Food and Drug Administration
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510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K191514

B Applicant

Access Bio, Inc.

C Proprietary and Established Names

CareStart Flu A&B Plus

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:
New device 510(k) clearance for the *CareStart* Flu A&B *Plus*

B Measurand:
Influenza A and influenza B viral nucleoprotein antigens

C Type of Test:
Qualitative Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

The CareStart Flu A&B Plus is an in vitro rapid immunochromatographic assay for the qualitative detection of influenza virus type A and B nucleoprotein antigens directly from nasopharyngeal swab specimens of symptomatic patients.

The test is intended for use as an aid in the rapid differential diagnosis of acute influenza type A and B viral infections. This test is intended to distinguish between influenza type A and/or B virus in a single test. This test is not intended to detect influenza type C viral antigens. Negative test results are presumptive and should 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 basis for treatment or other patient management decisions.

Performance characteristics for influenza A and B were established during the 2018-2019 influenza season when influenza A/H3N2 and A/H1N1pdm09, and B/Victoria were the predominant influenza viruses in circulation. When other influenza viruses are emerging, performance characteristics may vary.

If infection with a novel influenza 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 the state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to received and culture specimens.

B Indication(s) for Use:

Same as Intended Use(s)

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

N/A

IV Device/System Characteristics:

A Device Description:

The *CareStart* Flu A&B *Plus* test is an immunochromatographic assay for detection of extracted influenza type A and B virus nucleoprotein antigens in nasopharyngeal swab specimens.

The assay kit consists of 20 cassettes (each cassette contains one test strip), 20 extraction vials, 20 caps, 20 swabs for the collection of nasopharyngeal specimens, and three external control swabs, two positive control swabs and one negative control swab. One of the positive control swabs contains inactivated influenza A virus dried onto the swab and the other positive control

swab contains inactivated influenza B dried onto the swab. The negative control swab contains inactivated *Streptococcus* Group A dried onto the swab.

The kit may be stored at 1-30°C.

B Principle of Operation:

The *CareStart* Flu A&B *Plus* test is based on antigen-antibody binding technology. The virus antigens are released from the specimen during the extraction step. Three drops of the extracted sample are added to the cassette that contains a testing strip. During the migration through the strip, the viral antigens bind to anti-influenza A and anti-influenza B antibodies conjugated to indicator particles. The antigen-antibody complexes migrate through the test strip and are captured by reagents on the membrane to form test lines and a control line. There are two separate test lines, one detecting influenza A antigens and one detecting influenza B antigens. The control line is formed by absorbing residual sample and serves as the internal control for the assay. The test is visually read 10 minutes after the sample is added to the cassette.

Results Interpretation:

- The presence of two lines in the cassette, one for influenza A next to the letter "A" and one control line next to the letter "C", indicates influenza A positive result.
- The presence of two lines in the cassette, one for influenza B next to the letter "B" and one control line next to the letter "C", indicates influenza B positive result.
- The presence of three lines in the cassette, one for influenza A next to the letter "A", one for influenza B next to the letter "B", and one control line next to the letter "C", indicates a double positive for influenza A and influenza B result.
- The presence of the control line in the cassette next to the letter "C" without any other lines indicates a negative result.
- The absence of a C line, regardless of whether any other lines are present or absent, represents an invalid result.

The sample with invalid result should be retested using the remaining sample present in the extraction vial.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BD Veritor System for Rapid Detection of Flu A + B CLIA waived kit

B Predicate 510(k) Number(s):
K180438

C Comparison with Predicate(s):

Device & Predicate Device(s):	K191514	K180438
Device Trade Name	*CareStart* Flu A&B *Plus*	BD Veritor System for Rapid Detection of Flu A + B

General Device Characteristic Similarities		
Intended Use/Indications For Use	The *CareStart* Flu A&B *Plus* is an in vitro rapid immunochromatographic assay for the qualitative detection of influenza virus type A and B nucleoprotein antigens directly from nasopharyngeal swab specimens of symptomatic patients. The test is intended for use as an aid in the rapid differential diagnosis of acute influenza type A and B viral infections. This test is intended to distinguish between influenza type A and/or B virus in a single test. This test is not intended to detect influenza type C viral antigens. Negative test results are presumptive and should 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 basis for treatment or other patient management decisions. Performance characteristics for influenza A and B were established during the 2018-2019 influenza season when influenza A/H3N2 and A/H1N1pdm09, and B/Victoria were the predominant influenza viruses in circulation. When other influenza viruses are emerging, performance characteristics may vary. If infection with a novel influenza 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 the state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is	The BD Veritor System for Rapid Detection of Flu A+B is 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. The BD Veritor System for Rapid Detection of Flu A+B (also referred to as the BD Veritor System and BD Veritor System Flu A+B) 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 device. The test is to be used as an aid in the diagnosis of influenza A and B viral infections. A negative test is presumptive and it is recommended that these results be confirmed by viral culture or an FDA cleared influenza A and B molecular assay. Outside the U.S., a negative test is presumptive and it is recommended that these results be confirmed by viral culture or a molecular assay cleared for diagnostic use in the country of use. FDA has not cleared this device for use outside of the U.S. 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. The test is not intended to detect influenza C antigens. Performance characteristics for influenza A and B were established during January through March of 2011 when influenza viruses A/2009 H1N1, A/H3N2, B/Victoria lineage, and B/Yamagata lineage 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."

