100%	95.4 - 100%	10.0	10.3
Flu B HN	80/80	100%	95.4 - 100%	0.0	N/A
Negative	80/80	100%	95.4 - 100%	0.0	N/A

* Milli-absorbance units measured by the Acucy Reader.

Instrument-to-Instrument Precision Study

The seven-member panel was tested using five replicates on three different Acucy Readers at a manufacturer's internal site. Testing was conducted by one operator using one lot of the Acucy Influenza A&B Test in one day. Table 2 below shows the results of the instrument-to-instrument precision study.

Table 2: Instrument-to-Instrument Precision Study Results

	Reader #1		Reade #2		Reade #3		All Readers		
Sample	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**95% CI**	**Mean mAbs (%CV)**
Flu A MP	100% (5/5)	43.8 (4.1)	100% (5/5)	43.0 (4.7)	100% (5/5)	44.3 (7.4)	100% (15/15)	79.6 - 100%	43.7 (5.4)
Flu A LP	100% (5/5)	20.2 (11.2)	100% (5/5)	19.6 (3.8)	100% (5/5)	19.8 (6.4)	100% (15/15)	79.6 - 100%	19.9 (7.4)
Flu A HN	100% (5/5)	0.0 (N/A)	100% (5/5)	0.0 (N/A)	100% (5/5)	0.0 (N/A)	100% (15/15)	79.6 - 100%	0.0 (N/A)
Flu B MP	100% (5/5)	23.4 (9.1)	100% (5/5)	22.8 (4.3)	100% (5/5)	23.2 (4.4)	100% (15/15)	79.6 - 100%	23.1 (6.0)
Flu B LP	100% (5/5)	9.5 (7.4)	100% (5/5)	10.5 (5.7)	100% (5/5)	10.6 (10.5)	100% (15/15)	79.6 - 100%	10.2 (9.3)
Flu B HN	100% (5/5)	0.0 (N/A)	100% (5/5)	0.0 (N/A)	100% (5/5)	0.0 (N/A)	100% (15/15)	79.6 - 100%	0.0 (N/A)
Negative	100% (5/5)	0.0 (N/A)	100% (5/5)	0.0 (N/A)	100% (5/5)	0.0 (N/A)	100% (15/15)	79.6 - 100%	0.0 (N/A)

Lot-to-Lot Precision Study

The seven-member panel was tested using five replicates on three different Acucy Influenza A&B Test lots at a manufacturer's internal site. Testing was conducted by one operator using one Acucy Reader in over three days. Table 3 below shows the results of the lot-to-lot precision study.

Table 3: Lot-to-Lot Precision Study Results

	Lot #1		Lot #2		Lot #3		All Lots		
Sample	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**95% CI**	**Mean mAbs (%CV)**
Flu A MP	100% (15/15)	43.9 (6.9)	100% (15/15)	44.3 (12.3)	100% (15/15)	42.7 (5.7)	100% (45/45)	92.1 - 100%	43.7 (8.8)
Flu A LP	100% (15/15)	20.6 (9.3)	100% (15/15)	21.3 (4.8)	100% (15/15)	19.9 (9.5)	100% (45/45)	92.1 - 100%	20.6 (8.4)
Flu A HN	100% (15/15)	0.0 (N/A)	100% (15/15)	1.4 (N/A)	100% (15/15)	0.4 (N/A)	100% (45/45)	92.1 - 100%	0.6 (N/A)
Flu B MP	100% (15/15)	19.8 (6.0)	100% (15/15)	21.2 (6.3)	100% (15/15)	22.6 (9.5)	100% (45/45)	92.1 - 100%	21.2 (9.2)
Flu B LP	100% (15/15)	8.7 (6.6)	100% (15/15)	8.8 (7.6)	100% (15/15)	9.7 (7.8)	100% (45/45)	92.1 - 100%	9.1 (8.9)
Flu B HN	100% (15/15)	0.4 (N/A)	100% (15/15)	0.0 (N/A)	100% (15/15)	0.0 (N/A)	100% (45/45)	92.1 - 100%	0.1 (N/A)
Negative	100% (15/15)	0.0 (N/A)	100% (15/15)	0.3 (N/A)	100% (15/15)	0.0 (N/A)	100% (45/45)	92.1 - 100%	0.1 (N/A)

External Multi-Site User-to-User Reproducibility Study

The seven-member panel was tested twice per day with three replicates per testing period, for five none consecutive days over a period of two weeks, at three external

CLIA waived testing sites in the US. Testing at each site was conducted by two operators using one lot of the Acucy Influenza A&B Test and one Acucy Reader. The six operators participated in this study consisted of two nurses and four administrative assistants with no formal medical laboratory training. All operators had limited or no training or hands-on experience in conducting laboratory testing. Table 4 below shows the results of the multi-site user-to-user reproducibility study.

Table 4: External Multi-Site User-to-User Reproducibility Study Results

	Site #1		Site #2		Site #3		All Sites		
Sample	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**Mean mAbs (%CV)**	**Agreement (Count)**	**95% CI**	**Mean mAbs (%CV)**
Flu A MP	100% (30/30)	46.7 (7.3)	100% (30/30)	36.6 (10.2)	100% (30/30)	34.8 (20.6)	100% (90/90)	95.9 - 100%	39.4 (18.5)
Flu A LP	100% (30/30)	20.9 (7.9)	100% (30/30)	17.1 (13.1)	100% (30/30)	16.0 (17.6)	100% (90/90)	95.9 - 100%	18.0 (17.3)
Flu A HN	100% (30/30)	0.5 (N/A)	100% (30/30)	0.0 (N/A)	96.7% (29/30)	0.5 (N/A)	98.9% (89/90)	94.0 – 99.8%	0.3 (N/A)
Flu B MP	100% (30/30)	23.8 (13.1)	100% (30/30)	22.9 (10.8)	96.7% (29/30)	20.3 (15.3)	98.9% (89/90)	94.0 - 99.8%	22.4 (14.5)
Flu B LP	100% (30/30)	10.2 (15.2)	100% (30/30)	10.5 (11.7)	100% (30/30)	9.1 (14.7)	100% (90/90)	95.9 - 100%	9.9 (14.8)
Flu B HN	100% (30/30)	0.0 (N/A)	100% (30/30)	0.0 (N/A)	100% (30/30)	0.0 (N/A)	100% (90/90)	95.9 - 100%	0.0 (N/A)
Negative	100% (30/30)	0.0 (N/A)	100% (30/30)	0.0 (N/A)	100% (30/30)	0.2 (N/A)	100% (90/90)	95.9 - 100%	0.1 (N/A)

b. Linearity/assay reportable range:

Not applicable

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

External Quality Controls

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. Refer to the "Device Description" section of this summary for details regarding these controls.

The External Quality Controls are used to monitor 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.

During the prospective clinical study conducted during the 2017 to 2018 influenza season, quality control testing using the external quality controls was performed per the product Instructions for Use at all 16 CLIA waived study sites. 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%).

Nasal and Nasopharyngeal Swab Specimens Stability

Analytical studies were performed to evaluate the stability of clinical nasal swab (NS) and nasopharyngeal swab (NPS) specimens, when stored at room temperature and refrigerated temperature. Samples were prepared in Clinical Nasal Matrix (CNM). CNM was obtained from a vendor. The pooled matrix was prepared by the vendor from leftover negative clinical samples (confirmed negative for influenza A and influenza B with an FDA-cleared molecular test) from other studies. One influenza A strain (A/Hong Kong/4801/14) and one influenza B strain (B/Phuket/3073/13) were diluted in CNM to levels corresponding to 2x LoD to prepare the positive samples. Negative samples were CNM only. To prepare contrived NS and NPS samples for testing, 50 µL of virus dilution was applied to a NS or a NPS and then reinserted into the original swab packaging for storage at the specified temperatures.

