(continued)

Galphimia glauca	1%
Histaminum hydrochloricum	1%
Live intranasal influenza virus vaccine	1%
Benzocaine	1 mg/mL
Menthol	1 mg/mL
Zanamivir	1 mg/mL
Mupirocin	1 mg/mL
Tobramycin	1 mg/mL

CLIA Waiver Study

Clinical Study at CLIA Waived Sites

To evaluate the expected performance of the **BioSign® Flu A+B** test when used by operators at CLIA-waived sites, a prospective clinical study was performed using nasopharyngeal and nasal swab specimens at seven CLIA waived sites (non-laboratory study sites) from December 2014 to May 2016. A total of sixteen operators from seven intended user sites in the USA were involved in the study. All collected samples were tested with both **BioSign® Flu A+B** and an FDA-cleared NAAT. The total number of samples tested was 455, of which 148 samples were archived samples which were confirmed by PCR as Influenza A or Influenza B.

The combined data from all sites of the prospective study and archived samples are presented in the table below.

	Comparator (PCR) Results			
BioSign® Flu A+B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	124	2	126	PPA: 89.2% 95% CI: 83.0-93.4%
Flu A Negative	15	314	329	NPA: 99.4% 95% CI: 97.7-99.8%
Total	139*	316	455	

*The total number of Influenza A positive includes 27 archived samples.

	Comparator (PCR) Results			
BioSign® Flu A+B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	133	3	136	PPA: 86.4% 95% CI: 80.1-90.9%
Flu B Negative	21	298	319	NPA: 99.0% 95% CI: 97.1-99.7%
Total	154*	301	455	

*The total number of Influenza B positive includes 121 archived samples.

Performance with near cutoff Concentrations at CLIA Waived Sites

To determine the performance of operators at CLIA waived sites with the **BioSign® Flu A+B** test when tested with samples near the cutoff, this study was conducted using a sample panel consisting of high negative (C_5), weak positive (C_{95}) and moderate positive (3 x C_{95}) samples for influenza type A and B, and samples negative for both flu A and B (true negative). For influenza A and B positive samples, A/Denver/1/57 (H1N1) and B/Maryland/1/59 were used. The testing was performed over a period of 10 days using 90 coded samples for each of 6 operators (True negative: 50, High Negative: 15, Low Positive; 15, Moderate Positive; 10 samples respectively). The results are summarized in below table.

	Sample	Site 1 (2 operators)	Site 2 (2operators)	Site 3 (1 operator)	Site 4 (1 operator)	Agreement	95% CI
Flu A	Negative	100% (100/100)	97.0% (97/100)	100% (50/50)	100% (50/50)	99.0% (297/300)	97.1% -99.7%
	High Negative C_5	96.7% (29/30)	100% (29/29*)	93.3% (14/15)	100% (15/15)	97.8% (87/89*)	92.2% -99.4%
	Low Positive C_{95}	96.7% (29/30)	100% (30/30)	100% (15/15)	93.3% (14/15)	97.8% (88/90)	92.3% -99.4%
	Moderate Positive	100% (20/20)	100% (20/20)	100% (10/10)	100% (10/10)	100% (60/60)	94.0% -100%
Flu B	Negative	100% (100/100)	100% (99/99*)	100% (50/50)	100% (50/50)	100% (299/299*)	98.7% -100%
	High Negative C_5	100% (30/30)	96.7% (29/30)	93.3% (14/15)	100% (15/15)	97.8% (88/90)	92.3% -99.4%
	Low Positive C_{95}	100% (30/30)	93.3% (28/30)	93.3% (14/15)	100% (15/15)	96.7% (87/90)	90.7% -99.0%
	Moderate Positive	100% (20/20)	95.0% (19/20)	100% (10/10)	100% (10/10)	98.3% (59/60)	91.2% -99.7%

*One test result out of 30 tests was invalid affecting the total number.

Annual analytical reactivity testing results with CDC influenza panel can be found on our web site at: http://www.pbmc.com

References

1. Shaw MW, Arden NH and Massab HF. New aspects of influenza viruses. Clin. Microbiol. Rev. 5: 74-92 (1992)

2. WHO recommendations on the use of rapid testing for influenza diagnosis, July 2005.

3. Design Considerations for Pivotal Clinical Investigations for Medical Devices: Guidance for Industry, Clinical Investigators, Institutional Review Boards and Food and Drug Administration Staff, November 7, 2013 (Page 45)

Symbols

Instructions For Use (Read)	PRC Procedure Card
REF Catalog Number	Do Not Reuse
Store At	IVD For In Vitro Diagnostic Use
Expiration Date	LOT Lot Number
CONT Contents	CONT + Positive Control
SWAB Swab	CONT - Negative Control
DEV Test Device	Manufacturer
IFU Instructions For Use	MF Manufactured For
CAP Reagent Capsule	EC REP Authorized Representative
	CE CE Mark

MTD | INFLUENZA — An *in vitro* immunocromatographic assay for the qualitative detection of influenza type A and type B antigens directly from nasal specimens

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P-52100-A

BioSign® Flu A+B

Rapid Immunoassay for Direct Detection and Differential Diagnosis of Influenza Type A and Type B Antigens

For Rx Use Only

For *In Vitro* Diagnostic Use

PBM

Catalog No. BSP-510-25 25 Test Kit

CLIA Complexity:

Moderate Complexity when used with Nasal Wash/Aspirate Samples

CLIA Waived when used with Nasal and Nasopharyngeal Swabs

Intended Use

BioSign® Flu A+B is an in vitro rapid qualitative test that detects influenza type A and type B nucleoprotein antigens directly from nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash specimens obtained from patients with signs and symptoms of respiratory infection. It is intended to aid in the rapid differential diagnosis of influenza A and B viral infections.