	available to received and culture specimens.	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, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to the state or local health department for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens
Sample Type	Nasopharyngeal swab	Nasopharyngeal and nasal swab
Test Results	Qualitative	Same
Test Targets	Influenza A and influenza B nucleoprotein antigens	Same
Test Principle	Immunochromatographic	Same
Test Format	Lateral flow test	Same
General Device Characteristic Differences		
Detection format	Visual determination of presence or absence of colored line(s) for the test line(s) and a colored line on the test strip indicate the presence of influenza A and/or B antigen	An opto-electronic 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 on the LCD screen based on pre-set thresholds.

VI Standards/Guidance Documents Referenced:

1. 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
2. Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses, July 15, 2011

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A seven-member panel was used in all three precision/reproducibility studies described in the sections below. The panel was made using two virus strains. For the reproducibility study and the within laboratory repeatability study influenza A/Michigan/45/2015 (H1N1) pdm09 and influenza B/Phuket/3073/13 were used. For the lot-lo-lot reproducibility study influenza A/Perth/16/2009 and influenza B/Colorado/06/2017 were used. Each strain was diluted into Universal Transport Medium (UTM) at three different target levels: moderate positive (~3x LoD), low positive (~1x LoD), and high negative (~0.1x LoD). Panel members were formulated with only one target present (influenza A or influenza B). Negative samples did not contain influenza virus. Simulated nasal swabs were prepared by pipetting 25 µL of sample (virus dilution) onto the head of the swab. Each swab was allowed to dry using vacuum dryer and then sealed in a desiccated pouch. The swabs were removed from the pouches prior to testing and the testing was performed according to the instructions for use.

Within-Laboratory Repeatability

The within-laboratory precision of the *CareStart* Flu A&B *Plus* was evaluated in a study performed inhouse over 15 non-consecutive days, with two operators. One lot of devices was used to test in triplicate the seven-member panel described above.

The qualitative results from the within-laboratory precision study are summarized in Table 1 below.

Table 1. Within-Laboratory Precision Study Results

Sample	Operator #1 Agreement (Count)	Operator #2 Agreement (Count)	All Operators	
			Agreement (Count)	95% CI
Negative	100% (90/90)	100% (90/90)	100% (180/180)	97.9% – 100%
Flu A High Negative	100% (90/90)	100% (90/90)	100% (180/180)	97.9% – 100%
Flu A Low Positive	100% (90/90)	100% (90/90)	100% (180/180)	97.9% – 100%
Flu A Moderate Positive	100% (90/90)	100% (90/90)	100% (180/180)	97.9% – 100%
Flu B High Negative	100% (90/90)	100% (90/90)	100% (180/180)	97.9% – 100%
Flu B Low Positive	100% (90/90)	100% (90/90)	100% (180/180)	97.9% – 100%
Flu B Moderate Positive	100% (90/90)	100% (90/90)	100% (180/180)	97.9% – 100%

All samples tested in the within laboratory precision study demonstrated 100% agreement with the expected results.

Lot-to-lot Precision

The lot-to-lot precision of the *CareStart* Flu A&B *Plus* was evaluated in a study performed inhouse over three days, with three operators. Three lots of devices were used to test in triplicate the seven-member panel described above.

The qualitative results from the lot-to-lot precision study are summarized in Table 2 below.

Table 2. Lot-to-Lot Precision Study Results

Sample	Lot #1 Agreement (Count)	Lot #2 Agreement (Count)	Lot #3 Agreement (Count)
Negative	100% (9/9)	100% (9/9)	100% (9/9)
Flu A High Negative	100% (9/9)	100% (9/9)	100% (9/9)
Flu A Low Positive	100% (9/9)	100% (9/9)	100% (9/9)
Flu A Moderate Positive	100% (9/9)	100% (9/9)	100% (9/9)
Flu B High Negative	100% (9/9)	100% (9/9)	100% (9/9)
Flu B Low Positive	100% (9/9)	100% (9/9)	100% (9/9)
Flu B Moderate Positive	100% (9/9)	100% (9/9)	100% (9/9)

All samples tested in the lot-to-lot precision study generated 100% agreement with the expected results.

External Multi-Site User-to-User Reproducibility

The reproducibility of *CareStart* Flu A&B *Plus* assay was evaluated at three external sites with three operators per site (a total of nine operators) over five nonconsecutive days. The seven-member panel described above was tested in this study. The summary of results is presented in Table 3 below.