A set of contrived NS and NPS samples (three replicates per storage condition) were stored at room temperature (15° - 30°C) for the following time periods before extraction and testing: 0, 1, 2, 3, 8, and 9 hours. Another set of contrived NS and NPS samples (three replicates per storage condition) were stored at refrigerated temperature (2° - 8°C) for the following time periods before extraction and testing: 0, 1, 4, 8, 16, and 25 hours. After the specified storage interval, the contrived NS and NPS samples were extracted in Extraction Buffer and tested with the Acucy Influenza A&B Test according to the directions in the Instructions for Use. Testing was performed with one lot of reagents using the READ NOW mode.

The results for the contrived samples in CNM stored at room temperature (15° - 30°C) show 100% agreement with Time 0 at all time points (up to 9 hours) for both swab types (nasal and nasopharyngeal), supporting a stability claim of 8 hours at room temperature (15° - 30°C). The results for the contrived samples in CNM stored at refrigerated temperature (2° - 8°C) also show 100% agreement with Time 0 at all

time points (up to 25 hours) for both swab types (nasal and nasopharyngeal), supporting a stability claim of 24 hours at refrigerated temperature (2° - 8°C).

Extracted Specimens Stability

Analytical studies were performed to evaluate the stability of extracted specimens, when stored at room temperature, refrigerated, and frozen. Samples were prepared in the same CNM used in the NS and NPS Specimens Stability studies. One influenza A strain (A/Hong Kong/4801/14) and one influenza B strain (B/Phuket/3073/13) were diluted in CNM to levels corresponding to 2x LoD to prepare the positive samples. Negative samples were CNM only. To prepare contrived NS samples for testing, 50 µL of virus dilution was applied to a NS and dried for at least one minute prior to extraction. Sufficient number of contrived NS samples that allow testing three replicates per storage condition were prepared. Prepared samples were then extracted in Extraction Buffer according to the Acucy Influenza A&B Test Instructions for Use. Extracted samples were stored in the Extraction Buffer vials at the following specified temperature ranges and time points until they were tested:

- room temperature (15° - 30°C) for 0, 1, 2, 4, 8, 9, 12, 13, 16, and 25 hours
- refrigerated temperature (2° - 8°C) for 0, 1, 2, 4, 8, 16, and 25 hours
- frozen temperature (-5° to -25°C) for 0, 1, 7, 14, 21, and 31 days

Testing was performed with one lot of reagents using the READ NOW mode.

Extracted specimens stored at room temperature (15° to 30°C) show 100% agreement with Time 0 results up to 13 hours. Failures were observed at 16 and 25 hours. This data supports a stability claim of 12 hours at room temperature (15° - 30°C). Extracted specimens stored refrigerated (2° to 8°C) show 100% agreement with Time 0 results for the duration of the testing schedule of 25 hours, supporting a stability claim of 24 hours when refrigerated at temperature of 2° to 8°C. Extracted specimens stored frozen (-5° to -25°C) also show 100% agreement with Day 0 results for the duration of the testing schedule of 31 days, supporting a stability claim of 30 days when frozen at temperature -5° to -25°C.

Open Test Cassette and Extraction Buffer Stability

Analytical studies were conducted to determine the stability of open Acucy Influenza A&B Test cassettes held at room temperature at various time points and to determine the stability of open vials of Acucy Influenza A&B Test Extraction Buffer held at room temperature or in a refrigerator at various time points.

Acucy Influenza A&B Test cassettes were opened, removed from the pouch and incubated at room temperature (15° - 30°C). Acucy Influenza A&B Test Extraction Buffer vials were opened and incubated at either room temperature (15° - 30°C) or refrigerated at 2° - 8°C. At various time points (2, 5, 9, 17, and 25 hours), three test cassettes or Extraction Buffer vials were removed from storage and tested with

contrived influenza A and influenza B samples. At Time 0 and each subsequent time point, unopened test cassettes and extraction buffer vials representing all incubation conditions were run as control conditions.

Contrived test samples were prepared by diluting an influenza A strain (A/California/07/09) and an influenza B strain (B/Brisbane/60/08) to a concentration of 2x LoD in Viral Transport Media. Samples (50 µL) were applied to nasopharyngeal swabs, dried for at least one minute, and tested with the stored reagents using the READ NOW mode. One lot of reagents was tested.

There was 100% qualitative agreement between the open test cassette/extraction buffer and the control condition at all time points. The data support the stability of an opened Acucy Influenza A&B Test cassette for up to 24 hours at room temperature (15° - 30°C). The data also support the stability of an opened Acucy Influenza A&B Extraction Buffer vial for up to 24 hours at both room temperature (15° - 30°C) and refrigerated temperature (2° - 8°C).

Matrix Equivalency Study

In order to utilize Viral Transport Media (VTM) as a simulated nasal matrix in making contrived samples to be tested in precision/reproducibility studies and other analytical studies, an analytical study was performed to evaluate the performance using VTM as simulated nasal matrix against a true negative Clinical Nasal Matrix (CNM) with the Acucy Influenza A&B Test.

CNM was obtained from a vendor. The pooled matrix was prepared by the vendor from leftover negative clinical samples (confirmed negative for influenza A and influenza B with an FDA-cleared molecular test) from other studies. Thirty (30) contrived influenza A samples (A/Hong Kong/4801/14) and 30 contrived influenza B samples (B/Brisbane/60/2008) were prepared at approximately 2x LoD, using either pooled Clinical Nasal Matrix (CNM) or VTM as a diluent. VTM or CNM without addition of virus was used as a negative sample. Virus dilutions (50 µL) were coated onto nasopharyngeal swabs, allowed to dry for at least one minute, and tested using the Acucy Influenza A&B Test. Each was tested with three test cassette lots, for a total of 90 replicates for each sample. Testing was performed using the READ NOW mode.

Results of this study are presented in Table 5 below.

Table 5: Matrix Equivalency Study Results

Flu A	Lot 1		Lot 2		Lot 3	
	CNM	**VTM**	**CNM**	**VTM**	**CNM**	**VTM**
Percent agreement	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)
Mean mAbs	29.1	33.7	30.6	33.4	31.0	35.5
% CV	7.1%	6.6%	6.5%	9.1%	9.4%	8.1%
Flu B	**Lot 1**		**Lot 2**		**Lot 3**	
	CNM	**VTM**	**CNM**	**VTM**	**CNM**	**VTM**
Percent agreement	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)
Mean mAbs	21.9	21.2	18.7	19.5	21.2	21.1
% CV	9.5%	5.9%	7.8%	6.0%	10.1%	4.0%
Negative	**Lot 1**		**Lot 2**		**Lot 3**	
	CNM	**VTM**	**CNM**	**VTM**	**CNM**	**VTM**
Percent Agreement	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)
Mean mAbs	0.0	0.0	0.0	0.0	0.0	0.0
% CV	N/A	N/A	N/A	N/A	N/A	N/A

The data support equivalency between Clinical Nasal Matrix and Viral Transport Media for preparation of samples for the Acucy Influenza A&B Test analytical study and precision/reproducibility study.

Swab Equivalency Study

This study was performed to evaluate the use of both Copan nasal swabs (Copan part number 519CS01) and nasopharyngeal swabs (Copan part number 534CS01) with the Acucy Influenza A&B Test and to demonstrate equivalent performance of the test both swab types.

Twenty (20) influenza A contrived samples (A/Hong Kong/4801/14 diluted to 2x LoD in CNM), twenty influenza B contrived samples (B/Phuket/3073/13 diluted to 2x LoD in CNM), and twenty negative samples (CNM) were prepared. Each virus dilution (50 µL) was coated on both swab types. All samples were tested with one lot of the Acucy Influenza A&B Test. Testing was performed using the READ NOW mode.

Results of this study are presented in Table 6 below.

