510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I. Background Information:

A 510(k) Number

K241188

B Applicant

Sekisui Diagnostics, LLC

C Proprietary and Established Names

Acucy Influenza A&B Test with the Acucy 2 System

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PSZ	Class II	21 CFR 866.3328 - Influenza Virus Antigen Detection Test System	MI - Microbiology

II. Submission/Device Overview:

A Purpose for Submission:

To obtain 510(k) clearance of the Acucy Influenza A&B Test with the Acucy 2 System

B Measurand:

Influenza A and influenza B viral nucleoprotein antigens

C Type of Test:

Qualitative immunoassay using an optoelectronic reader

III. Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Acucy Influenza A&B Test is a rapid chromatographic immunoassay for the qualitative detection and differentiation of influenza A and B viral nucleoprotein antigens directly from anterior nasal and nasopharyngeal swabs from patients with signs and symptoms of respiratory infection. The test is intended for use with the Acucy or Acucy 2 Reader 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.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Acucy 2 Reader

IV. Device/System Characteristics:

A Device Description:

The Acucy Influenza A&B Test is a lateral flow immunochromatographic assay in the sandwich immunoassay format for the direct and qualitative detection and differentiation of influenza A and B viral nucleoprotein antigens from anterior nasal and nasopharyngeal swabs of symptomatic patients. The test is automatically analyzed on the Acucy 2 Reader and should not be visually read. The Acucy Influenza A&B Test components/reagents have not been modified from those originally cleared in K182001.

B Principle of Operation:

The Acucy Influenza A&B Test allows for the differential detection of influenza A and influenza B antigens, when used with the Acucy 2 Reader. The patient swab sample is placed in the Extraction Buffer vial, during which time the virus particles in the sample are disrupted,

exposing internal viral nucleoproteins. After disruption, the sample is dispensed into the Test Cassette's sample well where it solubilizes reagents necessary for the detection of the viral antigens. From the sample well, the sample migrates along the membrane surface. If influenza A or B viral antigens are present, they will form a complex with the respective solubilized colloidal gold conjugated mouse monoclonal antibody to influenza A and/or B nucleoproteins. The complex will then continue to migrate until it is bound by rat anti-influenza A and/or mouse anti-influenza B antibodies that are immobilized at the test lines of the cassette's nitrocellulose membrane.

Depending upon the operator's choice, the Test Cassette is either immediately placed inside the Acucy 2 Reader for automatically timed development mode (WALK AWAY Mode) or placed on the counter or bench top for a manually timed development and then placed into the Acucy 2 Reader to be scanned (READ NOW mode). In either mode, upon completion of the development time, the Acucy 2 Reader scans the Test Cassette to measure the absorbance intensity at the test and control lines. These absorbance units are then processed to generate a final qualitative result using a method-specific algorithms.

The Acucy 2 Reader then displays the test results on the screen as either POS (+) or NEG (-) for each viral analyte, or as INVALID. The results can also be automatically printed on the optional printer.

There are five possible results:

- FLU A POS (+)/FLU B NEG (-)
- FLU A NEG (-)/FLU B POS (+)
- FLU A NEG (-)/FLU B NEG (-)
- FLU A POS (+)/FLU B POS (+)
- INVALID

Any INVALID result should be retested with a new patient sample and a new Test Kit.

FLU A and FLU B single positive results, and FLU A and B dual positive results do not rule out co-infection with other pathogens, nor do they identify any specific Influenza A or B virus subtypes.

Co-infection with Influenza A and B (i.e., FLU A and B dual positive results) is rare and it is therefore recommended that a FLU A and FLU B dual positive sample be re-tested with a new sample and a new test kit. 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.

Materials Provided

- 25 Test Cassettes: individually foil pouched with desiccant
- 25 Sterile anterior nasal swabs
- 25 Extraction Buffer vials each containing 0.4 mL phosphate buffered salt solution with 0.09% sodium azide as a preservative
- 25 Extraction Vial Dropper Tips
- 1 Influenza A+/B- Control Swab (Formalin inactivated Influenza A containing 0.05% sodium azide. Inactivity confirmed by inability of virus to infect cell culture)