Table 3. Reproducibility by Study Site

Sample	Site #1 Agreement (Count)	Site #2 Agreement (Count)	Site #3 Agreement (Count)	All Sites	
				Agreement (Count)	95% CI
Negative	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135)	97.2% – 100%
Flu A High Negative	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135)	97.2% – 100%
Flu A Low Positive	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135)	97.2% – 100%
Flu A Moderate Positive	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135)	97.2% – 100%

Flu B High Negative	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135)	97.2% – 100%
Flu B Low Positive	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135)	97.2% – 100%
Flu B Moderate Positive	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135)	97.2% – 100%

All samples tested in the reproducibility study generated 100% agreement with the expected results.

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

Analytical Specificity (Cross-Reactivity)

A total of 43 microorganisms, including 31 bacteria and 15 viruses, were tested in the cross-reactivity study. Positive influenza swabs were prepared with either influenza A/Perth/16/2009 or influenza B/Colorado/06/2017. Viruses were diluted in UTM to approximately 2x LoD. Using a pipette, 25 µL of influenza sample and 50 µL of microorganism at the concentration presented below were applied to the swab. Negative samples did not contain influenza virus. Positive and negative swabs were tested in triplicate.

The concentrations of potentially interfering microorganisms tested and the results from the cross-reactivity study are presented in Table 4 below.

Table 4. Cross-Reactivity Study Results

Organism	Final Concentration Tested	Flu A Positive Replicates	Flu B Positive Replicates	Flu A/Flu B Negative Replicates
Acinetobacter calcoaceticus	10^7 CFU/mL	3/3	3/3	3/3
Adenovirus 1	$10^{5.2}$ $TCID_{50}$/mL	3/3	3/3	3/3
Adenovirus 7	$10^{5.45}$ $TCID_{50}$/mL	3/3	3/3	3/3
Bordetella pertussis	10^7 CFU/mL	3/3	3/3	3/3
Candida albicans	10^7 CFU/mL	3/3	3/3	3/3
Chlamydophila pneumoniae	10^7 CFU/mL	3/3	3/3	3/3
Coronavirus OC43	$10^{5.45}$ $TCID_{50}$/mL	3/3	3/3	3/3
Coronavirus 229E	$10^{4.45}$ $TCID_{50}$/mL	3/3	3/3	3/3
Coxsackie virus B4	$10^{5.45}$ $TCID_{50}$/mL	3/3	3/3	3/3
Corynebacterium diphtheriae	10^7 CFU/mL	3/3	3/3	3/3
Cytomegalovirus	$10^{4.95}$ $TCID_{50}$/mL	3/3	3/3	3/3
Enterococcus faecalis	10^7 CFU/mL	3/3	3/3	3/3
Escherichia coli	10^7 CFU/mL	3/3	3/3	3/3
Gardnerella vaginalis	10^7 CFU/mL	3/3	3/3	3/3
Haemophilus influenzae	10^7 CFU/mL	3/3	3/3	3/3
Klebsiella pneumoniae	10^7 CFU/mL	3/3	3/3	3/3

Lactobacillus casei	10^7 CFU/mL	3/3	3/3	3/3
Legionella pneumophila	10^7 CFU/mL	3/3	3/3	3/3
Listeria monocytogenes	10^7 CFU/mL	3/3	3/3	3/3
Measles virus	$10^{4.2}$ $TCID_{50}$/mL	3/3	3/3	3/3
Metapneumovirus	2.8×10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Moraxella catarrhalis	10^7 CFU/mL	3/3	3/3	3/3
Mumps virus (Enders)	$10^{5.2}$ $TCID_{50}$/mL	3/3	3/3	3/3
Mycobacterium tuberculosis	10^7 CFU/mL	3/3	3/3	3/3
Mycoplasma pneumoniae	1.5×10^3 CFU/mL	3/3	3/3	3/3
Neisseria gonorrhoeae	10^7 CFU/mL	3/3	3/3	3/3
Neisseria meningitidis	10^7 CFU/mL	3/3	3/3	3/3
Neisseria sicca	10^7 CFU/mL	3/3	3/3	3/3
Parainfluenza virus 1	$10^{5.86}$ $TCID_{50}$/mL	3/3	3/3	3/3
Parainfluenza virus 2	$10^{5.2}$ $TCID_{50}$/mL	3/3	3/3	3/3
Parainfluenza virus 3	$10^{5.2}$ $TCID_{50}$/mL	3/3	3/3	3/3
Proteus vulgaris	10^7 CFU/mL	3/3	3/3	3/3
Pseudomonas aeruginosa	10^7 CFU/mL	3/3	3/3	3/3
Respiratory Syncytial Virus B	$10^{5.2}$ $TCID_{50}$/mL	3/3	3/3	3/3
Rhinovirus 1A	$10^{5.2}$ $TCID_{50}$/mL	3/3	3/3	3/3
Rubella	$10^{5.2}$ $TCID_{50}$/mL	3/3	3/3	3/3
Staphylococcus aureus	10^7 CFU/mL	3/3	3/3	3/3
Staphylococcus epidermidis	10^7 CFU/mL	3/3	3/3	3/3
Serratia marcescens	10^7 CFU/mL	3/3	3/3	3/3
Streptococcus mutans	10^7 CFU/mL	3/3	3/3	3/3
Streptococcus pneumoniae	10^7 CFU/mL	3/3	3/3	3/3
Streptococcus pyogenes	10^7 CFU/mL	3/3	3/3	3/3
Streptococcus sp. Group B	10^7 CFU/mL	3/3	3/3	3/3
Streptococcus sp. Group C	10^7 CFU/mL	3/3	3/3	3/3
Streptococcus sp. Group F	10^7 CFU/mL	3/3	3/3	3/3
Streptococcus sanguinis	10^7 CFU/mL	3/3	3/3	3/3