Table 6: Swab Equivalency Study Results

Flu A	**Nasal Swab**	**Nasopharyngeal Swab**
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	28.5	35.5
% CV	14.5%	10.2%
Flu B	**Nasal Swab**	**Nasopharyngeal Swab**
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	15.9	20.9
% CV	22.9%	14.8%
Negative	**Nasal Swab**	**Nasopharyngeal Swab**
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	0.0	0.0
% CV	N/A	N/A

The data support equivalency of qualitative performance between the two swab types. This study supports the use of both swab types with the Acucy Influenza A&B Test.

Test Mode Equivalency

This study was performed to qualify the use of the READ NOW and WALK AWAY/NORMAL test modes when reading Acucy Influenza A&B Test results on the Acucy Reader by demonstrating equivalent test performance.
Forty (40) contrived influenza A (A/Hong Kong/4801/14) samples at approximately 2x LoD, 40 contrived influenza B (B/Brisbane/60/08) samples at approximately 2x LoD, and 40 negative samples were prepared in Viral Transport Media and coated (50 µL) onto nasopharyngeal swabs. Samples were tested with one lot of the Acucy Influenza A&B Test: 20 samples using the READ NOW mode and 20 samples using the WALK AWAY/NORMAL mode.

Results of this study are presented in Table 7 below.

Table 7: Test Mode Equivalency Study Results

Flu A	**READ NOW**	**WALK AWAY/NORMAL**
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	43.1	43.6
% CV	9.0%	10.6%
Flu B	**READ NOW**	**WALK AWAY/NORMAL**
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	24.4	22.6
% CV	8.2%	13.3%
Negative	**READ NOW**	**WALK AWAY/NORMAL**
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	0.0	0.0
% CV	N/A	N/A

The data support equivalent performance of the Influenza A&B Test using the two test modes. The data supports use of either test mode when conducting analytical

performance studies.

d. Detection limit:

Two strains of each viral type of influenza A (H1N1pdm09 and H3N2) and influenza B (Victoria and Yamagata lineages) were tested in a two-step approach that involved first testing a series of 10-fold dilutions (n=3) to determine the initial starting point for the second 2-fold dilution series with 20 replicates for each dilution. The 2-fold dilution series starting point was determined to be the last 10-fold dilution that produced a 100% detection rate (3/3) with the subsequent dilution producing a detection rate less than 100%. The Limit of Detection (LoD) results from the second dilution series were confirmed by testing an additional 40 replicates (20 replicates tested with two different reagent lots). The LoD for each strain was determined to be the dilution concentration (in $TCID_{50}$/mL) that produced a ≥ 95% detection rage for a given target.

Contrived samples were prepared by diluting viral strains in pooled Clinical Nasal Matrix (CNM), which was confirmed negative by PCR. For each test, 50 µL was pipetted onto the head of a new nasopharyngeal swab. The seeded swab was then extracted into 400 µL of Acucy Flu A&B Extraction Buffer. Five drops of extracted sample were applied to the sample well of the test cassette. Testing was performed using the READ NOW mode. The LoD confirmation study results are presented in Table 8 below.

Table 8: Acucy Influenza A&B Test Limit of Detection (LoD) Confirmation Study Results

Viral Strain	LoD ($TCID_{50}$/mL)	$TCID_{50}$/mL	# POS/ # TESTED		% Detected		Mean mAbs		%CV	
			Lot #1	Lot #2	Lot #1	Lot #2	Lot #1	Lot #2	Lot #1	Lot #2
A/California/07/09 (H1N1pdm09)	**$1.41x10^1$**	$2.83x10^1$	20/20	19/20	100%	95%	15.3	15.1	7.2%	25.7%
		$1.41x10^1$	**20/20**	**19/20**	**100%**	**95%**	**7.8**	**8.1**	**12.4%**	**15.8%**
		$7.06x10^0$	0/20	0/20	0%	0%	0.3	0.8	N/A	N/A
		$3.53x10^0$	0/20	0/20	0%	0%	0.0	0.0	N/A	N/A
A/Hong Kong/4801/14 (H3N2)	**$7.06x10^1$**	$2.83x10^2$	20/20	20/20	100%	100%	60.8	59.8	7.6%	5.3%
		$1.41x10^2$	20/20	20/20	100%	100%	28.6	28.4	8.2%	7.8%
		$7.06x10^1$	**20/20**	**20/20**	**100%**	**100%**	**11.0**	**11.5**	**7.6%**	**10.0%**
		$3.53x10^1$	0/20	0/20	0%	0%	0.0	0.0	N/A	N/A
B/Brisbane/60/08 (Victoria)	**$2.35x10^1$**	**$2.35x10^1$**	**20/20**	**20/20**	**100%**	**100%**	**12.0**	**11.1**	**12.5%**	**12.3%**
		$1.17x10^1$	18/20	15/20	90%	75%	6.9	4.9	13.3%	53.0%
		$5.87x10^0$	0/20	0/20	0%	0%	0.0	0.0	N/A	N/A
B/Phuket/3073/13 (Yamagata)	**$3.40x10^1$**	**$3.40x10^1$**	**20/20**	**20/20**	**100%**	**100%**	**12.7**	**11.6**	**11.0%**	**7.1%**
		$1.70x10^1$	16/20	13/20	80%	65%	5.7	5.2	44.1%	44.9%
		$8.49x10^0$	0/20	0/20	0%	0%	0.3	0.0	N/A	N/A
		$4.25x10^0$	0/20	Not Tested	0%	Not Tested	0.0	Not Tested	N/A	Not Tested

e. Analytical Reactivity

Analytical Inclusivity Study

This study was performed to demonstrate that the Acucy Influenza A&B Test can detect a wide range of well characterized influenza A and influenza B strains with global epidemiological representation. For this study, 28 characterized strains were tested, composed of 23 strains of influenza A (6 H1N1 strains, 5 H1N1pdm09 strains, 10 H3N2 strains, 1 H3N2v strain, and 1 H7N9 avian strain) and 5 strains of influenza B.

The influenza strains were obtained from vendors and repositories. Each strain was diluted in viral transport media at a concentration of approximately 1.5x LoD, and 50 µL was applied to each nasopharyngeal swab. Testing was performed in triplicate in the READ NOW mode. If fewer than three of the replicates were detected, additional testing was performed to determine the concentration at which 100% agreement (observed/expected) was achieved using six replicates.

Results of the analytical inclusivity study are presented in Table 9 below.

Table 9: Results for Analytical Inclusivity Study

Strain	Sub Type	Concentration Tested	Percent Agreement
A/Brisbane/59/07	H1N1	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/PR/8/34	H1N1	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Singapore/63/04	H1N1	$1.06x10^2$ $TCID_{50}$/mL	0/3 (0%) initial
		$2.04x10^3$ $TCID_{50}$/mL	6/6 (100%) repeat
A/Taiwan/42/06	H1N1	$1.06x10^2$ $TCID_{50}$/mL	0/3 (0%) initial
		$1.02x10^3$ $TCID_{50}$/mL	6/6 (100%) repeat
A/New Cal/20/99	H1N1	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Solomon Islands/03/06	H1N1	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/NY/02/09	H1N1pdm09	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Mexico/4108/09	H1N1pdm09	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Canada/6294/09	H1N1pdm09	$1.06x10^2$ $TCID_{50}$/mL	0/3 (0%) initial
		$2.12x10^3$ $TCID_{50}$/mL	6/6 (100%) repeat
A/NY/01/09	H1N1pdm09	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/NY/03/09	H1N1pdm09	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Brisbane/10/07	H3N2	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Victoria/361/11	H3N2	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/HK/8/68	H3N2	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Perth/16/09	H3N2	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Wisconsin/67/05	H3N2	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Rhode Island/01/2010	H3N2	$1.06x10^2$ $TCID_{50}$/mL	0/3 (0%) initial
		$5.0x10^5$ $TCID_{50}$/mL	6/6 (100%) repeat
A/New York/55/2004	H3N2	$1.06x10^2$ $TCID_{50}$/mL	0/3 (0%) initial
		$2.00x10^5$ $TCID_{50}$/mL	6/6 (100%) repeat
A/Florida/2/2006	H3N2	$1.06x10^2$ $TCID_{50}$/mL	0/3 (0%) initial
		$3.30x10^5$ $TCID_{50}$/mL	6/6 (100%) repeat
A/Texas/50/2012	H3N2	$1.06x10^2$ $TCID_{50}$/mL	3/3 (100%)
A/Texas/71/2007	H3N2	$1.06x10^2$ $TCID_{50}$/mL	0/3 (0%) initial
		$4.08x10^3$ $TCID_{50}$/mL	6/6 (100%) repeat
B/Malaysia/2506/04	B	$5.10x10^1$ $TCID_{50}$/mL	3/3 (100%)
B/Wisconsin/1/10	B	$5.10x10^1$ $TCID_{50}$/mL	3/3 (100%)
B/Massachusetts/2/12	B	$5.10x10^1$ $TCID_{50}$/mL	0/3 (0%) initial
		$5.10x10^2$ $TCID_{50}$/mL	6/6 (100%) repeat
B/Texas/6/11	B	$5.10x10^1$ $TCID_{50}$/mL	0/3 (0%) initial

