ACCESSBIO

For use with nasopharyngeal swab specimens

For *in vitro* diagnostic use only

CLIA complexity: MODERATE

Rx Use only

CareStart™ Flu A&B *Plus*

Rapid Diagnostic Test for Detection of Influenza A and B Antigen

Package Insert (Instructions for Use)

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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, 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.

Explanation of Influenza

Influenza is a highly contagious, acute viral infection of the respiratory tract with symptoms such as headache, chills, dry cough, body aches or fever. It is a communicable disease that can be easily transmitted through aerosolized droplets containing the live virus from coughing and sneezing. The causative agents of the disease are immunologically diverse single strand RNA viruses known as influenza viruses. Influenza type A viruses are typically more prevalent than influenza type B viruses and are associated with most serious influenza epidemics, while influenza type B virus infections are usually milder. Diagnosis of influenza virus infection is difficult because the initial symptoms are similar to those caused by other infectious agents. Accurate diagnosis and prompt treatment of patients can have a positive effect on public health. Rapid and accurate diagnosis of influenza viral infection can also help reduce the inappropriate use of antibiotics and gives the physician the opportunity to prescribe appropriate antiviral medications.

Test Principle and Summary

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

Nasopharyngeal swabs require a sample preparation step in which the sample is eluted and washed off into the extraction buffer solution. Extracted swab sample is added to the sample well of the test device to initiate the test. When the swab sample migrates in the test strip, influenza A or B viral antigens bind to anti-influenza antibodies conjugated to indicator particles in the test strip forming an immune complex. The immune complex is then captured by each test line and control line on the membrane as it migrates through the strip.

Test results are interpreted at 10 minutes. The presence of two colored lines, a purple-colored line in the control region “C” and a red-colored line in the influenza A test region “A”, indicates influenza A positive. The presence of two colored lines, a purple-colored line in the control region “C” and a blue-colored line in the influenza B test region “B”, indicates influenza B positive. The presence of three colored lines, a purple-colored line in the control region “C”, a red-colored line in the influenza A test region “A”, and a blue-colored line in the influenza B test region “B indicates, influenza A and B dual positive result. The absence of a line on both influenza A and B test regions with a purple-colored line in the control region “C” indicates negative. No appearance of a purple-colored line in the control region “C” indicates an invalid test.

Reagents and Materials Provided

Contents Name	Quantity (in a kit)	Description
Test device	20 each	Foil pouched test device containing one test strip which is encased on plastic device cassette.
Extraction vial / cap	20 vials and caps	The extraction vial contains 400 μL extraction buffer solution.
Nasopharyngeal swab	20 each	Swab for nasopharyngeal specimen collection.
Influenza A positive control swab	1 each	Influenza A positive and influenza B negative external control swab. Inactivated influenza A antigen is dried on the tip of the swab.
Influenza B positive control swab	1 each	Influenza A negative and influenza B positive external control swab. Inactivated influenza B antigen is dried on the tip of the swab.
Influenza Negative control swab	1 each	Influenza A negative and influenza B negative external control swab. Inactivated Group A, *Streptococcus* is dried on the tip of the swab.
Package insert	1 each	Instructions for use
Quick Reference Instructions (QRI)	1 each	Quick reference instructions

The following materials are needed but not provided:

- Pair of gloves
- Timer / Pen
- Biohazard or sharps container

Hazard and Precautionary Statements

Hazardous codes

Code	Descriptions	Code	Descriptions
H315v	Causes skin irritation	H335	May cause respiratory irritation

Precautionary codes

Code	Descriptions	Code	Descriptions
P261	Avoid breathing dust/fume/gas/mist/vapors/spray	P405v	Store locked up
P264	Wash hands thoroughly after handling	P302+352v	IF ON SKIN, Wash with plenty of soap and water
P273	Avoid release to the environment	P501v	Dispose of contents/container in accordance with local/regional/national regulations
P270	Do not eat, drink or smoke when using this product	P301+P312	IF SWALLOWED, Call to POISON CENTER or doctor/physician if you feel unwell
P280v	Wear protective gloves/protective clothing/eye protection/face protection		
P315	Get immediate medical advice/attention		
P330	Rinse mouth		

Precautions

IVD use

- Prescription use and *in vitro* diagnostic use only.
- As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
- In order to obtain accurate results, the test operator must follow this instruction for use.
- Excess blood or mucus on the swab specimen may interfere with test performance and may yield a false positive result. Avoid touching any bleeding areas of the nasopharynx when collecting specimens.
- Do not interpret the test result after 15 minutes.
- Do not use if the test device package is damaged.
- Do not use if nasopharyngeal swab package is damaged.