The study results showed no cross-reactivity of the above listed microorganisms with the *CareStart* Flu A&B *Plus* assay at the concentrations tested. All influenza negative samples gave negative results. In addition, all influenza A and influenza B samples tested positive in the presence of the above listed microorganisms showing no interference at the concentrations tested.

Competitive Interference

The study was performed to evaluate if the *CareStart* Flu A&B *Plus* assay can detect low levels of influenza A in the presence of high levels of influenza B and vice versa. Four influenza strains were used in this study: influenza A/Perth/16/2009, influenza A/Michigan/45/2015, influenza B/Colorado/06/2017, and influenza B/Phuket/3073/2013. For low concentration samples, influenza strains were diluted to approximately 1x LoD and for the high concentrations 5-fold dilutions of the virus stock were used. Using a pipette, 50 µL of each influenza sample was applied to a swab. The swabs were tested in triplicate. Low and high concentrations of the influenza strains used in this study are presented in Table 5 below.

Table 5. Influenza Strains and Concentrations Tested

Influenza stain	Low concentration	High concentration
A/Perth/16/2009	$3.2 \times 10^{4.9}$ EID_{50}/mL	$2.0 \times 10^{7.9}$ EID_{50}/mL
A/Michigan/45/2015	$3.2 \times 10^{4.2}$ EID_{50}/mL	$2.0 \times 10^{7.2}$ EID_{50}/mL
B/Colorado/06/2017	$1.6 \times 10^{6.4}$ EID_{50}/mL	$2.0 \times 10^{8.4}$ EID_{50}/mL
B/Phuket/3073/2013	$1.6 \times 10^{5.5}$ EID_{50}/mL	$2.0 \times 10^{7.5}$ EID_{50}/mL

The results of the competitive interference study are summarized in Table 6 below.

Table 6. Competitive Interference Study Results

Influenza A strain	Influenza B strain	Influenza A Positive Replicates	Influenza B Positive Replicates
A/Perth/16/2009 Low concentration	B/Colorado/06/2017 High concentration	3/3	3/3
A/Perth/16/2009 Low concentration	B/Phuket/3073/2013 High concentration	3/3	3/3
A/Michigan/45/2015 Low concentration	B/Colorado/06/2017 High concentration	3/3	3/3
A/Michigan/45/2015 Low concentration	B/Phuket/3073/2013 High concentration	3/3	3/3
A/Perth/16/2009 High concentration	B/Colorado/06/2017 Low concentration	3/3	3/3
A/Perth/16/2009 High concentration	B/Phuket/3073/2013 Low concentration	3/3	3/3
A/Michigan/45/2015 High concentration	B/Colorado/06/2017 Low concentration	3/3	3/3
A/Michigan/45/2015 High concentration	B/Phuket/3073/2013 Low concentration	3/3	3/3

The study results showed that *CareStart* Flu A&B *Plus* assay can detect low levels of influenza A in the presence of high levels of influenza B and low levels of influenza B in the presence of high levels of influenza A.

Potentially interfering substances

Positive influenza swabs were prepared for four viral strains: influenza A/Perth/16/2009, influenza A/Michigan/45/2015, influenza B/Colorado/06/2017, and influenza B/Phuket/3073/2013. Viruses were diluted in UTM to approximately 2x LoD and the potentially interfering substance was spiked to the concentration presented in the table below. The swabs were prepared by pipetting 50 µL of sample to the swab. The negative swabs contained UTM and potentially interfering substance only. Positive and negative samples were tested in triplicate.

The concentrations of potentially interfering substances tested and the results from the interference study are presented in Table 7 below.