		$7.24x10^2$ $TCID_{50}$/mL	6/6 (100%) repeat
B/Florida/07/04	B	$5.10x10^1$ $TCID_{50}$/mL	1/3 (33.3%) initial
		$2.55x10^2$ $TCID_{50}$/mL	6/6 (100%) repeat
A/Indiana/08/2011	H3N2v	$1.06x10^2$ $TCID_{50}$/mL	0/3 (0%) initial
		$2.12x10^2$ $TCID_{50}$/mL	4/6 (66.6%) repeat
		$5.30x10^2$ $TCID_{50}$/mL	6/6 (100%) repeat
A/Anhui/1/2013	H7N9 (Avian)	$1.00x10^8$ EID_{50}/mL	3/3 (100%)

CDC Human Influenza Virus Panel for 2017

This study was performed to determine the minimum reactive concentrations for the CDC Human Influenza Virus Panel for 2017 with the Acucy Influenza A&B Test. For this study, 10 characterized strains were used, composed of six strains of influenza A (2 H1N1 strains, 2 H3N2 strains, and 2 H1N1pdm09 strains) and 4 strains of influenza B (2 strains representing the Victoria lineage and 2 strains representing the Yamagata lineage). The strains were obtained from the CDC, as the Human Influenza Panel for 2017. The strains were prepared and tested according to the instructions provided by CDC.

Each strain was serially diluted 1:5 in viral transport media. For each replicate, 50 µL was applied to a nasopharyngeal swab and allowed to dry for one minute before being processed following the instructions for the Acucy Influenza A&B Test. Testing was performed in replicates of five using the READ NOW mode. Testing was performed up to the point where there was no reactivity at two consecutive five-fold dilutions, as shown by obtaining zero positive results for all five replicates at a tested concentration.

Table 10 below shows the minimum reactive concentration for the 10 influenza strains.

Table 10: CDC Human Influenza Virus Panel for 2017 Results

Influenza Virus	Strain ID	Influenza strain designation	Minimum Reactive Concentration (EID_{50}/mL)	Detection Rate
A (H1N1)	1	A/Brisbane/59/2007	$6.4x10^{4.3}$	20.0% (1/5)
	2	A/Fujian Gulou/1896/2009	$3.2x10^{5.2}$	100% (5/5)
A (H3N2)	3	A/Perth/16/2009	$8.0x10^{5.9}$	100% (5/5)
	4	A/Hong Kong/4801/2014	$6.4x10^{2.9}$	40.0% (2/5)
A (H1N1)pdm09	5	A/California/07/2009	$3.2x10^{5.5}$	60.0% (3/5)
	6	A/Michigan/45/2015	$1.6x10^{5.3}$	100% (5/5)
B (Victoria lineage)	7	B/Brisbane/60/2008	$3.2x10^{5.9}$	100% (5/5)
	8	B/Texas/02/2013	$1.6x10^{6.1}$	100% (5/5)
B (Yamagata lineage)	9	B/Wisconsin/01/2010	$1.6x10^{3.9}$	100% (5/5)
	10	B/Phuket/3073/2013	$1.6x10^{6.9}$	100% (5/5)

CDC Human Influenza Virus Panel for 2018

This study was performed to determine the minimum reactive concentration of the CDC Human Influenza Virus Panel for 2018 with the Acucy Influenza A&B Test. Eight (8) characterized strains were used in this study, composed of four strains of Influenza A (2 H1N1pdm09 strains and 2 H3N2 strains), and four strains of Influenza B (2 Victoria lineage strains and 2 Yamagata lineage strains). The strains were obtained from the CDC, as part of the Human Influenza Virus Panel for 2018. Each strain was serially diluted 1:5 in viral transport media. For each replicate, 50 µL was applied to a nasopharyngeal swab and allowed to dry for one minute before being processed following the instructions for the Acucy Influenza A&B Test. Testing was performed in replicates of five using the READ NOW mode. Testing was performed up to the point where there was no reactivity at two consecutive five-fold dilutions, as shown by obtaining zero positive results for all five replicates at a tested concentration.

Table 11 below shows the minimum reactive concentration for the eight Influenza strains.

Table 11: CDC Human Influenza Virus Panel for 2018 Results

Influenza Virus	Strain ID	Influenza strain designation	Minimum Reactive Concentration (EID_{50}/mL)	Detection Rate
A (H3N2)	1	A/Perth/16/2009	$6.4x10^{3.9}$	60% (3/5)
	2	A/Singapore/INFIMH-16-0019/2016	$1.6x10^{5.2}$	100% (5/5)
A (H1N1)pdm09	3	A/California/07/2009	$6.4x10^{3.9}$	100% (5/5)
	4	A/Michigan/45/2015	$6.4x10^{3.2}$	40% (2/5)
B (Victoria lineage)	5	B/Brisbane/60/2008	$1.6x10^{5.5}$	100% (5/5)
	6	B/Colorado/06/2017	$1.6x10^{6.4}$	100% (5/5)
B (Yamagata lineage)	7	B/Wisconsin/01/2010	$3.2x10^{4.9}$	100% (5/5)
	8	B/Phuket/3073/2013	$3.2x10^{4.5}$	100% (5/5)

f. Analytical specificity

Cross-Reactivity Study

This study was performed to evaluate the potential of the Acucy Influenza A&B Test to cross-react with non-target microbial organisms, representing common respiratory pathogens or flora, resulting in a false positive result. A total of 41 organisms (viral, bacterial, and fungal) and human DNA were tested in triplicate with the Acucy Influenza A&B Test. Potentially cross-reactive bacteria and yeast representing common respiratory pathogens or flora were tested at a final concentration of $\geq 1 \times 10^6$ Colony-Forming Units/mL (CFU/mL) or Color Changing Units/mL (CCU/mL), or highest concentrations available for testing. Potentially interfering common respiratory viral pathogens or flora were tested at a final test concentration of $\geq 1 \times 10^5$ 50% Tissue Culture Infective Dose/mL ($TCID_{50}$/mL), Particle Forming Units/mL (pfu/mL), or copies/mL, or highest concentrations available for testing. Human genomic DNA was tested at a final test concentration of 1×10^4 copies/mL. The diluted organisms or human DNA sample (10 uL) were added to the extraction buffer to yield the desired testing concentrations. Three replicates of each sample were tested. Testing was performed using the READ NOW mode.

Results of the cross-reactivity study are summarized in Table 12 below.