	• Other commercial controls have not been validated with this test and are not recommended. • 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 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.
Safety and handling	• Do not use the kit contents beyond the expiration date. • Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents. • Do not eat, drink or smoke in the area where the specimens and kit contents are handled. • Dispose of used contents as biohazardous waste in accordance with federal, state and local requirements. • Nitrile or latex gloves should be worn when performing this test. • If the extraction solution contacts the skin or eye, flush with copious amounts of water. • Handle all specimens as though they contain infectious agents. • Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. • Reagents contain sodium azide, which is harmful if inhaled, swallowed or exposed to skin. Contact with acids produces very toxic gas. If there is contact with skin, wash immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
Contamination and inhibition	• Do not interchange kit contents. • Do not re-use any contents in the kit.

Storage and Stability

- Store kit at 1~30°C.
- The *CareStart*™ Flu A&B *Plus* kit reagents and materials are stable until the expiration date printed on the outer packaging.
- Do not freeze any contents of the kit.

Quality Control

Internal Quality Control: The *CareStart*™ Flu A&B *Plus* contains a built-in internal procedural control that is included in the test device. A purple-colored line appearing in the control region "C" is designed as an internal procedural control. The appearance of a purple procedural control line indicates that sufficient flow has occurred, and the functional integrity of the test device has been maintained. If the purple procedural control line does not develop at 10 minutes, the test result is considered invalid and retesting with a new device is recommended. If the internal procedural control line did not appear in retest, please contact the manufacturer or distributor before testing further patient specimens with new devices.

External Quality Control: External control is used to demonstrate that the test device and test procedure perform properly. It is recommended that a positive and negative external control swabs are run once with every new lot, shipment, and each new user. External positive and negative control swabs are provided in the kit. The external control should be tested using the nasopharyngeal swab test procedure provided in this package insert or in the quick reference instruction card. If the external control results are invalid, please contact the manufacturer or distributor before testing patient specimens.

Specimen Type

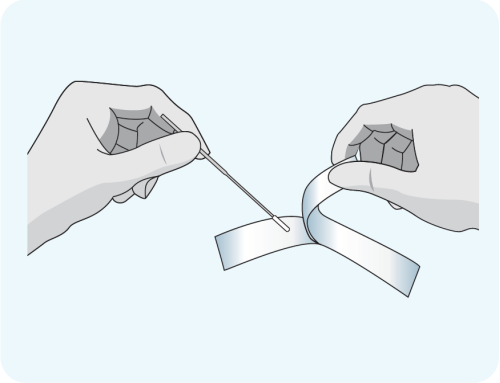
Acceptable specimen type for testing with the *CareStart*™ Flu A&B *Plus* are nasopharyngeal swabs which are collected from nasopharynx using nasopharyngeal swabs. It is essential that correct specimen collection and preparation methods be followed. Inadequate specimen collection, improper specimen handling and/or transport may yield a false negative result; therefore, specimen collection requires specific training and guidance due to the importance of specimen quality to obtain accurate test results.

Specimen Collection and Test Procedures

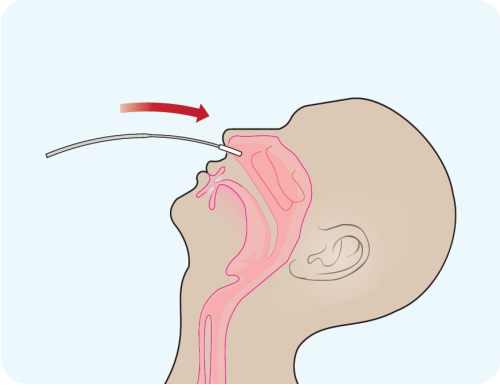
Nasopharyngeal Swab Sample Collection Procedure

Procedural Notes

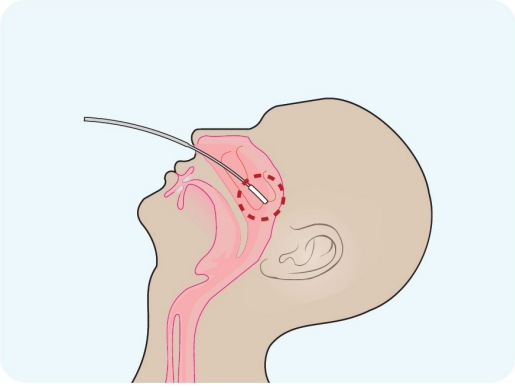
- Use only provided nasopharyngeal swab for specimen collection.
- Collect the specimen wearing safety gloves to avoid contamination.
- Do not touch the tip (specimen collection area) of the swab.
- Collect sample as soon as possible after onset of symptoms.
- Process the test sample immediately after collection.