- 1 Influenza A-/B+ Control Swab (Formalin inactivated Influenza A containing 0.05% sodium azide. Inactivity confirmed by inability of virus to infect cell culture)
- 2 separate Instructions for Use (IFU) documents for Acucy 2 Reader and Acucy Reader
- 2 separate Quick Reference Guides for Acucy 2 Reader and Acucy Reader (each containing instructions for READ NOW and WALK AWAY Modes)
- 2 separate External Quality Control (QC) Quick Reference Guides for the Acucy and the Acucy 2 Readers
- Workstation

Materials Required but Not Provided

- Acucy 2 Reader
- 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)

C Instrument Description Information:

1. Acucy 2 Reader Description:

   The Acucy 2 Reader is an optoelectronic reader that detects the insertion of an Acucy test cassette and reads the cassette barcode to detect the test information, prior to the drawer closing. Once it recognizes the test type, the user is prompted to close the drawer manually and the Acucy 2 Reader illuminates the cassette in the drawer and measures the intensity of the control and test lines.

   The Acucy 2 Reader uses a reflectance-based measurement method to evaluate the line signal intensities on the test strip, and a specific algorithm to determine the presence or absence of any target analyte(s). The Acucy 2 Reader determines the line intensity at each of the spatially-defined test and control line positions, interprets the results using a scoring algorithm, and reports a positive, negative, or invalid result based on pre-set thresholds. A liquid crystal display (LCD) on the Acucy 2 Reader communicates the testing progress and the final sample's test results to the operator.

   The Acucy 2 Reader supports the use of different assays by reading an assay-specific 2D barcode (2D) on the Test Cassette that identifies the test type (currently only Influenza A&B).

   The Reader has the ability to store and transfer test results via a USB memory drive.

2. Acucy 2 Reader Modes:

   Similar to the Acucy Reader, the Acucy 2 Reader has two modes of operation, the WALK AWAY and the READ NOW modes, described below:

   a. WALK AWAY Mode:

   In the WALK AWAY 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.

The following changes have been made to the "WALK AWAY" mode in the user interface:

- The user can now select WALK AWAY button from MAIN MENU
- All screens in WALK AWAY test mode have a blue progress bar at the top.
- The user does not log in to access this mode unlike Acucy 1.
- The user now enters the Patient ID or can, on the same screen, instruct the Acucy 2 to scan the barcode on the test cassette.
- The user now has a reference to the instructions for use (IFU) on the screen to access more details for sample preparation (if needed).
- The Analyzing Sample screen now has a progress bar indicating time to result (TTR).
- The user can run another test in WALK AWAY mode or return to MAIN MENU.

b. 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.

The following changes have been made on the "READ NOW" mode in the user interface.

- The user can now select READ NOW button from MAIN MENU
- All screens in READ NOW test mode have a blue progress bar at the top.
- The user does not log in to access this mode unlike the Acucy 1.

3. Calibration:

The calibration test is a required function for Acucy 2 Reader that checks the Reader optics and calculation systems using the calibration zone label affixed to the drawer. The calibration procedure is performed upon installation to activate the quality control and READ NOW/WALK AWAY functionality and is required every 30-days. The operator is prompted by the Reader to conduct calibration after 30-days has elapsed. The calibration procedure may also be performed as directed during troubleshooting or whenever the Reader date and time has been changed.

4. Quality Control:

There are three types of quality controls for the Acucy 2 and Acucy reader and Influenza A&B Test systems:

- Acucy 2 Reader calibration (described above)
- Internal control line integrated into the test strip of the Test Cassette (unchanged from K182001)
- External quality controls: Influenza A+/B- and A-/B+ Control Swabs (unchanged from K182001)

V. Substantial Equivalence Information:

A Predicate Device Name(s):

Acucy Influenza A&B Test with the Acucy System

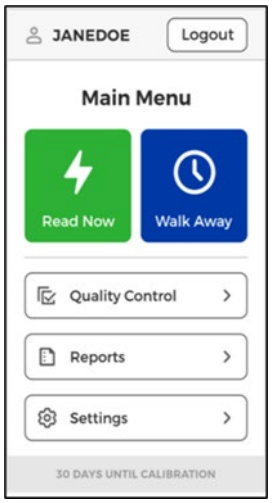
B Predicate 510(k) Number(s):

K182001

C Comparison with Predicate(s):

Device & Predicate Device(s):	K241188 (Candidate Device)	K182001 (Predicate Device)
Device Trade Name	Acucy Influenza A&B Test with the Acucy 2 Reader	Acucy Influenza A&B Test with the Acucy System
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Acucy Influenza A&B Test is a rapid chromatographic immunoassay for the qualitative detection and differentiation of influenza A and B viral nucleoprotein antigens directly from anterior nasal and nasopharyngeal swabs from patients with signs and symptoms of respiratory infection. The test is intended for use with the Acucy or Acucy 2 Reader 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.	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.

