April 18, 2025

Sekisui Diagnostics, LLC
Jason Bilobram
Principal Regulatory Affairs Specialist
6659 Top Gun Street
San Diego, California 92121

Re: K241188
Trade/Device Name: Acucy Influenza A&B Test with the Acucy 2 System
Regulation Number: 21 CFR 866.3328
Regulation Name: Influenza Virus Antigen Detection Test System
Regulatory Class: Class II
Product Code: PSZ
Dated: April 27, 2024
Received: April 29, 2024

Dear Jason Bilobram:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-

assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Silke Schlottmann -S

Digitally signed by Silke Schlottmann -S
Date: 2025.04.18 11:10:32 -04'00'

Silke Schlottmann, Ph.D.
Deputy Assistant Director
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K241188

Device Name
Acucy Influenza A&B Test with the Acucy 2 System

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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SEKISUI DIAGNOSTICS

510(k) Summary
(K231187)
Acucy Influenza A&B Test with the Acucy 2 System

As required by 21 CFR Section 807.92(c).

Submitted by:	Sekisui Diagnostics 6659 Top Gun Street San Diego, CA 92121 Phone: (858) 777-2600
Contact:	Jason Bilobram
Date of Preparation:	April 17, 2025
Trade name:	Acucy Influenza A&B Test with the Acucy 2 System
Common name:	Acucy 2 Reader with Influenza A/B
Type of Test:	Rapid chromatographic immunoassay for the qualitative detection of influenza A and B nucleoprotein antigens
Regulation number:	21 CFR 866.3328
Classification Name:	Influenza virus antigen detection test system
Product code:	PSZ
Classification:	Class II
Advisory Panel:	Microbiology (83)
Prescription Use:	Yes
Predicate Device Assay:	Acucy Influenza A&B Test with the Acucy System (K182001)

1. Device Description

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 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 sample well. 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 mouse monoclonal antibodies to influenza A and/or B nucleoproteins conjugated to colloidal gold. The complex will then be bound by a rat anti-influenza A and/or mouse anti-influenza B antibody coated on the nitrocellulose membrane.

Depending upon the operator's choice, the Test Cassette is either 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 Acucy 2 Reader to be scanned (READ NOW Mode).

The Acucy 2 Reader will scan the Test Cassette and measure the absorbance intensity by processing the results using method-specific algorithms. The Acucy 2 Reader will display the test results POS (+), NEG (-), or INVALID on the screen. The results can also be automatically printed on the optional Printer if this option is selected.

2. Device Intended 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 an 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.

Acucy Influenza A&B Kit Contents:

The Acucy Influenza A&B Test Kit contains all the materials needed to run a test, except for the Acucy 2 System, which is provided separately. The Acucy Influenza Test Kit contains the following components:

- 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)
- 25 Extraction Vial Dropper Tips
- 1 Influenza A+/B- Control Swab (Formalin inactivated influenza A containing 0.05% sodium azide)
- 1 Influenza A-/B+ Control Swab (Formalin inactivated influenza B containing 0.05% sodium azide)
- 2 Instructions for Use (IFU): 1 Acucy Reader, 1 Acucy 2 Reader
- 2 Quick Reference Guide: 1 Acucy Reader, 1 Acucy 2 Reader
- 2 External Quality Control (QC) Quick Reference Guide: 1 Acucy Reader, 1 Acucy 2 Reader
- 1 Workstation
- Note: Two extra test cassettes and reagents have been included in the kit for external quality control testing.

3. Substantial Equivalence

Attribute	Subject Device **Acucy 2 Reader with Influenza A/B (K241188)**	Predicate Device **Acucy Reader with Influenza A/B (K182001)**
Intended 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.	The Acucy® Influenza A&B Test for the rapid qualitative detection of influenza A&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 approved 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

Attribute	Subject Device **Acucy 2 Reader with Influenza A/B (K241188)**	Predicate Device **Acucy Reader with Influenza A/B (K182001)**
	Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	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. For near-patient, laboratory professional and healthcare professional in vitro diagnostic use only.
Regulation	Same	**21 CFR 866.3328**
Product Code	Same	PSZ
Device Class	Same	Device Class II
Technology/Detection	Same	The Acucy Influenza A&B Test allows for the differential detection of influenza A and influenza B antigens, when used with the Acucy Reader. The Acucy Reader will scan the test cassette and measure the absorbance intensity by processing the results using method-specific algorithms.
Assay Targets	Same	Influenza A, Influenza B
Specimen Type	Same	Nasal Swab (NS) Nasopharyngeal Swab (NPS)
Transport Media	Same	Viral Transport Media (VTM)
Test Format	Same	Lateral Flow, Cassette
Automation	Same	Detection and Results Interpretation
Assay Results	Same	Qualitative
Internal Control	Same	• Scan prevents use of expired or used cassettes • Scanning control for adequate flow • Scan prevents improper cassette insertions
Instrument Systems	Acucy 2 Instrument System	Acucy Instrument System
Time to Result	Same	15 minutes Read Time

The following performance data (analytical and clinical) were provided in support of the substantial equivalence determination.