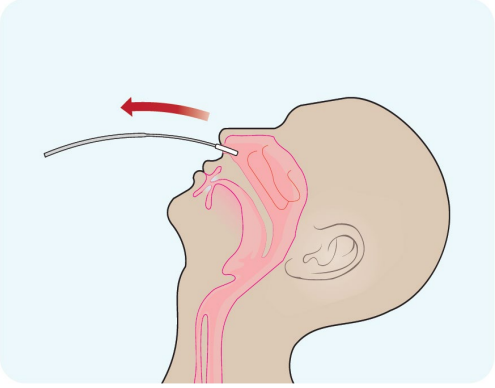
1. Remove a nasopharyngeal swab from the pouch.



2. Place the swab into one of the patient's nostrils until it reaches the posterior nasopharynx.



3. Slowly rotate 3-5 times the swab over the surface of the posterior nasopharynx.



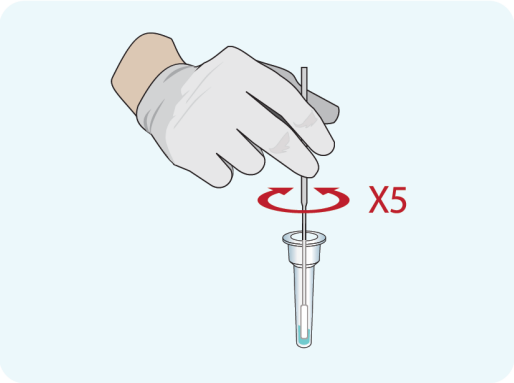
4. Remove the swab from the nostril.

Test Procedures

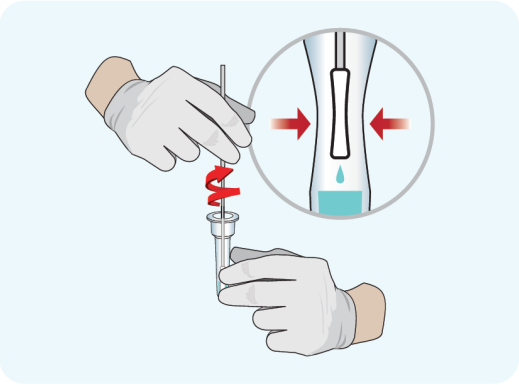
Procedural Notes

- Allow test device, reagents, specimens, and/or controls to equilibrate to room temperature (15~30°C) prior to testing.
- Remove the *CareStart*™ Flu A&B *Plus* test device and extraction vial from its foil pouch immediately before testing.
- The *CareStart*™ Flu A&B *Plus* kit IS INTENDED to be used only with nasopharyngeal swab specimens.
- The *CareStart*™ Flu A&B *Plus* kit IS NOT INTENDED for testing liquid samples such as wash or aspirate samples or swabs in transport media as results can be compromised by over dilution.

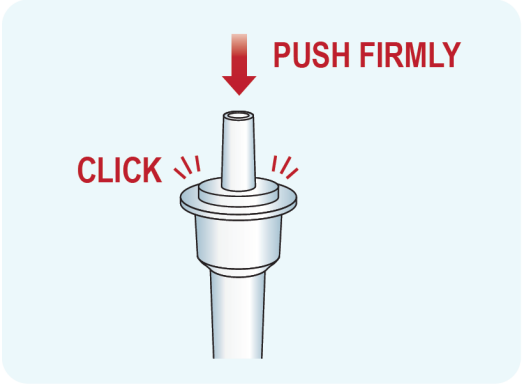
5. Peel off aluminum foil seal.



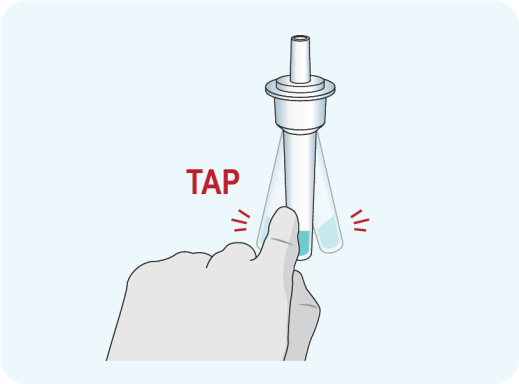
6. Place the swab into the extraction vial. Rotate the swab vigorously at least 5 times.