Negative test results are presumptive and it is recommended these results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions.

Performance characteristics for influenza A and B were established during the 2007-2009 and the 2014-2016 influenza seasons when influenza A/H1N1, A/H1N1 pandemic, A/H3N2, influenza B/Victoria lineage, and B/Yamagata lineage were the predominant influenza viruses in circulation according to the Flu Activity & Surveillance reports from the CDC. 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 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.

Summary and Explanation

Influenza is a highly contagious acute viral infection of the respiratory tract. It is a communicable disease easily transmitted from person to person through aerosol droplets excreted when sneezing and coughing. Common symptoms include high fever, chills, headache, cough, sore throat and malaise. The type A influenza virus is more prevalent and is the primary pathogen associated with serious epidemics. The type B virus causes a disease that is generally not as severe as that caused by the type A virus.

An accurate diagnosis of influenza based on clinical symptoms is difficult because the initial symptoms of influenza are similar to those of numerous other illnesses. Therefore, it can be confirmed only by laboratory diagnostic testing.[1] Early differential diagnosis of influenza type A or type B can allow for proper treatment with appropriate antiviral therapy while reducing the incidence of inappropriate treatment with antibiotics. Early diagnosis and treatment is of particular value in a clinical setting where accurate diagnosis can assist the healthcare professional with management of influenza patients who are at risk for complications.[2] **BioSign® Flu A+B** is a rapid immunoassay to be used as an aid for the differential diagnosis of influenza type A and type B.

Principle of Procedure

BioSign® Flu A+B utilizes the chemical extraction of viral antigens followed by solid-phase immunoassay technology for the detection of extracted antigen, influenza A and/or B. In the test procedure, a specimen is collected and placed for one minute into the Extraction Well of the test device containing extraction solution, during which time antigen is extracted from disrupted virus particles. The test device is then raised, tapped and laid back down onto a level surface to allow the solution in the Extraction Well to migrate through the pads containing detector antibodies conjugated to gold dye and then through the test membrane. If influenza antigens are present in the specimen, they will react with anti-influenza antibody coupled to gold dye particles, migrate through the membrane as antigen-antibody-dye complexes, bind to the immobilized anti-influenza antibody on the membrane, and generate a colored line in the Test line position (A and/or B). The rest of the sample and unbound/bound dye complexes continue to migrate to the Control line position (C), where antibody to the anti-influenza antibody is immobilized, and forms the Control line. Formation of the Control line serves as an internal control to demonstrate that antibodies in the dye pad have been hydrated and that sufficient sample has been applied to allow for migration to the Test line and beyond. If the Control line does not appear within the designated incubation time, the result is invalid and the test should be repeated.

BioSign® Flu A+B has two Test lines, one for influenza A and one for influenza B. The two Test lines allow for the separate and differential identification of influenza A and/or B from the same specimen. If either Test line appears in the test result window, together with the Control line, the test result is positive for influenza.

Reagents

Materials Provided

Each **BioSign® Flu A+B** kit contains enough reagents and materials for 25 tests. The following components are included in a kit.

- **BioSign® Flu A+B** test devices (25): The test strip in each device contains mouse monoclonal antibodies to nucleoprotein (NP) of influenza A and influenza B. The device is individually pouched.
- Extraction Reagent in capsules (25): For use with swab samples, 300 µL of Phosphate buffer with detergents and preservative
- Sterile Swabs (25): For swab samples
- Positive Control Swab (1): Influenza A and B antigens (non-infective recombinant nucleoprotein)
- Negative Control Swab (1): Inactivated Group B Streptococcus antigen (non-infective)
- Package Insert /Instructions for use (1)
- Procedure Card (1)

Materials Required, But Not Provided

For Aspirate Samples only (available separately; Catalog No. : BSP-510AS)

- Extraction Reagent in a bottle (5 mL): Phosphate buffer with detergents and 0.09% sodium azide
- Disposable Transfer Pipettes (50): Buffer and sample transfer
- Procedure card for aspirate samples

For All Sample types:

- Timer
- Latex gloves

Precautions/Warnings

- For *in vitro* diagnostic use only.
- Do not use after the expiration date.
- Use only the swabs provided for collecting swab samples. Other swabs may not work properly.
- Two forms of Extraction Reagent are available. Use Extraction Reagent in capsules to test swab samples, and Extraction Reagent in a bottle to test nasopharyngeal wash/aspirate samples.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Extraction Reagent is slightly caustic. Avoid contact with eyes, sensitive mucous membranes, cuts, abrasions, etc. If the reagent comes in contact with skin or eyes, flush with a large volume of water.
- Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterwards.
- All specimens should be handled as if they are capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens and test devices.
- The **BioSign® Flu A+B** test device should remain in its original sealed pouch until ready for use. Do not use the test if the seal is broken or the pouch is damaged.
- Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses emerge, 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, specimen should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Storage and Stability

The **BioSign® Flu A+B** test may be stored at 2-30°C (35-86°F) in the original sealed pouch, away from direct sunlight. Kit contents are stable until the expiration date printed on the pouch or box.