Table 7. Interference Study Results

Interfering Substance	Concentration Tested	Influenza A Positive Replicates		Influenza B Positive Replicates		Negative Samples
		A/Perth/ 16/2009	A/Michigan/ 45/2015	B/Colorado /06/2017	B/Phuket/ 3073/2013	
Acetaminophen	10 mg/mL	3/3	3/3	3/3	3/3	0/3
Acetyl salicylic acid	15 mg/mL	3/3	3/3	3/3	3/3	0/3
Beclomethasone	0.5 mg/mL	3/3	3/3	3/3	3/3	0/3
Benzocaine	5 mg/mL	3/3	3/3	3/3	3/3	0/3
Budesonide	2 mg/mL	3/3	3/3	3/3	3/3	0/3
Chlorpheniramine maleate	5 mg/mL	3/3	3/3	3/3	3/3	0/3
Dexamethasone	1 mg/mL	3/3	3/3	3/3	3/3	0/3
Dextromethorphan HBr	2 mg/mL	3/3	3/3	3/3	3/3	0/3
Diphenhydramine HCl	5 mg/mL	3/3	3/3	3/3	3/3	0/3
Ephedrine HCl	10 mg/mL	3/3	3/3	3/3	3/3	0/3
Flunisolide	5 mg/mL	3/3	3/3	3/3	3/3	0/3
Fluticasone	1 mg/mL	3/3	3/3	3/3	3/3	0/3
Guaiacol Glyceryl Ether	20 mg/mL	3/3	3/3	3/3	3/3	0/3
Histamine Dihydrochloride	10 mg/mL	3/3	3/3	3/3	3/3	0/3
Menthol	10 mg/mL	3/3	3/3	3/3	3/3	0/3
Mometasone	1 mg/mL	3/3	3/3	3/3	3/3	0/3
Mucin	2%	3/3	3/3	3/3	3/3	0/3
Mupirocin	1 mg/mL	3/3	3/3	3/3	3/3	0/3
OTC Throat drop (Halls)	15%	3/3	3/3	3/3	3/3	0/3
OTC Throat drop (Ricola)	15%	3/3	3/3	3/3	3/3	0/3
OTC Nasal spray (Afrin)	15%	3/3	3/3	3/3	3/3	0/3
OTC Nasal spray (Vicks Sinex)	15%	3/3	3/3	3/3	3/3	0/3
OTC Nasal spray (Zicam)	15%	3/3	3/3	3/3	3/3	0/3
Oxymetazoline HCl	10 mg/ml	3/3	3/3	3/3	3/3	0/3
Phenylephrine HCl	5 mg/ml	3/3	3/3	3/3	3/3	0/3
Phenylpropanol-amine	5 mg/ml	3/3	3/3	3/3	3/3	0/3
Tobramycin	1 mg/ml	3/3	3/3	3/3	3/3	0/3
Triamcinolone	1 mg/ml	3/3	3/3	3/3	3/3	0/3
Whole Blood	2%	3/3	3/3	3/3	3/3	0/3
Zanamivir	1 mg/ml	3/3	3/3	3/3	3/3	0/3

The study results showed no interference with positive or negative results for the *CareStart* Flu A&B *Plus* assay at the concentrations tested in the presented study.

Biotin Interference

Positive influenza swabs were prepared for four viral strains: influenza A/Perth/16/2009, influenza A/Michigan/45/2015, influenza B/Colorado/06/2017, and influenza B/Phuket/3073/2013. Viruses were diluted in UTM to approximately 2x LoD and biotin was spiked to the levels presented in the table below. The swabs were prepared by pipetting 50 µL of sample to the swab. The negative swabs contained UTM and biotin only. Positive and negative samples were tested in triplicate.

The results from the biotin interference study are summarized in Table 8 below.

Table 8. Biotin Interference Study Results

Biotin Concentration Tested	Influenza A Positive Replicates		Influenza B Positive Replicates		Negative Samples
	A/Perth/16/ 2009	A/Michigan/ 45/2015	B/Colorado/ 06/2017	B/Phuket/ 3073/2013	
2 µg/mL	0/3	0/3	3/3	3/3	0/3
1 µg/mL	0/3	0/3	3/3	3/3	0/3
500 ng/mL	3/3	3/3	3/3	3/3	0/3
250 ng/mL	3/3	3/3	3/3	3/3	0/3
200 ng/mL	3/3	3/3	3/3	3/3	0/3
125 ng/mL	3/3	3/3	3/3	3/3	0/3

Biotin concentrations up to 500 ng/mL did not lead to false results. Biotin concentrations >500 ng/mL may cause false negative influenza A results with the *CareStart* Flu A&B *Plus*. Due to the relatively high level of biotin tolerance and the specimen type, which is unlikely to have biotin concentrations as high as what is observed in serum/plasma specimens, no limitation in the labeling regarding the biotin interference was necessary.

4. Assay Reportable Range:

Not applicable.