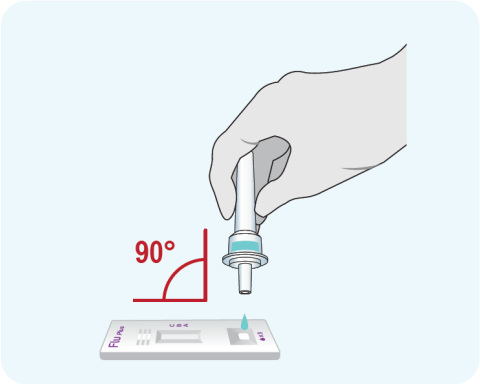
7. Remove the swab with rotating against the extraction vial while squeezing the sides of the vial to release the liquid from the swab. Properly discard the swab.



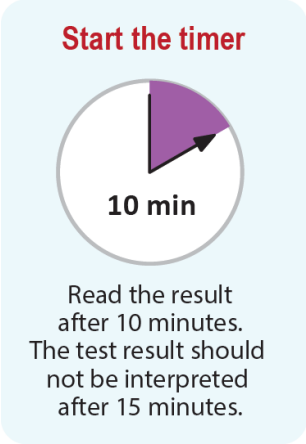
8. Close the vial with the provided cap and push firmly onto the vial.



9. Mix thoroughly by flicking the bottom of the tube.



10. Invert the extraction vial and hold the sample vertically above the sample well. Squeeze the vial gently. Allow three (3) drops of sample to fall into the sample well.



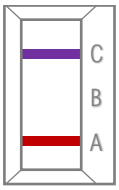
11. Read and interpret the test result after 10 minutes. The test result should not be read and interpreted after 15 minutes.

Interpretation of Results

NOTE: The test result should not be read and interpreted after 15 minutes. Do not interpret the result using any instruments.

Influenza Antigen Positive:

Influenza A infection: two distinct colored lines appear.

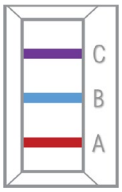


One purple-colored line next to “C” and one red-colored line next to “A” indicates influenza A positive result.

Influenza B infection: two distinct colored lines appear.

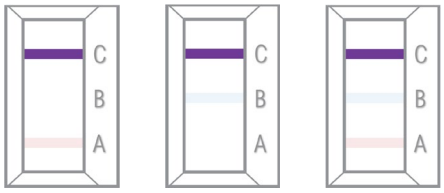
One purple-colored line next to “C” and one blue-colored line next to “B” indicates influenza B positive result.

Influenza A and B infection: three distinct colored lines appear.



One purple-colored line next to “C”, one blue-colored line next to “B”, and one red-colored line next to “A” indicates influenza A and B co-infection positive result.

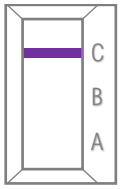
Result with faint colored line(s):



The color intensity in the test region will vary depending on the amount of influenza virus type A and/or B antigen present in the sample. Any faint colored line(s) in the test region(s) should be considered as positive.

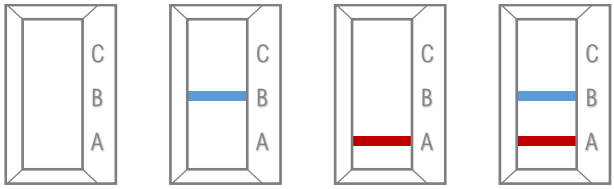
NOTE: The control line “C” may appear as a bluish-purple color in the case of influenza A strong positive and a reddish-purple color in the case of the influenza B strong positive.

Negative:



One purple-colored line only next to “C” indicates a negative result.

Invalid:



If the purple-colored line in the control region “C” is not visible, the result is invalid. Re-run the test one time using the remaining specimen in the extraction vial if an invalid result is obtained during initial testing.

Limitations

1. This test will indicate the presence of influenza virus type A and B nucleoprotein antigens in the specimen from both viable and non-viable influenza virus.
2. The detection of viral antigen is dependent upon proper specimen collection, handling, transportation, storage, and preparation, including extraction. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
3. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.
4. Results from the device should be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
5. This device has been evaluated for use with human specimen material only.
6. False negative results may occur if the number of organisms in the clinical specimen is below the detection limits of the device.
7. This device is a qualitative test and does not provide information on the viral load present in the specimen.
8. The performance of this device has not been evaluated for patients without signs and symptoms of influenza infection.