Device & Predicate Device(s):	K241188 (Candidate Device)	K182001 (Predicate Device)
	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	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.
Assay Results	Qualitative	Same
Assay Targets	Influenza A and B nucleoprotein antigens	Same
Assay Format	Lateral flow test cassette	Same
Sample Types	Anterior nasal swab and nasopharyngeal swab	Same
Assay Antibodies	Monoclonal antibodies to influenza A and B nucleoproteins.	Same
Additional Reagents	Extraction buffer with detergents	Same
Sample Transfer Method	Dropper tip applied to extraction vial to transfer extracted sample onto test cassette.	Same
Reporting of Results	Reader displays results on screen; or may be printed	Same
Time to Results	15 Minutes	Same
Instrument Modes	READ NOW or WALK AWAY	Same
Storage Temperature	Room Temperature	Same
Assay Controls	Internal procedural control	Same
Instrument Quality Control Features	• Scanning procedural control zone for adequate flow • Reader prevents use of used cassettes. • Reader prevents use of expired cassettes. • Reader prevents improper cassette insertion	Same
General Device Characteristic Differences		
Calibrator	External Calibration Device	Internal Calibration device.
Imaging Mode	Photodiode	CMOS Camera
Printer	Included	Optional

Device & Predicate Device(s):	K241188 (Candidate Device)	K182001 (Predicate Device)
LCD Display/Graphic User Interface		
Cybersecurity	• Removal of Master Password Card replaced with FORGOT PIN. • Allows adjustment of time of Auto-logout at supervisor level access.	• Operator/supervisor passwords • Unique Master Password Card • Auto-logout after 60 minutes

VI. Standards/Guidance Documents Referenced:

Document	Title	Publisher	Applicable Study
Special Controls 21 CFR 862.2160	Special Controls for Influenza virus antigen detection test systems	FDA/CDRH	All Studies
CLSI EP17-A2	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition	CLSI	Detection Limit
CLSI EP12-A2	Evaluation of Qualitative, Binary Output Examination Performance - 3rd Edition	CLSI	Analytical Studies
IEC/EN 61326-1Ed.3.0 b:2020	Electrical equipment for measurement, control, and laboratory use - EMC requirements - Part 1: General requirements	IEC/EN	EMC
ISO 11135:2014	Sterilization of health care products - Ethylene oxide Requirements for	ISO	Sterility

Document	Title	Publisher	Applicable Study
	development, validation and routine control of a sterilization process for medical devices.		
ISO 10993-7:2008	Biological evaluation of medical devices - Part 7: Ethylene oxide sterilization residuals	ISO	Sterility

VII. Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility

a. Precision/Reproducibility Panel:

In three different studies, the same seven-member sample panel was used to evaluate the Acucy Influenza A&B Test with the Acucy 2 Reader for repeatability, instrument-to-instrument precision, and between site reproducibility. Each panel member was made with two viral strains: Influenza A/Michigan/45/2015 Strain and Influenza B/Colorado/06/2017. Each strain was diluted into negative clinical matrix (NCM) at three different target levels (see Table 1 below):

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 for one (1) minute before testing per IFU was performed.

For MP and LP samples, the expected result is detection of virus (positive result). For HN and N samples, the expected result is the absence of virus (negative result) is, as summarized in Table 1 below:

Table 1. Sample Panel Tested in the Precision Study with Expected Detection Rates

Panel Member	Target Concentration		Target Positive Detection Rate
Negative	(N)	0X LoD	0%
High Negative Flu A or Flu B	(HN)	0.05X LoD	0-5%
Low Positive Flu A or Flu B	(LP)	0.95X LoD	≥95 %
Moderate Positive Flu A or Flu B	(MP)	2X LoD	100%

b. Repeatability (Within-Laboratory Precision):

The seven-member panel was tested at one internal site two (2) times per day (2 runs), each with two (2) replicates per run and for twenty (20) non-consecutive days. The testing was conducted by one (1) operator using one (1) lot of the Acucy Influenza A&B Test and one Acucy 2 Reader. All samples produced expected results as summarized in Table 2 below:

Table 2. Within-Laboratory Repeatability Study Results

Sample	Count	Agreement	95% CI
Flu A MP	80/80	100%	95.4% - 100%
Flu A LP	80/80	100%	95.4% - 100%
Flu A HN	80/80	100%	95.4% - 100%
Flu B MP	80/80	100%	95.4% - 100%
Flu B LP	80/80	100%	95.4% - 100%
Flu B HN	80/80	100%	95.4% - 100%
Negative	80/80	100%	95.4% - 100%

c. Instrument-to-Instrument Precision:

For between-instrument precision, the seven-member panel was tested on three (3) different Acucy 2 Readers over the course of five (5) days at the manufacturer's internal site. Testing was conducted by one operator using one lot of the Acucy Influenza A&B Test testing five (5) replicates per panel member and run. Twenty-five (25) replicates were obtained per panel member per instrument (1 operator x 5 replicates x 5 days), for a total of 75 replicates per panel member (1 operator x 5 replicates x 3 instruments x 5 days). All samples produced expected results as summarized in Table 3 below:

Table 3. Instrument-to-Instrument Precision Study Results

Sample	Reader #1	Reader #2	Reader #3	Overall (All Readers)	
	%Agreement (Count)	%Agreement (Count)	%Agreement (Count)	Agreement (Count)	95% CI
Flu A (MP)	100% (25/25)	100% (25/25)	100% (25/25)	100% (75/75)	95.1% - 100%
Flu A (LP)	100% (25/25)	100% (25/25)	100% (25/25)	100% (75/75)	95.1% - 100%
Flu A (HN)	100% (25/25)	100% (25/25)	100% (25/25)	100% (75/75)	95.1% - 100%
Flu B (MP)	100% (25/25)	100% (25/25)	100% (25/25)	100% (75/75)	95.1% - 100%
Flu B (LP)	100% (25/25)	100% (25/25)	100% (25/25)	100% (75/75)	95.1% - 100%
Flu B (HN)	100% (25/25)	100% (25/25)	100% (25/25)	100% (75/75)	95.1% - 100%
Negative	100% (25/25)	100% (25/25)	100% (25/25)	100% (75/75)	N/A

d. Multi-Site Reproducibility Study:

To assess between-site reproducibility, the seven-member panel was tested at three testing sites for five (5) non-consecutive days over a period of two (2) weeks. At each site, testing was conducted by two (2) operators using the same Acucy Influenza A&B Test lot and testing three (3) replicates per panel member and run. Each site had their own unique Acucy 2 Reader. A total of ninety (90) replicates for each panel member were tested across three (3) sites to evaluate reproducibility (2 operators x 3 replicates x 5 days x 3 sites).

This study was used to supplement the original reproducibility study used to support the clearance of the Acucy Influenza A&B Test with the Acucy System (K182001) to demonstrate that performance remains equivalent. Results were acceptable, and are summarized in Table 4 below:

Table 4. Multi-Site Reproducibility Study Results

Sample	Site #1	Site #2	Site #3	Overall (All Sites)	
	Agreement (Count)	Agreement (Count)	Agreement (Count)	Agreement (Count)	95% CI
Flu A (MP)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9-100.0%
Flu A (LP)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9-100.0%
Flu A (HN)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9-100.0%
Flu B (MP)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9-100.0%
Flu B (LP)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9-100.0%
Flu B (HN)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9-100.0%
Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9-100.0%

2. Analytical Specificity/Interference:

The analytical specificity and interference testing (Inclusivity, Cross Reactivity, Microbial Interference, Endogenous/Exogenous Interference, Competitive Interference) were conducted in support of K182001 and were leveraged for this submission. Refer to the Decision Summary of K182001 for further details.

3. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a. Instrument Calibration

The Acucy 2 Reader's optics and calculation systems are calibrated using the calibration zone label affixed to the drawer. This calibration is performed upon installation to activate the instrument's functionality and is required every 30-days. The operator is prompted by the Reader to conduct calibration after 30-days has elapsed. The calibration procedure may also be performed as directed during troubleshooting or whenever the Reader date and time has been changed.

b. Internal Quality Control

The Acucy Influenza A&B Test Cassette contains a built-in internal control feature. Each time a test is run in the Acucy 2 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/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.

c. External Quality Controls

The Acucy Influenza A&B Test with Acucy 2 Reader includes one Influenza A+/B- control swab (Red Label) and one Influenza A-/B+ control swab, each of which contains inactivated virus, for external quality control testing. These controls are identical to those used with the Acucy Reader and were validated in K182001.

d. Swab Equivalency

The test is intended for use with anterior nasal (ANS) and nasopharyngeal (NP) swabs. A swab equivalency test was performed in K182001 that demonstrated equivalent performance between ANS and NP swabs and was also used in support of this submission.

e. Test Mode Equivalency:

This study was performed to validate equivalent performance of the READ NOW and WALK AWAY test modes when reading Acucy Influenza A&B Test results on the Acucy 2 Reader.

Contrived positive Influenza A (A/Michigan/45/2015) and Influenza B (B/Colorado/06/2017) samples were prepared at 2X LoD, and negative samples were prepared in NCM. For each sample replicate, 50 µL of contrived sample was pipetted onto a swab and tested per the IFU of the test. Testing was conducted with one lot of the Acucy Influenza A&B Test and two unique Acucy 2 Readers.

Twenty (20) replicates of each contrived sample were tested using the READ NOW mode and 20 replicates of each contrived sample were also tested using the WALK AWAY mode. Results of the test mode equivalency study are shown in Table 5 below. The samples showed 100% results concurrence in both modes and similar measured mean absorbance (mAbs) values for all test lines. This data supports use of either test mode for analytical testing.

Table 5. Test Mode Equivalency Study Results

	READ NOW	WALK AWAY
Flu A		
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	39.0 mAbs	39.4 mAbs
% CV	9.6%	9.8%
Flu B		
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	45.2 mAbs	44.8 mAbs
% CV	18.2%	18.4%

	READ NOW	WALK AWAY
Negative		
Percent Agreement	100% (20/20)	100% (20/20)
Mean mAbs	0 mAbs	0 mAbs
% CV	NA	NA

4. Limit of Detection (LoD):

The LoD of the test for two strains of Influenza A (A/Michigan/45/2015 (H1N1) and A/Singapore/INFIMH-16-0019/2016 (H3N2) and two strains of Influenza B (B/Phuket/3073/2013 (Yamagata Lineage) and B/Colorado/06/2017 (Victoria Lineage) was determined using two lots of Acucy Influenza A and B test cassettes on each, the Acucy reader and the Acucy 2 reader. The LoD was determined as the lowest virus concentration that was detected ≥ 95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive). The limit of detection was established in two (2) phases: a range finding and a confirmatory LoD study.

a. *Range Finding LoD Study*:

Contrived samples were prepared by diluting Influenza A or B virus in PCR negative NCM. Five (5) replicates of a 1:10 dilution series were tested on two lots of Acucy cassettes, using both Acucy 1 and Acucy 2 readers. For each test, 50 µL was pipetted onto the head of an anterior nares swab (ANS). Swabs were then processed per the IFU, using the READ NOW mode. The lowest concentration of each lot with 4/5 positive results was selected as a preliminary LoD for confirmation. The preliminary LoD study results are shown in Table 6.

Table 6: Preliminary Testing Results for LoD Study

Viral Strain	Concentration ($TCID_{50}$/mL)	Acucy 1 Reader		Acucy 2 Reader	
		Test Lot 1	Test Lot 2	Test Lot 1	Test Lot 2
Influenza A/Michigan/45/2015 (H1N1)	1.41E+04	5/5	5/5	5/5	5/5
	1.41E+03	5/5	5/5	5/5	5/5
	1.41E+02	**5/5**	**5/5**	**5/5**	**5/5**
	1.41E+01	0/5	0/5	0/5	0/5
	1.41E+00	0/5	0/5	0/5	0/5
	1.41E-01	0/5	0/5	0/5	0/5
Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2)	3.16E+05	5/5	5/5	5/5	5/5
	3.16E+04	5/5	5/5	5/5	5/5
	3.16E+03	**5/5**	**5/5**	**5/5**	**5/5**
	3.16E+02	0/5	0/5	0/5	0/5
	3.16E+01	0/5	0/5	0/5	0/5
	3.16E+00	0/5	0/5	0/5	0/5
Influenza B/Phuket/3073/2013 (Yamagata Lineage)	4.17E+04	5/5	5/5	5/5	5/5
	4.17E+03	5/5	5/5	5/5	5/5
	4.17E+02	**5/5**	**5/5**	**5/5**	**5/5**
	4.17E+01	0/5	0/5	0/5	0/5
	4.17E+00	0/5	0/5	0/5	0/5
	4.17E-01	0/5	0/5	0/5	0/5