Table 12: Summary Results of the Cross-Reactivity Study

Organism	**Final Concentration Tested**	**Flu A Positive Replicates**	**Flu B Positive Replicates**
Adenovirus type 1	1 X 10^5 $TCID_{50}$/mL	0/3	0/3
Adenovirus type 7A	1 X 10^5 $TCID_{50}$/mL	0/3	0/3
Bordetella pertussis	1 X 10^6 CFU/mL	0/3	0/3
Candida albicans	1 X 10^6 CFU/mL	0/3	0/3
Coronavirus	2.71 X 10^4 $TCID_{50}$/mL	0/3	0/3
Corynebacterium ulcerans	> 1.11 X 10^3 CFU/mL	0/3	0/3
Coxsackie virus	1 X 10^5 $TCID_{50}$/mL	0/3	0/3
Cytomegalovirus (CMV)	8 X 10^4 $TCID_{50}$/mL	0/3	0/3
Epstein-Barr Virus (EBV)	1 X 10^5 copies/mL	0/3	0/3
Escherichia coli	1 X 10^6 CFU/mL	0/3	0/3
Haemophilus influenza	1 X 10^6 CFU/mL	0/3	0/3
Human Herpes Virus 6 (HHV6), Z29	1 X 10^5 copies/mL	0/3	0/3
Human Herpes Virus 7 (HHV7), SB Strain	1 X 10^5 $TCID_{50}$/mL	0/3	0/3
Klebsiella pneumoniae	1 X 10^6 CFU/mL	0/3	0/3
Lactobacillus acidophilus Z048	1 X 10^6 CFU/mL	0/3	0/3
Legionella pneumoniae	1 X 10^6 CFU/mL	0/3	0/3
Moraxella catarrhalis	1 X 10^6 CFU/mL	0/3	0/3
Mycoplasma hominis	1 X 10^6 CFU/mL	0/3	0/3
Mycoplasma pneumoniae	1.37 X 10^6 CCU/mL	0/3	0/3
Neisseria meningitides	1 X 10^6 CFU/mL	0/3	0/3
Neisseria gonorrhoeae	1 X 10^6 CFU/mL	0/3	0/3
Parainfluenza virus 1	8 X 10^4 $TCID_{50}$/mL	0/3	0/3

Parainfluenza virus 2	1 X 10^5 $TCID_{50}$/mL	0/3	0/3
Parainfluenza virus 3	1 X 10^5 $TCID_{50}$/mL	0/3	0/3
Pseudomonas aeruginosa *	1 X 10^6 CFU/mL	0/3	0/3
Staphylococcus aureus (MRSA)	1 X 10^6 CFU/mL	0/3	0/3
Staphylococcus aureus (MSSA)	1 X 10^6 CFU/mL	0/3	0/3
Staphylococcus epidermidis (MRSE)	1 X 10^6 CFU/mL	0/3	0/3
Streptococcus pneumoniae	2.13X 10^6 CFU/mL	0/3	0/3
Streptococcus salivarius	1 X 10^6 CFU/mL	0/3	0/3
Measles virus	4.70 X 10^4 $TCID_{50}$/mL	0/3	0/3
Mumps virus	9.34 X 10^4 $TCID_{50}$/mL	0/3	0/3
Metapneumovirus 3 type B1	8.98 X 10^4 $TCID_{50}$/mL	0/3	0/3
Metapneumovirus 9 type A1	1.61 X 10^5 $TCID_{50}$/mL	0/3	0/3
Rhinovirus	3.26 X 10^4 $TCID_{50}$/mL	0/3	0/3
Human genomic DNA	1 X 10^4 copies/mL	0/3	0/3
Chlamydia pneumoniae	> 9.60 X 10^2 pfu/mL	0/3	0/3
Enterovirus	1.11 X 10^5 $TCID_{50}$/mL	0/3	0/3
Mycobacterium tuberculosis (avirulent)	1 X 10^6 CFU/mL	0/3	0/3
Respiratory Syncytial virus type A2 (RSVA)	1 X 10^5 $TCID_{50}$/mL	0/3	0/3
Respiratory Syncytial virus type B (RSVB)	1 X 10^5 $TCID_{50}$/mL	0/3	0/3
Streptococcus pyogenes	> 1.92 X 10^3 CFU/mL	0/3	0/3

* Required re-testing.

The results show there is no observed cross-reactivity with the organisms or human genomic DNA at the concentrations tested. *Pseudomonas aeruginosa* was re-tested after the original data showed 1 of the 3 replicates positive for Flu B. The re-test confirmed that the microorganism is not cross-reactive in the Acucy Influenza A&B Test. Upon investigation, it was determined that the false positive replicate was caused by particulate matter on the Read Window of the Test Cassette.

Microbial Interference Study

Microbial interference was assessed for the Acucy Influenza A&B Test using the same 42-member test panel as the one used in the Cross-Reactivity Study above. The organism or human genomic DNA was mixed with either influenza A or influenza B in the same sample to determine if the presence of the non-target organism or human genomic DNA would interfere with the detection of the influenza analyte using the Acucy Influenza A&B Test.

Contrived samples with Influenza A (A/Hong Kong 4801/14) or Influenza B (B/Phuket/3073/13) were prepared at a concentration of approximately 2x LoD. Influenza samples were prepared in viral transport media (VTM), and 50 uL was applied to nasopharyngeal swabs, which were allowed to dry for one hour prior to testing. The diluted organisms (10 uL) were added to the extraction buffer to yield the desired concentration for testing before introducing the contrived sample swab. Three replicates of each sample were tested. Testing was performed using the READ NOW

mode.

Results of the Microbial Interference Study are summarized in Table 13 below.

Table 13: Summary Results of the Microbial Interference Study

Organism	Final Concentration Tested	Flu A Positive Sample (2xLoD)		Flu B Positive Sample (2xLoD)	
		Flu A Positive Replicates	Flu B Positive Replicates	Flu A Positive Replicates	Flu B Positive Replicates
Adenovirus type 1	1 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Adenovirus type 7A	1 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Bordetella pertussis	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Candida albicans	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Coronavirus	2.71 X 10^4 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
*Corynebacterium ulcerans**	> 1.11 X 10^3 CFU/mL	3/3	0/3	0/3	3/3
Coxsackie virus	1 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Cytomegalovirus (CMV)	8 X 10^4 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Epstein-Barr Virus (EBV)	1 X 10^5 copies/mL	3/3	0/3	0/3	3/3
Escherichia coli	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Haemophilus influenza	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Human Herpes Virus 6 (HHV6), Z29	1 X 10^5 copies/mL	3/3	0/3	0/3	3/3
Human Herpes Virus 7 (HHV7), SB Strain	1 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Klebsiella pneumoniae	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Lactobacillus acidophilus Z048	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Legionella pneumoniae	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Moraxella catarrhalis	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Mycoplasma hominis	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Mycoplasma pneumoniae	1.37 X 10^6 CCU/mL	3/3	0/3	0/3	3/3
Neisseria meningitides	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Neisseria gonorrhoeae	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Parainfluenza virus 1	8 X 10^4 $TCID_{50}$/mL	3/3	0/3	0/3	3/3

Parainfluenza virus 2	1 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Parainfluenza virus 3	1 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Pseudomonas aeruginosa	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Staphylococcus aureus (MRSA)	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Staphylococcus aureus (MSSA)	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Staphylococcus epidermidis (MRSE)	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Streptococcus pneumoniae	2.13X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Streptococcus salivarius	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Measles Virus	4.70 X 10^4 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Mumps	9.34 X 10^4 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Metapneumovirus 3 type B1	8.98 X 10^4 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Metapneumovirus 9 type A1	1.61 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Rhinovirus	3.26 X 10^4 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Human genomic DNA	1 X 10^4 copies/mL	3/3	0/3	0/3	3/3
Chlamydia pneumoniae	> 9.60 X 10^2 pfu/mL	3/3	0/3	0/3	3/3
Enterovirus	1.11 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Mycobacterium tuberculosis (avirulent)	1 X 10^6 CFU/mL	3/3	0/3	0/3	3/3
Respiratory Syncytial virus type A2 (RSVA)	1 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Respiratory Syncytial virus type B (RSVB)	1 X 10^5 $TCID_{50}$/mL	3/3	0/3	0/3	3/3
Streptococcus pyogenes	> 1.92 X 10^3 CFU/mL	3/3	0/3	0/3	3/3

* Required re-testing.