9. The performance of this device has not been evaluated for monitoring treatment of influenza infection.
10. The performance of this device has not been evaluated for the screening of blood or blood products for the presence of influenza.
11. This test cannot rule out diseases caused by other bacterial or viral pathogens.
12. The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described below can lead to erroneous results.
13. The performance of this device has not been evaluated for patients receiving intranasally administered influenza vaccine. Individuals who received nasally administered influenza vaccine may have positive test results for up to three days after vaccination.
14. The performance of this device has not been evaluated for immunocompromised individuals.
15. Additional testing is required to differentiate any specific influenza A subtypes or strains, in consultation with state or local public health departments.
16. Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing specimens from adults may have lower sensitivity than testing specimens from children.
17. Positive and negative predictive values are highly dependent on prevalence of infection. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low.
18. Monoclonal antibodies may fail to detect or detect with less sensitivity, influenza viruses that have undergone minor amino acid changes in the target epitope region.

Expected Values

The prevalence of influenza varies from year to year, with outbreaks occurring during the fall and winter months. The influenza positivity rate is dependent upon many factors, including specimen collection, test method, and geographic location. Prevalence varies throughout the flu season and from location to location. The *CareStart*™ Flu A&B *Plus* prospective clinical study was conducted during the 2018-2019 influenza season. The following tables show the number of influenza A and influenza B positive cases and its percentage in four subject age categories, as observed during the clinical study.

Age Group	Positivity Rates for Influenza A with the *CareStart*™ Flu A&B *Plus* during the Clinical Study		
	Number of Nasopharyngeal Swab Specimens	Number of **Influenza A** Positives	**Influenza A** Positivity Rate
≤5 Years of Age	174	61	35.1%
6-21 Years of Age	333	143	42.9%
22-59 Years of Age	394	101	25.6%
≥60 Years of Age	43	11	25.6%
Total	944	316	33.5%

Age Group	Positivity Rates for Influenza B with the *CareStart*™ Flu A&B *Plus* during the Clinical Study		
	Number of Nasopharyngeal Swab Specimens	Number of **Influenza B** Positives	**Influenza B** Positivity Rate
≤5 Years of Age	174	2	1.1%
6-21 Years of Age	333	7	2.1%
22-59 Years of Age	394	5	1.3%
≥60 Years of Age	43	1	2.3%
Total	944	15	1.6%

Performance Characteristics

Clinical Study

2018-2019 Prospective Clinical Study in the U.S

The clinical performance characteristics of *CareStart*™ Flu A&B *Plus* were evaluated in a multi-site prospective study during the 2018-2019 influenza season in the U.S. against an FDA-cleared influenza A and B molecular assay. A total of 10 Point-of-Care investigational sites throughout the U.S. participated in the study. To be enrolled in the study, patients had to be presenting at the participating study centers with flu-like symptoms and meet inclusion/exclusion criteria.

Two nasopharyngeal swabs were collected from one nostril from each subject using standard collection methods. At all sites, the first collected nasopharyngeal swab was eluted in 3 mL of viral transport media and transported to the central laboratory for testing using the FDA-cleared

molecular assay as a comparator method. The second collected nasopharyngeal swab was tested directly on *CareStart*™ Flu A&B *Plus* according to product instructions.

A total of 955 subjects were enrolled in this study. Of those, 11 specimens are unevaluable (i.e., four samples failed to meet inclusion criteria, two samples were not collected due to subject refusal after enrollment, one sample was transported to the central laboratory in damaged and leaking state, and four samples transported to the central laboratory were mislabeled). A total of 944 nasopharyngeal swab specimens were considered evaluable. The performance of the *CareStart*™ Flu A&B *Plus* for influenza A and influenza B as compared to the comparator method is presented in the tables below.

All discrepant results were investigated by testing using an alternative FDA-cleared molecular assay at the same central laboratory. The results of this testing are captured in the footnotes but were not included in the calculations of the performance estimates shown below.

CareStart™ Flu A&B *Plus* Influenza A Performance against the Comparator Method

CareStart™ Flu A&B *Plus* – Influenza A	Molecular Comparator		
	Positive	Negative	Total
Positive	307	9[a]	316
Negative	77[b]	551	628
Total	384	560	944
Positive Percent Agreement (PPA)	79.9% (95% CI: 75.7% – 83.7%)		
Negative Percent Agreement (NPA)	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*

CareStart™ Flu A&B *Plus* Influenza B Performance against the Comparator Method

CareStart™ Flu A&B *Plus* – Influenza B	Molecular Comparator		
	Positive	Negative	Total
Positive	15	0	15
Negative	2[a]	927	929
Total	17	927	944
Positive Percent Agreement (PPA)	88.2% (95% CI: 65.7% – 96.7%)		
Negative Percent Agreement (NPA)	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*