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

Internal Quality Control

The *CareStart* Flu A&B *Plus* assay contains built in internal assay control. The control line contains control antibodies which bind residual sample. The appearance of the control line on the test ensures that sufficient flow of the sample occurred during the assay.

External Quality Controls

The *CareStart* Flu A&B *Plus* kit contains two positive external control swabs (one for influenza A and one for influenza B) and one negative external control swab that allow for

monitoring of the performance of the assay. Influenza A positive external control contains inactivated influenza A virus, influenza B positive external control contains inactivated influenza B virus. Negative control swab contains inactivated *Streptococcus* sp. Group A.

External Controls Lot-to-Lot Reproducibility

The lot-to-lot reproducibility for the external positive and negative control swabs was evaluated using three lots of external controls with three operators. The results from the study are summarized in Table 9 below.

Table 9. External Controls Lot-to-Lot Reproducibility Study Results

Lot No.	Influenza A Positive Control Results Agreement (Count)	Influenza B Positive Control Results Agreement (Count)	Negative Control Results Agreement (Count)
1	100% (9/9)	100% (9/9)	100% (9/9)
2	100% (9/9)	100% (9/9)	100% (9/9)
3	100% (9/9)	100% (9/9)	100% (9/9)

All lots of external controls generated 100% agreement with the expected results.

6. Detection Limit:

The limit of detection (LoD) of the assay was determined with four strains of influenza A virus and four strains of influenza B virus in three step process. First, the initial LoD range was established using a series of five-fold dilutions of virus stocks prepared in UTM. Using a pipette, 50 µL of each dilution was applied to a swab. The swabs were tested in triplicate. The lowest concentration that resulted in 100% detection (3/3) was used to test 20 replicates. The dilution that resulted in ≥95% detection was considered initial LoD range. In the next step, a series of two-fold dilutions in negative nasal matrix near the initial LoD range were prepared. Using a pipette, 50 µL of each dilution were applied to a swab and tested in triplicate until a negative result was obtained for at least one of the replicates. The estimated LoD was confirmed by testing 20 replicates. The results from the study are presented in Table 10 below.

Table 10. Limit of Detection (LoD)

Influenza virus type/subtype	Strain	LoD (EID_{50}/mL)[1]	Percent Positive Results (# positive/# tested)
A (H3N2)	A/Perth/16/2009	$3.2 \times 10^{4.9}$	100% (20/20)
	A/Singapore/INFIMH-16-0019/2016	$2.0 \times 10^{5.2}$	100% (20/20)
A (H1N1) pdm09	A/California/07/2009	$3.2 \times 10^{4.9}$	100% (20/20)
	A/Michigan/45/2015	$3.2 \times 10^{4.2}$	100% (20/20)
B (Victoria lineage)	B/Brisbane/60/2008	$2.0 \times 10^{5.5}$	100% (19/20)
	B/Colorado/06/2017	$1.6 \times 10^{6.4}$	100% (20/20)

B (Yamagata lineage)	B/Wisconsin/01/2010	$4.0 \times 10^{4.9}$	100% (20/20)
	B/Phuket/3073/2013	$1.6 \times 10^{5.5}$	100% (20/20)

[1]EID_{50} = 50% Egg Infectious Dose

7. Analytical Reactivity (Inclusivity)

A total of 15 influenza A strains and 10 influenza B strains were tested in the inclusivity study. 10-fold dilutions of virus stocks were prepared in UTM and 50 µL were pipetted onto the swabs. The swabs were tested in triplicate in this study. The lowest dilution of each strain that resulted in 100% detection (3/3) is presented in Table 11 below.