The results show that the tested organisms and human genomic DNA at the concentrations tested in this study did not interfere with the detection of influenza A or influenza B samples at close to the LoD concentrations. One sample for the Microbial Interference study was re-tested. *Corynebacterium ulcerans* had 1 of 3 replicates negative for the Influenza B positive sample. The sample was re-tested per protocol and resulted in 3/3 positive.

g. Competitive Interference Study

Since the Acucy Influenza A&B Test detects two analytes (influenza A and B), a competitive interference study was conducted to determine whether viral target analytes at a high viral concentration would interfere with the detection of a second target at near LoD concentrations in co-infected samples.

A high-titer target at a medically relevant level (≥1 x 10^4 $TCID_{50}$/mL), or VTM only, was mixed with the second target at a low-titer level (approximately 4x LoD). Viral strains used in this study were A/California/07/09 (H1N1pdm09), A/Hong Kong/4801/14 (H3N2), and B/Phuket/3073/13. Testing was performed in triplicate with the Acucy Influenza A&B Test to determine whether detection of the low-titer targets was affected by the presence of the high-titer strain. The contrived samples were prepared according to the scheme shown in Table 14 below. High-titer strains or VTM were mixed in a tube with low-titer strains to the concentrations in Table 14. Then 50 µL was applied to a swab and allowed to dry for 1 hour before testing. Testing was performed using the READ NOW mode.

Table 14: Competitive Interference Study Testing Scheme

Sample	**Source 1 (High Titer Strain or VTM)**		**Source 2 (Low Titer Strain)**	
	Virus	**$TCID_{50}$/mL**	**Virus**	**Multiples of LoD**
1	Flu A (H1N1pdm09)	2 x 10^4	Flu B	4x LoD
2	Flu B	2 x 10^4	Flu A (H1N1pdm09)	4x LoD
3	VTM	N/A	Flu A (H1N1pdm09)	4x LoD
4	VTM	N/A	Flu B	4x LoD
5	Flu A (H3N2)	2 x 10^4	Flu B	4x LoD
6	Flu B	2 x 10^4	Flu A (H3N2)	4x LoD
7	VTM	N/A	Flu A (H3N2)	4x LoD

Results of the Competitive Interference Study using high and low viral concentration combinations are shown in Table 15 below. No interference was observed at the concentrations tested in this study.

Table 15: Summary of Competitive Interference Results

Sample	**Source 1**	**Source 2**	**Flu A Positive Replicates**	**Flu B Positive Replicates**
1	Flu A (H1N1pdm09) High Concentration	Flu B Low Concentration	3/3	3/3
2	Flu B High Concentration	Flu A (H1N1pdm09) Low Concentration	3/3	3/3
3	VTM	Flu A (H1N1pdm09) Low Concentration	3/3	0/3
4	VTM	Flu B Low Concentration	0/3	3/3
5	Flu A (H3N2) High Concentration	Flu B Low Concentration	3/3	3/3

6	Flu B High Concentration	Flu A (H3N2) Low Concentration	3/3	3/3
7	VTM	Flu A (H3N2) Low Concentration	3/3	0/3

h. Potentially Interfering Substances Study

A total of 23 potentially interfering substances, either naturally present in respiratory specimens or artificially introduced into the nasal cavity or nasopharynx, were tested in this study to evaluate the susceptibility of the Acucy Influenza A&B Test to interference when elevated levels of these substances were added to influenza A and influenza B positive samples.

Positive influenza A and influenza B contrived samples were prepared by diluting viral strains (A/Hong Kong/4801/14 and B/Phuket/3073/13) in Viral Transport Media (VTM) to approximately 2x LoD. The potentially interfering substances were spiked into the influenza A and influenza B samples at elevated levels. Two controls were also run, samples spiked with VTM only and samples spiked with no interfering substances. Samples (50 µL) were applied to nasopharyngeal swabs and tested in triplicate with the Acucy Influenza A&B Test. Testing was performed using the READ NOW mode.

Concentrations of potentially interfering substances tested and the study results are summarized in Table 16 below.

Table 16: Summary Results of the Potentially Interfering Substances Study

Potentially Interfering Substance	**Active Ingredient**	**Concentration Tested**	**Flu A Positive Sample (2xLoD)**		**Flu B Positive Sample (2xLoD)**	
			Flu A Positive Replicates	**Flu B Positive Replicates**	**Flu A Positive Replicates**	**Flu B Positive Replicates**
No substance (Control)	N/A	N/A	3/3	0/3	0/3	3/3
VTM (Control)	VTM	N/A	3/3	0/3	0/3	3/3
Mucus (Bovine)	Mucin protein	19 mg/mL	3/3	0/3	0/3	3/3
Whole Blood with EDTA	N/A	5% v/v	3/3	0/3	0/3	3/3
Tylenol	Acetaminophen	0.1 mg/mL	3/3	0/3	0/3	3/3
NSAIDs	Aspirin	16.2 mg/mL	3/3	0/3	0/3	3/3
	Ibuprofen	40 mg/mL	3/3	0/3	0/3	3/3
	Naproxen	110 mg/mL	3/3	0/3	0/3	3/3
Nasal Corticosteroids	Dexamethasone (injection)	3 mg/mL	3/3	0/3	0/3	3/3

	Dexamethasone (oral)	0.5 mg/mL	3/3	0/3	0/3	3/3
	Fluticasone	50 ug/mL	3/3	0/3	0/3	3/3
	Mometasone furoate	2.5 µg/mL	3/3	0/3	0/3	3/3
	Budesonide	25 µg/mL	3/3	0/3	0/3	3/3
	Flunisolide	68.75 µg/mL	3/3	0/3	0/3	3/3
	Triamcinolone acetonide	5.5 µg/mL	3/3	0/3	0/3	3/3
	Beclomethasone	16 µg/mL	3/3	0/3	0/3	3/3
Nasal Sprays	Oxymetazoline	0.025% v/v	3/3	0/3	0/3	3/3
	Phenylephrine	0.5% v/v	3/3	0/3	0/3	3/3
	Sodium chloride	0.325% v/v	3/3	0/3	0/3	3/3
Nasal Gel	Galphima glauca, Luffa operculate	4x, 4x	3/3	0/3	0/3	3/3
Antiviral	Oseltamivir	5 mg/mL	3/3	0/3	0/3	3/3
Antibacterial, systemic	Tobramycin	40.0 µg/mL	3/3	0/3	0/3	3/3
Throat Lozenges	Benzocaine	2.5% soln.	3/3	0/3	0/3	3/3
Antibiotic Nasal Ointment	Mupirocin	0.15 mg/mL	3/3	0/3	0/3	3/3
Allergy Medicine	Histaminum hydrochloricum	1% solution	3/3	0/3	0/3	3/3

The study results shows that no interference with the Acucy Influenza A&B Test was observed in the presence of potentially interfering substances at the concentrations tested in this study.

i. Carry-Over Study

This study evaluated the potential of sample carry-over between test cassettes when high-titer (1 x 10^5 $TCID_{50}$/mL) samples were run in an alternating fashion with negative samples.

High titer influenza A samples (n=30) were prepared using the A/Hong Kong/4801/14 strain diluted in VTM to a concentration of 1 x 10^5 $TCID_{50}$/mL. High titer influenza B samples (n=30) were prepared using the B/Phuket/3073/13 strain diluted in VTM to a concentration of 1 x 10^5 $TCID_{50}$/mL. Negative samples (n=60) were prepared using VTM. Dilutions (80 µL) were applied to nasal swabs, allowed to dry for up to one minute, and tested using the Acucy Influenza A&B Test using the READ NOW mode. Positive and negative samples were alternated so that a negative sample was always

run immediately after a positive sample. A false positive result for a negative sample would suggest contamination from the previous test.