4. Performance Studies

4.1 Analytical Performance

Precision Study Summary

The purpose of this study was to evaluate the consistency of performance of the Acucy 2 reader when using the Acucy Influenza A&B Test as part of the Acucy 2 System. The studies were designed to assess Within-Laboratory Repeatability over multiple days and Instrument-to-Instrument Variability.

On each day of testing, contrived positive samples at 0.95x (Low Positive), 2x LoD (Moderate positive), and 0.05x LoD (High negative) were prepared. Contrived swabs were prepared by adding 50µL of sample to head of swab. Negative samples were prepared by adding 50µL of sample to the head of swab. Swabs were dried for 1 minute. All further testing is performed as per Acucy Influenza A&B Instructions for Use (IFU, PN 3248).

Table 1: Expected results for sample panel tested in precision studies

Panel member	Target Concentration	Target Positive Detection Rate
Negative (N)	0x LoD	0%
High Negative (HN) Flu A ***or*** Flu B	0.05x LoD	0-5%
Low Positive (L) Flu A ***or*** Flu B	0.95x LoD	≥95%
Moderate Positive (M) Flu A ***or*** Flu B	2x LoD	100%

Within Laboratory Repeatability Study

The precision panel tested comprised of a total of 7 panel members one (1) negative sample, two (2) High Negative samples (0.05x LoD – one each for Flu A and Flu B), two (2) low positive samples (0.95x LoD – one each for Flu A and Flu B), and two (2) moderate positive samples (2x LoD – one each for Flu A and Flu B). All testing was done with Influenza A Michigan and Influenza B Colorado. In order to evaluate the repeatability of the testing within a lab, one (1) lot of Acucy Influenza A&B test was tested by one (1) operator using the seven-member

precision panel twice per day for twenty days (n=2). Over the course of 20 days, 80 replicates were run per panel member (1 operator x 20 days x 2 runs x 2 replicates).

Table 2a: Acucy, 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 AB HN	80/80	100%	95.4% – 100%
Negative	80/80	100%	95.4% – 100%

Table 2b: Acucy 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 AB HN	80/80	100%	95.4% – 100%
Negative	80/80	100%	95.4% – 100%

Instrument to Instrument Precision Study

To evaluate the instrument-to-instrument precision, one (1) lot of Acucy Influenza A&B was tested by one (1) operator. The seven-member precision panel was tested in five (5) replicates each on three (3) different Acucy 2 readers on five days as described above. Over the course of 5 days, 25 replicates were obtained per panel member per instrument (1 operator x 5 replicates x 5 days). In total there are 75 replicates per panel member on 3 different instruments (1 operator x 5 replicates x 3 instruments x 5 days). All the results were as expected on all days as per the acceptance criteria listed below. The acceptance criteria for the seven (7) member panel and the results are shown in Table 3.

Table 3: Instrument to Instrument Precision Study

Test Organism	Day	Flu A			Flu B			N/A
Sample Type		M	L	HN	M	L	HN	N
Concentration Tested (x LoD)		2	0.95	0.05	2	0.95	0.05	Neg
Reader # 100015	1	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	2	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	3	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	4	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	5	5/5	5/5	0/5	5/5	5/5	0/5	0/5
Reader # 100017	1	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	2	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	3	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	4	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	5	5/5	5/5	0/5	5/5	5/5	0/5	0/5
Reader # 100022	1	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	2	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	3	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	4	5/5	5/5	0/5	5/5	5/5	0/5	0/5
	5	5/5	5/5	0/5	5/5	5/5	0/5	0/5
Total		75/75	75/75	0/75	75/75	75/75	0/75	0/75
Pass(P)/Fail(F)		P	P	P	P	P	P	P

4.2 Test Mode Equivalency

The purpose of this study was to qualify the two available test modes of the Acucy 2 System (READ NOW and WALK AWAY) and confirm that they function equivalently. The study was performed by testing twenty (20) replicates each of contrived positive Flu A and Flu B at a concentration of 2x LoD and twenty (20) Flu A and Flu B negative samples (matrix alone) using two (2) Acucy 2 Readers at the same time. One reader was in WALK AWAY mode, and the other in READ NOW mode. One (1) lot of Acucy Influenza A&B kits were used for testing. All testing was done with Influenza A/Michigan and Influenza B/Colorado. On each day of testing, contrived positive samples at 2x LoD (Moderate positive), were prepared by dilution in clinical matrix.