Table 11. Analytical Reactivity

Subtype	Influenza Strain	Concentration tested	Flu A Positive Replicates	Flu B Positive Replicates
A (H3N2)	A/Alaska/232/2015	2.6×10^6 $CEID_{50}$/mL	3/3	0/3
	A/California/02/2014	5.8×10^2 $TCID_{50}$/mL	3/3	0/3
	A/Hong Kong/4801/2014	9.6×10^5 $CEID_{50}$/mL	3/3	0/3
	A/Michigan/15/2014	9.3×10^4 FFU/mL	3/3	0/3
	A/Texas/71/2017	9.3×10^4 FFU/mL	3/3	0/3
A (H3N2)v	A/Indiana/08/2011	8.1×10^2 $TCID_{50}$/mL	3/3	0/3
	A/Minnesota/11/2010	2.2×10^4 $CEID_{50}$/mL	3/3	0/3
A (H1N1) pdm09	A/Bangladesh/3002/2015	1.3×10^5 $CEID_{50}$/mL	3/3	0/3
	A/Dominican Republic/7293/2013	5.0×10^3 $TCID_{50}$/mL	3/3	0/3
	A/Iowa/53/2015	2.9×10^6 $CEID_{50}$/mL	3/3	0/3
	A/Massachusetts/15/2013	1.6×10^6 $CEID_{50}$/mL	3/3	0/3
	A/Michigan/272/2017	9.6×10^3 $TCID_{50}$/mL	3/3	0/3
	A/New Hampshire/02/2010	1.8×10^6 $CEID_{50}$/mL	3/3	0/3
	A/South Carolina/2/2010	2.5×10^5 $CEID_{50}$/mL	3/3	0/3
	A/St. Petersburg/61/2015	9.3×10^5 $CEID_{50}$/mL	3/3	0/3
B (Victoria lineage)	B/New Jersey/1/2012	8.8×10^4 $TCID_{50}$/mL	0/3	3/3
	B/Colorado/6/2017	1.6×10^6 $CEID_{50}$/mL	0/3	3/3
	B/Florida/78/2015	1.7×10^6 $CEID_{50}$/mL	0/3	3/3
	B/Hong Kong/286/2017	2.7×10^3 $TCID_{50}$/mL	0/3	3/3
	B/Maryland/15/2016	1.3×10^3 $TCID_{50}$/mL	0/3	3/3
B (Yamagata lineage)	B/Guangdong-Liwan/1133/2014	1.8×10^6 $CEID_{50}$/mL	0/3	3/3
	B/Massachusetts/2/2012	1.0×10^7 $CEID_{50}$/mL	0/3	3/3
	B/Phuket/3073/2013	1.1×10^6 $CEID_{50}$/mL	0/3	3/3
	B/Texas/06/2011	6.2×10^6 $CEID_{50}$/mL	0/3	3/3
	B/Utah/09/2014	6.3×10^4 $CEID_{50}$/mL	0/3	3/3

8. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable. Performance of the *CareStart* Flu A&B *Plus* was evaluated in a clinical study against an FDA-cleared molecular assay.

2. Matrix Comparison:

Matrix Equivalency

Matrix equivalency study was conducted to determine if UTM can be used as a substitute to natural negative clinical matrix in analytical studies performed to evaluate the *CareStart* Flu A&B *Plus* assay. Influenza A/Michigan/45/2015 and influenza B/Colorado/06/2017 were diluted in natural nasal swab matrix or UTM. The pooled natural matrix was prepared by eluting nasal swabs in 3 mL of UTM and combing matrix obtained from 17 healthy volunteers. The negativity for influenza A and B of the pooled matrix was confirmed by testing with an FDA-cleared molecular assay. The swabs were prepared by pipetting 50 µL of sample onto each swab. A series of 5-fold dilutions of each influenza strain were tested in triplicate until at least one negative result was obtained. The lowest dilution that generated 100% positive results (3/3) was confirmed by testing 20 replicates. The results from the matrix equivalency study are presented in Table 12 below.

Table 12. Matrix Equivalency Study Results

Influenza virus type/subtype	Matrix	LoD (EID_{50}/mL)	Percent Positive Results (# positive/# tested)
A (H1N1) pdm09 A/Michigan/45/2015	Clinical matrix	$3.2 \times 10^{4.2}$	100% (20/20)
	UTM	$3.2 \times 10^{4.2}$	100% (20/20)
B (Victoria lineage) B/Colorado/06/2017	Clinical matrix	$1.6 \times 10^{6.4}$	100% (19/20)
	UTM	$1.6 \times 10^{6.4}$	100% (20/20)

The results of the study support equivalency between the clinical matrix and the Universal Transport Media (UTM) for preparation of samples for the analytical studies and the reproducibility study.

C Clinical Studies:

1. Clinical Sensitivity and Specificity

Clinical performance characteristics of the *CareStart* Flu A&B *Plus* were evaluated in a multi-center prospective clinical study in the U.S. during 2018-2019 influenza season. A total of 10 clinical sites representing point-of-care participated in the study. To participate in the clinical study, the patients had to show flu-like symptoms, meet other inclusion criteria and sign informed consent. Demographics of the patients who participated in the clinical study are presented in Table 13 below.

Table 13. Demographics of Clinical Study Participants

Age group	Number	Percent of Patients
≤ 5 years	174	18.4%
6 to 21 years	333	35.3%
22 to 59 years	394	41.7%
≥ 60 years	43	4.6%
Total	944	100%
Sex	**Number**	**Percent of Patients**
Male	409	43.3%
Female	535	56.7%
Total	944	100%

The performance of the *CareStart* Flu A&B *Plus* assay was evaluated against an FDA-cleared molecular assay. Two nasopharyngeal swabs were collected from the same nostril of each patient participating in the clinical study. The first swab was tested with an FDA-cleared molecular assay and the second swab was tested with the *CareStart* Flu A&B *Plus*. The discrepant results were further analyzed by testing with an alternative FDA-cleared molecular assay. The performance estimates for influenza A and B compared to an FDA-cleared molecular assay are presented in Tables 14-15 below. The results of discrepant analyses are presented as footnotes and were not included in the calculation of the performance estimates.