CareStart™ Flu A&B _Plus_ Influenza A and B Performance against the Comparator Method (Percent Agreement) by Age Group

		Prospective Study during the 2018-2019 influenza season	
		Influenza A	Influenza B
≤5 Years of Age	PPA	83.3% (55/66) 95% CI: 72.6% – 90.4%	100% (2/2) 95% CI: 34.2% – 100%
	NPA	94.4% (102/108) 95% CI: 88.4% – 97.4%	100% (172/172) 95% CI: 97.8% – 100%
6-21 Years of Age	PPA	82.5% (141/171) 95% CI: 76.1% – 87.4%	87.5% (7/8) 95% CI: 52.9% – 97.8%
	NPA	98.8% (160/162) 95% CI: 95.6% – 99.7%	100% (325/325) 95% CI: 98.8% – 100%
22-59 Years of Age	PPA	74.1% (100/135) 95% CI: 66.1% – 80.7%	83.3% (5/6) 95% CI: 43.7% – 97.0%
	NPA	99.6% (258/259) 95% CI: 97.8% – 99.9%	100% (388/388) 95% CI: 99.0% – 100%
≥60 Years of Age	PPA	91.7% (11/12) 95% CI: 64.6% – 98.5%	100% (1/1) 95% CI: 20.7% – 100%
	NPA	100% (31/31) 95% CI: 89.0% – 100%	100% (42/42) 95% CI: 91.6% – 100%

Retrospective Study

Due to extremely low prevalence of influenza B observed during the 2018-2019 influenza season in the U.S., the prospective clinical study performance data were supplemented with data from a retrospective study testing 162 swab samples prepared from archived respiratory specimens that were obtained from patients with influenza-like symptoms and were confirmed positive or negative by an FDA-cleared molecular assay for influenza A and influenza B. These swab samples (112 samples positive for influenza B and 50 negative samples) were distributed (blinded and randomized) among four of the investigational sites and the testing was incorporated into the daily workflow at each site during the prospective clinical study period.

All of the 162 swab specimens enrolled in the retrospective study were considered evaluable to supplement the prospective clinical performance data for influenza B. The performance of the _CareStart_™ Flu A&B _Plus_ for influenza B with archived samples, as compared to the FDA-cleared molecular method is presented in the table below.

CareStart™ Flu A&B *Plus* Influenza B (swab specimens prepared from frozen archived respiratory specimens) Performance against the Comparator Method

CareStart™ Flu A&B *Plus* – Influenza B (swab specimens prepared from frozen archived respiratory specimens)	Molecular Comparator		
	Positive	Negative	Total
Positive	113	1	114
Negative	4	44	48
Total	117	45	162
Positive Percent Agreement (PPA)	96.6% (95% CI: 91.5% – 98.7%)		
Negative Percent Agreement (NPA)	97.8% (95% CI: 88.4% – 99.6%)		

Reproducibility study

The reproducibility study was performed to evaluate the reproducibility of the *CareStart*™ Flu A&B *Plus* with contrived swab samples at three Point-of-Care sites in the U.S. The test sample panels consisted of seven samples at various virus concentrations, including near the respective LoD (i.e., true negative, high negative influenza A, low positive influenza A, moderate positive influenza A, high negative influenza B, low positive influenza B, and moderate positive influenza B). The sample panel was tested in a blinded manner by three operators at each of three test sites on five non-consecutive days. Agreement of obtained results with expected results was 100% across all sites, operators, and days.

Reproducibility by Study Site

Sample Category	Site 1		Site 2		Site 3		Overall % Agreement and 95% CI
	%	Count	%	Count	%	Count	
True Negative[a] (no virus)	100.0%	45/45	100.0%	45/45	100.0%	45/45	100.0% (135/135) (97.2% - 100.0%)
High Negative A[a] (0.1x LoD)	100.0%	45/45	100.0%	45/45	100.0%	45/45	100.0% (135/135) (97.2% - 100.0%)
Low Positive A (1x LoD)	100.0%	45/45	100.0%	45/45	100.0%	45/45	100.0% (135/135) (97.2% - 100.0%)
Moderate Positive A (3x LoD)	100.0%	45/45	100.0%	45/45	100.0%	45/45	100.0% (135/135) (97.2% - 100.0%)
High Negative B[a] (0.1x LoD)	100.0%	45/45	100.0%	45/45	100.0%	45/45	100.0% (135/135) (97.2% - 100.0%)
Low Positive B (1x LoD)	100.0%	45/45	100.0%	45/45	100.0%	45/45	100.0% (135/135) (97.2% - 100.0%)
Moderate Positive B (3x LoD)	100.0%	45/45	100.0%	45/45	100.0%	45/45	100.0% (135/135) (97.2% - 100.0%)

[a] The expected results for true negative and high negative samples are negative results.