Contrived swabs were prepared by adding 50µL of sample (either contrived positive or negative) to the head of swab. Swabs were dried for 1 minute. All further testing was performed as per Acucy Influenza A&B Instructions for Use (IFU, PN 3248). All tests showed the expected results, positives read as positives in both modes and negatives read as negatives whether in the READ NOW or WALK AWAY mode. The two available test modes function equivalently. The results of the testing are shown in Table 4.

Table 4: Test mode Equivalency Study Results

Mode →	**READ NOW**		**WALK AWAY**	
Sample	**Flu A Result**	**Flu B Result**	**Flu A Result**	**Flu B Result**
Flu A+/B-	20/20 POS	20/20 NEG	20/20 POS	20/20 NEG
Flu A-/B+	20/20 NEG	20/20 POS	20/20 NEG	20/20 POS
Flu A-/B- Negative	20/20 NEG	20/20 NEG	20/20 NEG	20/20 NEG

4.3 Limit of Detection (LoD)

A comparison of the analytical sensitivity of the Acucy and Acucy 2 readers side by side was performed in a limit of detection (LoD) experiment. For comparing the LoD, two (2) lots of Acucy Influenza A&B assay devices were tested with two (2) strains each of Influenza A (Michigan and Singapore) and two (2) strains each of Influenza B (Phuket and Colorado). A range finding experiment using 5 replicates and 10-fold dilutions of the stock was undertaken on day one (1). After determining the target dilution, for each strain serial two (2) fold dilutions were made corresponding to five low level samples around the expected LoD. These concentrations were tested over three days for a total of 20 replicates per concentration and the lowest concentration at with ≥ 95% of samples were positive was designated the LoD for that lot for the strain tested. Following completion of testing of all strains and both lots the lowest concentration that agrees across both lots was selected as the LoD for that strain.

Confirmation testing was done with 20 replicates once the expected LoD was established. Pooled negative clinical matrix samples were used for preparing dilutions. To create the negative clinical matrix, nasal swab samples were collected from healthy donors and confirmed Flu negative by PCR. Each Flu negative-confirmed nasal swab sample was extracted in 3.0 mL of NCM as per the testing that was done for Acucy™ Influenza A&B Test with the Acucy™ System (K182001). Swab extracts are combined and mixed thoroughly to create a pool of negative clinical matrix to be used as diluent. Contrived swabs in all cases were prepared by adding 50µL of sample to head of swab and dried for one (1) minute. All further testing was performed as per Acucy Influenza A&B Instructions for Use. The results of the LoD testing are shown in Tables 5-7. The results are displayed as Positive detection rate (Number of positives/ Total tested). The LoD for all strains tested on both lots of devices were identical whether tested using Acucy or Acucy 2, confirming that the performance of the Acucy 2 is equivalent to the Acucy.

Table 5: Range finding experiment to determine the target concentration

Strain	Concentration (TCID50/mL)	Device A		Device B	
		Acucy	Acucy 2	Acucy	Acucy 2
Influenza A/Michigan Strain (Stock Conc = 1.41E+05)	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 Strain (Stock Conc = 3.16E+06)	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 Strain (Stock Conc = 4.17E+05)	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
Influenza B/Colorado Strain (Stock Conc = 1.41E+05)	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	4/5	0/5	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

Table 6: Acucy 2 Influenza A&B Test Limit of Detection (LoD) Confirmation Results