Table 14. Performance of the *CareStart* Flu A&B *Plus* for Influenza A

CareStart* Flu A&B *Plus	**Molecular Comparator**		
	Positive	**Negative**	**Total**
Positive	307	9[a]	316
Negative	77[b]	551	628
Total	384	560	944
Sensitivity: 79.9% (95% CI: 75.7% – 83.7%)			
Specificity: 98.4% (95% CI: 97.0% – 99.2%)			

[a] Influenza A was detected in 2/9 false positive specimens using an alternative FDA-cleared molecular influenza A/B assay

[b] Influenza A was not detected in 15/77 false negative specimens using an alternative FDA-cleared molecular influenza A/B assay

Table 15. Performance of the *CareStart* Flu A&B *Plus* for Influenza B (Prospectively Collected Specimens)

CareStart* Flu A&B *Plus	**Molecular Comparator**		
	Positive	**Negative**	**Total**
Positive	15	0	15
Negative	2[a]	927	929
Total	17	927	944
Sensitivity: 88.2% (95% CI: 65.7% – 96.7%)			
Specificity: 100.0% (95% CI: 99.6% – 100.0%)			

[a] Influenza B was detected in 2/2 false negative specimens using an alternative FDA-cleared molecular influenza A/B assay

Due to the low prevalence of influenza B during 2018-2019 flu season in the U.S., additional archived specimens were tested with *CareStart* Flu A&B *Plus* assay. A total of 162 swabs (112 samples positive for influenza B and 50 negative samples) prepared from archived frozen respiratory specimens were tested with *CareStart* Flu A&B *Plus* assay and an FDA-cleared molecular assay. The performance estimates for the archived specimens are presented in Table 16 below.

Table 16. Performance of the *CareStart* Flu A&B *Plus* for Influenza B (Archived Specimens)

CareStart* Flu A&B *Plus	**Molecular Comparator**		
	Positive	**Negative**	**Total**
Positive	113	1	114
Negative	4	44	48
Total	117	45	162
Sensitivity: 96.6% (95% CI: 91.5% – 98.7%)			
Specificity: 97.8% (95% CI: 88.4% – 99.6%)			

Performance of the *CareStart* Flu A&B *Plus* against the molecular comparator (percent agreement) by the age group for influenza A is presented in Table 17 below.

Table 17. Performance of the *CareStart* Flu A&B *Plus* for Influenza A Stratified by Age Group

Age group	**Influenza A Sensitivity 95% CI**	**Influenza A Specificity 95% CI**
≤ 5 years	83.3% (55/66) 72.6% – 90.4%	94.4% (102/108) 88.4% – 97.4%
6 to 21 years	82.5% (141/171) 76.1% – 87.4%	98.8% (160/162) 95.6% – 99.7%
22 to 59 years	74.1% (100/135) 66.1% – 80.7%	99.6% (258/259) 97.8% – 99.9%
≥60 years	91.7% (11/12) 64.6% – 98.5%	100% (31/31) 89.0%– 100.0%

Performance of the *CareStart* Flu A&B *Plus* against the molecular comparator (percent agreement) by the age group for influenza B prospectively collected specimens is presented in Table 18 below.

Table 18. Performance of the *CareStart* Flu A&B *Plus* for Influenza B (Prospectively Collected Specimens) Stratified by Age Group

Age group	Influenza B Sensitivity 95% CI	Influenza B Specificity 95% CI
≤ 5 years	100% (2/2) 34.2% – 100%	100% (172/172) 97.8% – 100%
6 to 21 years	87.5% (7/8) 52.9% – 97.8%	100% (325/325) 98.8% – 100%
22 to 59 years	83.3% (5/6) 43.7% – 97.0%	100% (388/388) 99.0% – 100%
≥60 years	100% (1/1) 20.7% – 100%	100% (42/42) 91.6% – 100%

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

In the *CareStart* Flu A&B *Plus* prospective clinical study performed during 2018-2019 influenza season, a total of 944 nasopharyngeal swabs were evaluated. The number of positives and positivity rate of influenza A and influenza B specimens per age group are presented in Tables 19-20 below.

Table 19. Influenza A Positives by *CareStart* Flu A&B *Plus*

Age group	Number of Specimens	Number of Influenza A Positives	Influenza A Positivity Rate
≤ 5 years	174	61	35.1%
6 to 21 years	333	143	42.9%
22 to 59 years	394	101	25.6%
≥60 years	43	11	25.6%
Total	944	316	33.5%

Table 20. Influenza B Positives by *CareStart* Flu A&B *Plus*

Age group	Number of Specimens	Number of Influenza B Positives	Influenza B Positivity Rate
≤ 5 years	174	2	1.5%
6 to 21 years	333	7	2.1%
22 to 59 years	394	5	1.3%
≥60 years	43	1	2.3%
Total	944	15	1.6%

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.