Viral Strain	Concentration ($TCID_{50}$/mL)	Acucy 1 Reader		Acucy 2 Reader	
		Test Lot 1	Test Lot 2	Test Lot 1	Test Lot 2
Influenza B/Colorado/06/2017 (Victoria Lineage)	1.41E+04	5/5	5/5	5/5	5/5
	1.41E+03	5/5	**5/5**	5/5	**5/5**
	1.41E+02	**4/5**	0/5	**4/5**	1/5
	1.41E+01	0/5	0/5	0/5	0/5
	1.41E+00	0/5	0/5	0/5	0/5
	1.41E-01	0/5	0/5	0/5	0/5

b. *Confirmatory LoD Study*:

After determining the target dilution for each strain, serial 2-fold dilutions were made corresponding to five (5) low level samples around the LoD ranges determined in the preliminary studies. These concentrations were tested over three (3) days for a total of 20 replicates per concentration and the lowest concentration with ≥ 95% of samples generating positive results was designated as the LoD for that lot for the strain tested, as shown in Table 7 below.

Table 7. Final Testing for LoD Study

Viral Strain	Preliminary LoD ($TCID_{50}$/mL)	Tested Concentration ($TCID_{50}$/mL)	Acucy 1 Reader		Acucy 2 Reader	
			Test Lot 1	Test Lot 2	Test Lot 1	Test Lot 2
Influenza A/Michigan/45/ 2015 (H1N1)	**1.41E+02**	**2.82E+02**	**20/20**	**20/20**	**20/20**	**20/20**
		1.41E+02	17/20	18/20	17/20	18/20
		7.05E+01	5/20	2/20	4/20	4/20
		3.53E+01	2/20	0/20	0/20	0/20
		1.76E+01	0/20	0/20	0/20	0/20
Influenza A/Singapore/IN FIMH-16-0019/2016 (H3N2)	**3.16E+03**	6.32E+03	20/20	20/20	20/20	20/20
		3.16E+03	**20/20**	**20/20**	**20/20**	**20/20**
		1.58E+03	14/20	9/20	15/20	10/20
		7.90E+02	0/20	0/20	0/20	0/20
		3.95E+02	0/20	0/20	0/20	0/20
Influenza B/Phuket/3073/ 2013 (Yamagata Lineage)	**4.17E+02**	8.34E+02	20/20	20/20	20/20	20/20
		4.17E+02	20/20	**20/20**	20/20	**20/20**
		2.09E+02	**19/20**	13/20	**20/20**	17/20
		1.04E+02	14/20	0/20	18/20	5/20
		5.02E+01	0/20	0/20	0/20	0/20
Influenza B/Colorado/06/ 2017 (Victoria Lineage)	**1.41E+02 (Lot 1)**	**2.82E+02**	**19/20**	NA	**20/20**	NA
		1.41E+02	9/20	NA	17/20	NA
		7.05E+01	1/20	NA	3/20	NA
		3.53E+01	0/20	NA	0/20	NA
		1.76E+01	0/20	NA	0/20	NA
	1.41E+03 (Lot 2)	2.82E+03	NA	20/20	NA	20/20
		1.41E+03	NA	20/20	NA	20/20
		7.05E+02	NA	**20/20**	NA	**20/20**
		3.53E+02	NA	17/20	NA	17/20
		1.76E+02	NA	3/20	NA	5/20

The established LoDs for the Acucy and Acucy 2 Reader are identical for each cassette lot, indicating that the performance of the predicate and candidate instruments is similar.

5. Assay Cut-Off:

The initial assay threshold of 5.0 milli-absorbance units (mAbs) was established as a default setting for the Acucy 2 Reader by the instrument developer and validated with a limit of blank study.

The Limit of Blank (LoB) of the Acucy Influenza A&B Assay on the Acucy 2 System was determined by testing sixty (60) replicates of a blank sample (pooled NCM negative for Influenza A and B) on 2 lots of Acucy Influenza A/B cartridges. All blank samples tested on Acucy 2 Reader showed 0 mAbs for both the Influenza A and Influenza B lines.