Strain	Concentration (TCID50/mL)	Device A		Device B	
		Acucy	Acucy 2	Acucy	Acucy 2
Influenza A/Michigan Strain (Stock Conc = 1.41E+05)	2.82E+02	20/20	20/20	20/20	20/20
	1.41E+02	17/20	17/20	18/20	18/20
	7.05E+01	5/20	4/20	2/20	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 Strain (Stock Conc = 3.16E+06)	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	15/20	9/20	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 Strain (Stock Conc = 4.17E+05)	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	20/20	13/20	17/20
	1.04E+02	14/20	18/20	0/20	5/20
	5.20E+01	0/20	0/20	0/20	0/20
Influenza B/Colorado Strain (Stock Conc = 1.41E+05)	2.82E+02	19/20	20/20	NA	NA
	1.41E+02	9/20	17/20	NA	NA
	7.05E+01	1/20	3/20	NA	NA
	3.53E+01	0/20	0/20	NA	NA
	1.76E+01	0/20	0/20	NA	NA
	2.82E+03	NA	NA	20/20	20/20
	1.41E+03	NA	NA	20/20	20/20
	7.05E+02	NA	NA	20/20	20/20
	3.53E+02	NA	NA	17/20	17/20
	1.76E+02	NA	NA	3/20	5/20

Table 7: Confirmation testing for LoD

Influenza Strain used	Test Device	LoD Concentration ($TCID_{50}$/mL)	Acucy	Acucy 2
Influenza A/Michigan Strain	Device A	2.82E+02	20/20	20/20
	Device B	2.82E+02	20/20	20/20
Influenza A/Singapore Strain	Device A	3.16E+03	20/20	20/20
	Device B	3.16E+03	20/20	20/20
Influenza B/Phuket Strain	Device A	2.09E+02	20/20	20/20
	Device B	4.17E+02	20/20	20/20
Influenza B/Colorado Strain	Device A	2.82E+02	20/20	20/20
	Device B	7.05E+02	20/20	20/20

4.4 Analytical Cutoff Study

The purpose of this study was to establish the Limit of Blank (LoB) of the Acucy Influenza A&B Assay on the Acucy 2 System by testing sixty (60) replicates of a blank sample per lot to determine the LoB of each lot. Two different device lots were used in testing. Pooled negative clinical matrix samples were used for preparing these samples. 50 µL of contrived specimen was pipetted onto the head of a new nasal swab and dried for one (1) minute. All testing was done per the IFU.

All blank samples showed 0 mABS. In the original study on the Acucy system, the analytical cut-off values were determined to be 6.4 and 5.4 mABS for the Flu A Line and Flu B Line respectively obtained by comparing the assay thresholds of two (2) lots of Acucy Influenza A&B Assay. Acucy 2 was designed to replace Acucy and is expected to be similar to the latter. Under these circumstances the analytical cut-off for Acucy 2 has been set to match the previously established cut-off of 6.4 and 5.4 mABS for Flu A Line and Flu B Line respectively.

4.5 Cross Contamination Study

The purpose of this study was to evaluate if there is any target carryover between test cassettes leading to cross-contamination between samples when testing the Acucy Influenza A&B test on the Acucy 2 system. The cross-contamination study was evaluated by alternately testing samples at a high titer (1.0 x105 TCID50/ml) and negative samples. This study used one (1) strain of Influenza A and one (1) strain of Influenza B for high titer positive samples and tested on one (1) lot of the Acucy Influenza A&B Test with Acucy 2 System. 120 samples were tested in all, 60 negatives and 30 each of Flu A and Flu B samples. Sample dilutions were prepared. Contrived swabs were prepared by adding 50µL of sample to head of swab. Negative samples were prepared by adding 50µL of negative clinical matrix to the head of a swab. Swabs were dried for 1 minute. All further testing is performed as per Acucy Influenza A&B Instructions for Use (IFU, PN 3248). All testing was done with Influenza A Michigan and Influenza B Colorado. All tests showed expected results as shown in Table 8, positives read as positives and negatives read as negatives.

Table 8: Results of cross contamination study

Strain	Test ID	Result	Pass/Fail
Flu A	Flu A High Positive (1 - 30)	30/30	Pass
Flu B	Flu B High Positive (1 - 30)	30/30	Pass
Negative	No Flu A/B, matrix	60/60	Pass

4.6 Method Comparison - Acucy Reader vs. Acucy 2 Reader

A comparison study was conducted to establish performance equivalence between the Acucy and Acucy 2 Readers. Thirty (30) samples each of PCR confirmed Flu A positive and Flu B positive clinical samples and thirty (30) Flu A and Flu B negative clinical samples were tested using one (1) Acucy Reader and one (1) Acucy 2 Reader with one (1) lot of Acucy Influenza A&B test kits. The Flu A positive samples were considered negatives for Flu B, and those positive for Flu B were considered negative for Flu A. Together, including double negative samples, there were a total of 60 negatives for each group. These samples were representative of a range of virus levels with RT-PCR values ranging from Ct 11.5 - 32.4.