All influenza A and influenza B samples gave positive results as expected. All negative samples gave negative results, as summarized in Table 17 below.

Table 17: Summary of Carry-Over Study Results

Sample	**Flu A Positive Replicates**	**Flu B Positive Replicates**	**Negative Replicates**
Influenza A High Positive	30/30	0/30	0/30
Negative	0/30	0/30	30/30
Sample	**Flu A Positive Replicates**	**Flu B Positive Replicates**	**Negative Replicates**
Influenza B High Positive	0/30	30/30	0/30
Negative	0/30	0/30 *	30/30 *

* One of the 30 negative replicates tested in the influenza B Carry-Over Study initially generated a false positive Flu B result. This replicate generated a negative result upon retesting using a new test cassette.

j. Assay cut-off

Primary Cut-off

The initial cutoff of 5.0 milli-absorbance units (mAbs) was established as a default setting for the Acucy Reader by the instrument developer.

Sixty (60) negative samples, 60 influenza A positive (1x LoD) samples, and 60 influenza B positive samples (1x LoD) were tested with two lots of the Acucy Influenza A&B Test to verify the initial assay cutoff. All contrived samples were prepared in pooled Clinical Nasal Matrix (CNM). Negative samples were CNM only. An influenza A strain (A/Hong Kong/4801/14) was diluted to approximately 1x LoD in CNM. An influenza B strain (B/Brisbane/60/2008) was diluted to approximately 1x LoD in CNM. Clinical nasal matrix was obtained from a vendor. The pooled matrix was prepared by the vendor from leftover negative samples (from a clinical study for another investigational device) that were confirmed negative for influenza A and influenza B with an FDA-cleared molecular test. For each replicate, 50 µL of each contrived sample were applied to a nasopharyngeal swab and dried one minute before extraction and testing. Testing was performed using the READ NOW mode.

Data from the Positive influenza A and B samples in this study showed separation of the positive signal above the cutoff and no examples of false positives. Data from the negative samples in this study were used to calculate the Limit of Blank (LoB) using the Classical Approach (non-parametric analysis) decribed in the CLSI EP17-A2, Evaluation of Detection Capability for the Clinical Laboratory Measurement Procedures; Approved Guideline – Second Ed. June 2012. The calculated LoB values from this study, 4.71 mAbs for influenza A and 3.51 for influenza B, represented the maximal values determined for influenza A and influenza B, respectively. These data

supported the initial cutoff of 5.0 mAbs for both the influenza A line and the influenza B line.

Clinical validation of the initial 5.0 mAbs cutoff was performed using the clinical dataset from a previously conducted clinical study during the 2016-2017 influenza season. In total, 1252 clinical samples were analyzed for both test lines in the Receiver Operator Characteristic (ROC) analysis. From this ROC analysis, the optimal cutoff for influenza A was determined to be 6.4 mAbs, with a sensitivity of 88.6% and a specificity of 95.4%. For influenza B, the optimal cutoff was determined to be 5.4 mAbs with a sensitivity of 84.4% and a specificity of 96.5%.

Secondary Cut-off

The Acucy Reader uses a secondary cutoff in the event that one analyte has a result that is ≥ 350 mAbs. This cutoff was set to 26.0 mAbs for influenza A and B, as high titer samples have been shown to generate higher levels of non-specific binding at the second test line, resulting in signals greater than 5.0 mAbs and a false positive result for the second analyte. A study was performed to verify the secondary cutoff. Different concentrations of influenza A and influenza B were tested alone or combined (simulating a co-infected sample).

Contrived samples were prepared by diluting an influenza A viral strain (A/Hong Kong/4801/14) and/or an influenza B viral strain (B/Brisbane/60/2008) in VTM. Samples were prepared at three levels: a concentration to produce a result of > 350 mAbs (High); a concentration to produce a result of 30-50 mAbs (Mid); and a concentration to produce a result of < 25 mAbs (Low).

Data from this study and the Carry-Over Study validated the effectiveness of this secondary cutoff in preventing the reporting of false positive results for the second analyte when one analyte is present in very high concentrations.

Cut-off Validation

Both the primary and the secondary cut-offs were further validated during the prospective clinical study conducted during the 2017-2018 influenza season.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      Not applicable. Performance of the Acucy Influenza A&B Test was evaluated against a compostite reference result, which was calculated from the results of three reference methods: two FDA-cleared molecular methods and cell culture, in a multi-site prospective clinical study.

   b. *Matrix comparaison:*

Not applicable.

3. Clinical Studies:

Clinical performance characteristics of the Acucy Influenza A&B Test with the Acucy Reader were evaluated in a multi-center prospective study during the 2017-2018 influenza season in the U.S. To be enrolled in the study, patients had to present at the participating study centers with influenza-like symptoms as defined in the clinical study protocol, and to provide informed consents.

Patient subjects were randomized to have either two nasal swabs or two nasopharyngeal swabs collected according to standard collection methods (i.e., even numbered subjects had two nasal swabs collected and odd numbered subjects had two nasopharyngeal swabs collected). The paired nasal swabs or nasopharyngeal swabs were obtained from each subject from the same nostril. The paired swabs were collected in no set order for testing under the clinical study protocol (i.e., one for Acucy Influenza A&B Test and the other for reference methods testing). Any swab specimens required for standard of care testing were collected prior to the specimens for this investigation; nasal washes/aspirates for standard of care testing excluded a patient from study participation.

Sixteen (16) geographically diverse Point of Care (POC) clinical sites (CLIA waived sites) participated in the study. At least one, but no more than three untrained CLIA waived operators performed testing at each site. Testing personnel were blinded to the standard of care test results for the specimens they tested. Two reference labs participated in the study to perform reference methods testing of the samples. Samples for testing by the reference labs were prepared as directed by the reference testing laboratories and sent by courier on the day of sample collection to the reference lab for receipt the following day. Testing at the reference laboratories was performed by trained laboratory personnel.

One of the paired nasal or nasopharyngeal swab was used for immediate testing with the Acucy Influenza A&B Test per the instructions for use. Each site was supplied with two Acucy Readers. One Acucy Reader was used to test specimens in the "READ NOW" mode and the second in the "WALK AWAY/NORMAL" mode. The other nasal or nasopharyngeal swab of the pair was rotated in 3.0 mL of viral transport media (VTM) for 10 seconds within one hour of specimen collection. The swab was subsequently removed and discarded. The sample eluted in VTM was stored at 2-8ºC until transport was made on the same day as specimen collection on ice-packs in an insulated container. The samples collected in VTM were tested by the reference methods within the allowable time frames of specimen collection per the standard reference laboratory cell culture procedure or the Instructions for Use for the FDA-cleared molecular tests.

The performance of the Acucy Influenza A&B Test with the Acucy Reader was evaluated against a compostite reference result, which was calculated from the results of three reference methods: two FDA-cleared molecular methods and cell culture. The composite reference result for each specimen tested by all three reference

methods was based on the following algorithm:

- At least two out of three reference methods positive = positive result for either Flu A or B;
- One out of three reference methods positive = negative result for either Flu A or B;
- At least two out of three reference methods negative = negative for either Flu A or B.

Nasal or nasopharyngeal swab specimens 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. Additional nine samples generated a final invalid result with the Acucy Influenza A&B Test, resulting in a total of 1003 samples with valid results.

Patient age and gender distribution for the 1003 prospective specimens is presented in Table 18 below.