Lot-to-Lot Precision

Three different lots of the *CareStart*™ Flu A&B *Plus* were evaluated for precision. Agreement of observed results with expected results was 100%. No variability was observed between reagent lots.

Sample Category	Reagent Lot 1		Reagent Lot 2		Reagent Lot 3		Overall % Agreement and 95% CI
	%	Count	%	Count	%	Count	
True Negative[a] (no virus)	100%	9/9	100%	9/9	100%	9/9	100% (27/27) (87.5% - 100%)
High Negative A[a] (0.1x LoD)	100%	9/9	100%	9/9	100%	9/9	100% (27/27) (87.5% - 100%)
Low Positive A (1x LoD)	100%	9/9	100%	9/9	100%	9/9	100% (27/27) (87.5% - 100%)
Moderate Positive A (3x LoD)	100%	9/9	100%	9/9	100%	9/9	100% (27/27) (87.5% - 100%)
High Negative B[a] (0.1x LoD)	100%	9/9	100%	9/9	100%	9/9	100% (27/27) (87.5% - 100%)
Low Positive B (1x LoD)	100%	9/9	100%	9/9	100%	9/9	100% (27/27) (87.5% - 100%)
Moderate Positive B (3x LoD)	100%	9/9	100%	9/9	100%	9/9	100% (27/27) (87.5% - 100%)

[a] The expected results for true negative and high negative samples are negative results.

Analytical Sensitivity: Limit of Detection

Four influenza A strains and four influenza B strains were tested at varying concentrations to determine and confirm the Limit of Detection (LoD).

Influenza Virus		LoD (EID_{50}/ml)	% Reactive (No. of positive / Total No. of Replicates)
Type/Subtype	Strain		
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	$2.0 \times 10^{5.5}$	95% (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)

EID_{50}= 50% Egg Infectious Dose

Analytical Sensitivity: Reactivity (Inclusivity)

Analytical reactivity (inclusivity) was evaluated for the *CareStart*™ Flu A&B *Plus* by testing a panel of 15 influenza A strains and 10 influenza B strains. Viral strains were diluted to a concentration near the LoD and tested in triplicate with the *CareStart*™ Flu A&B *Plus*. All viruses were detected in all three replicates at the concentrations shown below.

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

$CEID_{50}$= 50% Chicken Embryo Infectious Dose
$TCID_{50}$= 50% Tissue Culture Infectious Dose
FFU= Focus Forming Assay Unit

Analytical Specificity: Cross Reactivity (Exclusivity) and Microbial Interference

The potential cross-reactivity (exclusivity) of a panel of common organisms was evaluated with influenza negative samples using the *CareStart*™ Flu A&B *Plus*. Potential microbial interference was evaluated with samples containing influenza A or influenza B at approximately 2x LoD. A total of 31 bacteria were tested at a target concentration of approximately 10^7 cfu/ml with the exception of *Mycoplasma pneumoniae*, which was tested at a final concentration of 1.5×10^3 cfu/ml. The 15 non-influenza viruses were tested at concentrations between $10^{5.86}$ and

$10^{4.2}$ $TCID_{50}$/ml. All influenza negative samples gave negative results at the concentrations of the potentially cross-reactive common organisms tested showing no cross-reactivity with *CareStart*™ Flu A&B *Plus* assay. All samples with influenza A or influenza B tested positive showing no microbial interference at the concentrations of the potentially interfering common organisms tested.

Bacteria		Viruses
Acinetobacter calcoaceticus	*Neisseria gonorrhoeae*	Adenovirus 1
Bodetella pertussis	*Neisseria meningiditis*	Adenovirus 7
Candida albicans	*Neisseria sicca*	Coronavirus (OC43)
Chlamydophila pneumoniae	*Proteus vulgaris*	Coronavirus (229E)
Corynebacterium diphtheriae	*Pseudomonas aeruginosa*	Cytomegalovirus
Enterococcus faecalis	*Staphylococcus aureus*	Human coxsackievirus B4
Escherichia coli	*Staphylococcus epidermidis*	Human metapneumovirus
Gardnerella vaginalis	*Serratia marcescens*	Measles
Haemophilus influenza	*Streptococcus mutans*	Mumps (Enders)
Klebsiella pneumoniae	*Streptococcus pneumoniae*	Parainfluenza virus type 1
Lactobacillus casei	*Streptococcus pyogenes*	Parainfluenza virus type 2
Legionella pneumophila	*Streptococcus* sp. Group B	Parainfluenza virus type 3
Listeria monocytogenes	*Streptococcus* sp. Group C	Respiratory Syncytial Virus Type B
Moraxella catarrhalis	*Streptococcus* sp.Group F	Rhinovirus 1A
Mycobacterium tuberculosis	*Streptococcus sanguinis*	Rubella
Mycoplasma pneumoniae		