Table 9: Influenza A, Method Comparison Study Results

Test: Acucy Influenza A&B Test with Acucy System			
Comparative: Acucy Influenza A&B Test with Acucy 2 System	**Flu A Positive**	**Flu A Negative**	**Total**
Flu A Positive	30	1*	31
Flu A Negative	0	59	59
Total	30	60	90
Performance	PPA: 100%	NPA: 98.3%	

* Confirmed Flu B positive sample generated a double positive result.

Table 10: Influenza B, Method Comparison Study Results

Test: Acucy Influenza A&B Test with Acucy System			
Comparative: Acucy Influenza A&B Test with Acucy 2 System	**Flu B Positive**	**Flu B Negative**	**Total**
Flu B Positive	30	0	30
Flu B Negative	0	60	60
Total	30	60	90
Performance	PPA: 100%	NPA: 100%	

5. CLIA Waiver Studies

5.1 Flex Studies

These studies were carried out to characterize operator workflow and usability risks identified with use of the Acucy Influenza A&B test on the Acucy 2 System by simulating conditions where the test was performed outside the intended use instructions and environment and the Acucy 2 Reader differed from the Acucy Reader. Each flex study was conducted using sample panel (Negative, Low Positive (2x LoD) Flu A, and Low Positive (2x LoD) Flu B) tested in five (5) replicates for every flex condition using one (1) lot of Acucy Influenza A&B kit.

The following flex studies were performed:

- Temperature and Humidity
- Vibrations from surrounding instruments
- Environmental lighting
- Air Draft conditions
- Operational altitude/barometric pressure
- Non-level reader position
- Contamination of Cassette Read Window
- Movement of Acucy 2 Reader in WALK AWAY mode
- Movement of test cassette and vertical incubation
- Positioning of Acucy test in reader drawer

Based on the results of the flex testing, all the hazards and sources of potential error have been controlled to reasonably acceptable levels through design features and appropriate labelling mitigations.

5.2 Reproducibility

The reproducibility of the Acucy Influenza A&B Test was evaluated on the Acucy Reader in testing performed at three point-of-care (POC) sites. A panel of swabs including negative (no virus), high negative (below the limit of detection), low positive (at or near the limit of detection) and moderate positive (at or near 2x the limit of detection) for influenza A and B were coded, randomized, and masked to the operators. The study was conducted with two operators per site over five non-consecutive days. As seen in the table below, the Acucy Influenza A&B Test produces reproducible results when tested by multiple intended users, at multiple sites, over multiple days.

Table 11: Acucy Influenza A, External Multi-Site Reproducibility Study Results

Sample	Site 1	Site 2	Site 3	Total
Influenza A (H3N2) High Negative	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90)
Influenza A (H3N2) Low Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Influenza A (H3N2) Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)

Table 12: Acucy Influenza B, External Multi-Site Reproducibility Study Results

Sample	Site 1	Site 2	Site 3	Total
Influenza B High Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Influenza B Low Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Influenza B Moderate Positive	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90)
Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)

Supplementary Reproducibility Study

The reproducibility of the Acucy Influenza A&B Test was evaluated on the Acucy 2 Reader in testing performed at three laboratory sites. A panel of swabs including negative (no virus), high negative (below the limit of detection), low positive (at or near the limit of detection) and moderate positive (at or near 2x the limit of detection) for influenza A and B were coded, randomized, and masked to the operators. The study was conducted with two operators per site over five non-consecutive days. As seen in the tables below, the Acucy Influenza A&B Test produces reproducible results when tested by multiple intended users, at multiple sites, over multiple days.

Table 13: Acucy 2 Influenza A, External Multi-Site Reproducibility Study Results

Sample	Site 1	Site 2	Site 3	Total
Influenza A High Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Influenza A Low Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Influenza A Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)

Table 14: Acucy 2 Influenza B, External Multi-Site Reproducibility Study Results

Sample	Site 1	Site 2	Site 3	Total
Influenza B High Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Influenza B Low Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Influenza B Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)

Table 15: Acucy 2 Negative, External Multi-Site Reproducibility Study Results

Sample	Site 1	Site 2	Site 3	Total
Influenza A & B Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)

Conclusion

The results of the studies summarized above demonstrate that the Acucy 2 Instrument with Influenza A/B is substantially equivalent to the stated predicate device.