Table 18: Patient Demographics

Age Group	**Number**	**Percent of Patients**
≤ 5 years	326	32.5%
6 to 21 years	406	40.5%
22 to 59 years	209	20.8%
≥ 60 years	62	6.2%
Total	1003	100%
Sex	**Number**	**Percent of Patients**
Male	514	51.2%
Female	489	48.8%
Total	1003	100%

Compared to the composite reference algorithm described above, the performance estimates of the Acucy Influenza A&B Test for influenza A and influenza B are presented in Table 19 below.

Table 19: Influenza A and B Performance (All Nasal and Nasopharyngeal Swab Specimens; Both READ NOW and WALK/AWAY NORMAL Modes)

Flu A	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	216	31	247
Acucy Negative	8	748	756
Total	224	779	1003
Sensitivity = 96.4% (216/224), 95%CI: 93.1% to 98.2%			
Specificity = 96.0% (748/779), 95%CI: 94.4% to 97.2%			
Flu B	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	130	16	146
Acucy Negative	28	829	857
Total	158	845	1003
Sensitivity = 82.3% (130/158), 95%CI: 75.6% to 87.4%			
Specificity = 98.1% (829/845), 95%CI: 96.9% to 98.8%			

Compared to the composite reference algorithm, the performance of the Acucy Influenza A&B Test for influenza A and influenza B stratified by swab types are presented in Table 20 and Table 21 below.

Table 20: Influenza A and B Performance (Nasal Swab Specimens Only; Both READ NOW and WALK/AWAY NORMAL Modes)

Flu A	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	96	16	112
Acucy Negative	4	379	383
Total	100	395	495
Sensitivity = 96.0% (96/100), 95%CI: 90.2% to 98.4%			
Specificity = 95.9% (379/395), 95%CI: 93.5% to 97.5%			
Flu B	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	61	9	70
Acucy Negative	14	411	425
Total	75	420	495
Sensitivity = 81.3% (61/75), 95%CI: 71.1% to 88.5%			
Specificity = 97.9% (411/420), 95%CI: 96.0% to 98.9%			

Table 21: Influenza A and B Performance (Nasopharyngeal Swab Specimens Only; Both READ NOW and WALK/AWAY NORMAL Modes)

Flu A	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	120	15	135
Acucy Negative	4	369	373
Total	124	384	508
Sensitivity = 96.8% (120/124), 95%CI: 92.0% to 98.7%			
Specificity = 96.1% (369/384), 95%CI: 93.7% to 97.6%			
Flu B	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	69	7	76
Acucy Negative	14	418	432
Total	83	425	508
Sensitivity = 83.1% (69/83), 95%CI: 73.7% to 89.7%			
Specificity = 98.4% (418/425), 95%CI: 96.6% to 99.2%			

Compared to the composite reference algorithm, the performance of the Acucy Influenza A&B Test for influenza A and influenza B stratified by read modes are presented in Table 22 and Table 23 below.

Table 22: Influenza A and B Performance (All Nasal and Nasopharyngeal Swab Specimens; READ NOW Mode Only)

Flu A	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	101	13	114
Acucy Negative	6	372	378
Total	107	385	492
Sensitivity = 94.4% (101/107), 95%CI: 88.3% to 97.4%			
Specificity = 96.6% (372/385), 95%CI: 94.3% to 98.0%			
Flu B	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	68	13	81
Acucy Negative	15	396	411
Total	83	409	492
Sensitivity = 81.9% (68/83), 95%CI: 72.3% to 88.7%			
Specificity = 96.8% (396/409), 95%CI: 94.6% to 98.1%			

Table 23: Influenza A and B Performance (All Nasal and Nasopharyngeal Swab Specimens; WALK/AWAY NORMAL Mode Only)

Flu A	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	115	18	133
Acucy Negative	2	376	432
Total	117	394	511
Sensitivity = 98.3% (115/117), 95%CI: 94.0% to 99.5%			
Specificity = 95.4% (376/394), 95%CI: 92.9% to 97.1%			
Flu B	Composite Reference Positive	Composite Reference Negative	Total
Acucy Positive	62	3	65
Acucy Negative	13	433	446
Total	75	436	511
Sensitivity = 82.7% (62/75), 95%CI: 72.6% to 89.6%			
Specificity = 99.3% (433/436), 95%CI: 98.0% to 99.8%			

Compared to the composite reference algorithm, the performance of the Acucy Influenza A&B Test for influenza A and influenza B stratified by patient age groups are presented in Table 24 below.

Table 24: Influenza A and B Performance Stratified by Patient Age Groups

Influenza Type	**≤ 5 Years of Age (n=326)**		**6 to 21 Years of Age (n=406)**		**≥ 22 Years of Age (n=271)**	
Influenza A	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	98.6%	94.9%	96.8%	96.5%	89.3%	96.7%
	(71/72)	(241/254)	(120/124)	(272/282)	(25/28)	(235/243)
	92.5% - 99.8%	91.4% - 97.0%	92.0% - 98.7%	93.6% - 98.1%	72.8% - 96.3%	93.6% - 98.3%
Influenza B	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	96.6%	97.3%	83.5%	98.4%	70.5%	98.7%
	(28/29)	(289/297)	(71/85)	(316/321)	(31/44)	(224/227)
	82.8% - 99.4%	94.8% - 98.6%	74.2% - 89.9%	96.4% - 99.3%	55.8% - 81.8%	96.2% - 99.5%

Compared to the composite reference algorithm, the performance of the Acucy Influenza A&B Test for influenza A and influenza B in the prospective clinical study was also analyzed by study site. The Acucy Influenza A&B Test demonstrated similar performance across the 16 study sites for influenza A and influenza B, respectively.

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

The Acucy Influenza A&B Test detected three mixed influenza A and B infections in this prospective clinical evaluation. Of the three samples tested positive for influenza A and influenza B by the Acucy Influenza A&B Test, the composite reference result for one of the three sample was negative for influenza A and influenza

B, and the composite reference results for two of the three samples were influenza B positive only.

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   In the Acucy Influenza A&B Test prospective clinical study (described in the "Clinical Studies" section above), a total of 1003 nasal or nasopharyngeal swab specimens were determined to be evaluable during the 2017-2018 influenza season. The number and percentage of influenza A and influenza B positive cases per specified age group, as determined by the Acucy Influenza A&B Test, are presented in Table 25 and Table 26 below, respectively.

Table 25: Influenza A Positives by the Acucy Influenza A&B Test per Patient Age Group

Age Group	Number of Nasal or Nasopharyngeal Swab Specimens	Number of Influenza A Positives	Influenza A Positivity Rate
≤ 5 years	326	84	25.8%
6 to 21 years	406	130	32.0%
≥ 22 years	271	33	12.2%
Total	1003	247	24.6%

Table 26: Influenza B Positives by the Acucy Influenza A&B Test per Patient Age Group

Age Group	Number of Nasal or Nasopharyngeal Swab Specimens	Number of Influenza B Positives	Influenza B Positivity Rate
≤ 5 years	326	36	11.0%
6 to 21 years	406	76	18.7%
≥ 22 years	271	34	12.5%
Total	1003	146	14.6%

N. Instrument Name:

Acucy Reader

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes ___X_____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No __X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes _____X___ or No _____

3. Specimen Identification:

   Operator labels the Extraction Buffer vial with the extracted patient sample with patient identification. Operator enters the patient identification into the Acucy 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. Operator then opens the Test Cassette foil pouch, labels the Test Cassette with the patient ID. Test Cassette scanned automatically by the reader once it is inserted in the reader.

4. Specimen Sampling and Handling:

   An operator first collects a patient NS or NPS sample. The operator removes the cap off an Extraction Buffer vial, and inserts the NS or NPS sample into the Extraction Buffer vial, and vigorously mixes against the side of the vial 10 times while submerged. The operator then removes the swab while squeezing the vial to remove the liquid from the swab, properly discards the swab, adds dropper tip to the Extraction Buffer vial, presses to seal the vial, and labels 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. 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. 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. 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. 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.

5. 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.

6. Quality Control:

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

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable.

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.