Interfering Substances Effect

To assess substances with the potential to interfere with the performance of the *CareStart*™ Flu A&B *Plus*, influenza A positive, influenza B positive, and negative samples were tested with the addition of potentially interfering substances. The influenza concentration in the positive samples was approximately 2x LoD. All samples tested produced expected results, demonstrating that the *CareStart*™ Flu A&B *Plus* test performance was not affected by any of the 31 potentially interfering substances listed in the table below at the concentrations tested.

Interfering Substances	Concentration	Interfering Substances	Concentration
Acetaminophen	10 mg/ml	Mometasone	1 mg/ml
Acetyl salicylic acid	15 mg/ml	Mucin	2%
Beclomethasone	0.5 mg/ml	Mupirocin	1 mg/ml
Benzocaine	5 mg/ml	OTC Throat drop (Halls)	15%
Budesonide	2 mg/ml	OTC Throat drop (Ricola)	15%
Chlorpheniramine maleate	5 mg/ml	OTC Nasal spray (Afrin)	15%
Dexamethasone	1 mg/ml	OTC Nasal spray (Vicks Sinex)	15%
Dextromethorphan HBr	2 mg/ml	OTC Nasal spray (Zicam)	15%

Interfering Substances	Concentration	Interfering Substances	Concentration
Diphenhydramine HCl	5 mg/ml	Oxymetazoline HCl	10 mg/ml
Ephedrine HCl	10 mg/ml	Phenylephrine HCl	5 mg/ml
Flunisolide	5 mg/ml	Phenylpropanolamine	5 mg/ml
Fluticasone	1 mg/ml	Tobramycin	1 mg/ml
Guaiacol Glyceryl Ether	20 mg/ml	Triamcinolone	1 mg/ml
Histamine Dihydrochloride	10 mg/ml	Whole Blood	2%
Menthol	10 mg/ml	Zanamivir	1 mg/ml

The interfering effects of biotin concentrations ranging between 125 ng/mL and 2 µg/mL were tested in a separate study. Biotin concentrations up to 500 ng/ml did not lead to false results. Biotin concentrations >500 ng/ml can cause false negative influenza A results with the *CareStart*™ Flu A&B *Plus*.

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4. Williams KM, Jackson MA, Hamilton M. Rapid diagnostic testing for URIs in children: impact on physician decision making and cost. Infect Med. 2002;19(3):109-111

Technical Support

For questions, or to report a problem, please call Technical Support at +1-888-898-1270 (Available Hours: Mon. to Fri.: 8 a.m. – 5 p.m.) or TShelp@accessbio.net (24/7 available).

Test system problems may also be reported to the FDA using the MedWatch reporting system (phone: 1-800 FDA-1088; fax: 1-800 FDA-1078: or http://www.fda.gov/medwatch).

Description of Symbols

Symbol	Descriptions
	Consult instructions for use Indicates the need for the user to consult the instructions for use.
	Manufacturer Indicates the medical device manufacturer.
	Batch code Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Do not re-use Indicates a medical device that is intended for one use, or for use on a single patient during a single procedure.
	Use by date Indicates the date after which the medical device is not to be used.
	Contains sufficient for <n> tests Indicates the total number of IVD tests that can be performed with the IVD.
	Keep away from sunlight Indicates a medical device that needs protection from light sources.
	Keep dry Indicates a medical device that needs to be protected from moisture.
	Authorized representative in EU

Symbol	Descriptions
	Catalog number Indicates the manufacturer's catalog number so that the medical device can be identified.
	Caution Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
	Date of manufacture Indicates the date when the medical device was manufactured.
	Temperature limit Indicates the temperature limits to which the medical device can be safely exposed.
	Do not use if the package is damaged Indicates a medical device that should not be used if the package has been damaged or opened.
	Positive control Indicates a control material that is intended to verify the results in the expected positive range.
	Negative control Indicates a control material that is intended to verify the results in the expected negative range.
	CE marking

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