The analytical instrument cut-offs for influenza A and B that were original established for the Acucy system were determined to be 6.4 and 5.4 mAbs, respectively. As such, the Acucy Influenza A and B Test specific cut-offs for the Acucy 2 Reader are not significantly different from those previously established for the Acucy reader. Since the Acucy 2 reader uses similar detection technology than the Acucy reader, the assay specific cut-off for the Acucy 2 reader were set to the same values previously established for the Acucy reader.

6. Carry-Over:

This study evaluated the potential of sample carry-over between test cassettes when high-titer samples were run in an alternating fashion with negative samples.

Thirty (30) high titer influenza A samples were prepared using Influenza A/Michigan and Influenza B/Colorado strains diluted in NCM to a concentration of 1 x 105 TCID50/mL. Thirty (30) negative samples were prepared using NCM. For each contrived sample, 50 µL was applied to nasal swabs, allowed to dry for up to one minute, and tested according to the device's IFU 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 positive samples gave positive results as expected. All negative samples gave negative results, as summarized in Table 8 below:

Table 8. Summary of Carry-Over Study Results

Sample	Flu A Positive	Flu B Positive	Negative
Influenza A High Positive	30/30 (100%)	0/30 (0%)	0/30 (0%)
Influenza B High Positive	0/30 (0%)	30/30 (100%)	0/30 (0%)
Negative	0/60 (0%)	0/60 (0%)	60/60 (100%)

B Comparison Studies:

Method Comparison with Predicate Device:

This study evaluated the systemic differences between the Acucy and the Acucy 2 Reader using the same samples.

Aliquots of 50µL of known Influenza A or Influenza B positive or Influenza A and B negative nasopharyngeal swab (NPS) remnant samples were spiked onto sterile swabs and tested in parallel on both instruments per the IFU of the test. These samples were representative of a range of virus levels with RT-PCR values ranging from Ct 11.5 - 32.4.

Testing was performed as per Acucy Influenza A&B instructions for use. Thirty each of Influenza A and Influenza B samples and 30 Influenza A /B negative clinical samples were tested using one lot of Acucy Influenza A&B test kits. Each cassette was read on both the Acucy Reader and an Acucy 2 Reader. The Influenza A positive samples are considered as negatives for Influenza B and those positive for Influenza B are considered negative for Influenza A. Thus, together with the double negative samples, for each group, there are a total of sixty (60) negative results. All the positive samples showed up as positive on both readers. Results for the performance of the Acucy Influenza A&B Test for Influenza A and Influenza B stratified by the two Acucy Readers are in Tables 9 and 10 below:

Table 9. Influenza A performance with Archived Nasopharyngeal Swab Specimens Using READ NOW Mode on Acucy 2 Reader Compared to Acucy Reader.

Flu A by Acucy 2 Reader	**Flu A by Acucy Reader**	
	Positive	Negative
Positive	30	1*
Negative	0	59
PPA = 100.0 % (95% CI: 88.7% - 100.0 %) **NPA = 98.3 % (95% CI: 91.1% - 99.7 %)**		
*One of the Flu B positive samples returned a double positive result (positive for Flu A and Flu B) on Acucy 2 Reader but not on the Acucy Reader.		

For Influenza A, the results of the method comparison with the predicate demonstrated 100% PPA (95% CI: 88.7% -100%) and 98.3% NPA (95% CI: 91.1% - 99.7 %) One of the Flu B positive samples returned a positive result for Flu A as well (double positive) when read on Acucy 2 Reader.

Table 10. Influenza B performance with Archived Nasopharyngeal Swab Specimens Using READ NOW Mode on Acucy 2 Reader Compared to Acucy Reader

Flu B by Acucy 2 Reader	**Flu B by Acucy Reader**	
	Positive	Negative
Positive	30	0
Negative	0	60
PPA = 100.0 % (95% CI: 88.7% - 100.0 %) **NPA = 100.0 % (95% CI: 94.0.% - 100.0 %)**		

For Influenza B, the results of the method comparison with predicate device demonstrated 100.0% PPA (95% score CI: 88.7% -100.0%) and 100% NPA (95% score CI: 94.0% -100.0%) as compared to the Acucy 2 reader.

C Clinical Studies:

The clinical performance was previously reviewed under K182001 and leveraged to support performance for the Acucy Influenza A&B Test with the Acucy 2 System.

D Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII. Proposed Labeling:

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

IX. Conclusion:

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

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