Specimen Collection and Preparation

- Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false negative test results. Training in specimen collection is highly recommended because of the importance of specimen quality.
- To collect nasopharyngeal or nasal swab specimens, the swab provided in the **BioSign® Flu A+B** test kit should only be used.
- Using 2.5 mL of sterile saline solution is recommended to collect wash/aspirate specimens.
- Use fresh samples for best performance. Freshly collected specimens should be tested immediately. If necessary, aspirate specimens may be stored for up to 8 hrs at room temperature or up to 24 hrs at 2-8°C, and swab samples for up to 4 hrs at room temperature or up to 8 hrs at 2-8°C. Aspirate samples can be frozen for up to 7 days.
- If transport of the samples is required, the following transport media have been tested and shown not to interfere with the performance of the test.

BD™ Universal Viral Transport medium	Saline solution
Veal Infusion Broth	Puritan UTM medium
Copan UTM-RT medium	Hank's Balanced Salt Solution
Tryptose Phosphate Broth	Bartel ViraTrans™ medium
PBS	PBS + 0.5% BSA
M4 medium	M5 medium M6 medium
BD™ Eswab collection kit (Buffer only)	Puritan Amies Transport medium

***Note**: Using one milliliter (1 mL) or less of transport media is recommended for optimal test performance, as dilution of the sample may result in decreased test sensitivity.

Flu A+B Specimen Collection Procedures

Good sample collection is the most important first step for an accurate test result. Therefore, follow below instruction carefully to obtain as much secretion as possible.

Nasal Swab Specimen:

Using a flocked swab provided in the **BioSign® Flu** kit, gently insert the swab approximately 1/4" into the anterior nares (just inside the nasal orifice). Rotate the swab a few times, and repeat in the second nostril, using the same swab.

Nasopharyngeal Swab Specimen:

Using a flocked swab provided in the **BioSign® Flu A+B** kit, insert the swab into the nostril, gently rotating the swab inward until resistance is met at the level of the turbinates. Rotate the swab a few times against the nasopharyngeal wall and then withdraw the swab..

Nasopharyngeal Aspirate Specimen:

With the patient's head slightly hyper-extended, instill 2.5 mL or less (the minimal volume of saline required per patient's size and age) of sterile saline into the patient's nostril. Gently thread the tube through the external nostril, into the nasopharynx. Aspirate wash solution by gentle suction with rotating movement.

NOTE: Catheter should remain in nasopharynx no longer than 10 seconds. Repeat the procedure until adequate sample volume (2.5ml) is obtained.

Nasopharyngeal Wash Specimen:

Adults and Older Children:

Position the patient comfortably in a sitting position, with the neck slightly hyper-extended. Prior to the procedure, have the patient blow their nose.

Using a sterile syringe, introduce 2.5 ml of sterile saline into one nostril. If possible, have the patient retain the saline for a few seconds. Place specimen container directly under the nose with slight pressure on the upper lip. Tilt the head forward and allow the fluid to flow into the specimen container. Repeat the procedure on other nostril, collecting fluid into the same container.

Infants and Younger Children:

The parent should wrap one arm around the child in a manner that will restrain the child's body and arms. Fill a bulb syringe with 2.5 ml of sterile saline, depending on the size of the patient, and instill saline into one nostril, while the head is tilted back. Release the pressure on the bulb to aspirate the specimen back into the bulb. Transfer the specimen into specimen container. Repeat the procedure on other nostril, transferring the second specimen into the same specimen container.Test Procedure

Test Procedure

Procedural Notes

- The test procedure below must be followed to obtain accurate and reproducible results.
- Reagents, specimens, and devices must be at room temperature (18-30°C) for testing.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.
- Label the device with the patient identification or control to be tested.
- Place test device on a level surface.

Swab Sample Procedure

1. Tear the tab off the Extraction Reagent capsule.
2. Squeeze the Extraction Reagent capsule to dispense all of the solution into the Extraction Well of the test device.
3. Insert the specimen swab on the Swab Stand in the Extraction Well. Rotate swab 3 times to mix the specimen.
4. Incubate 1 minute with the swab in Extraction Well.
5. Rotate swab 3 times to mix the specimen. Remove and discard the swab.
6. Raise the device upright (see picture).
7. Let it stand for 1-2 seconds. Gently tap the device to ensure that the liquid flows into the hole.
8. Lay the device back down onto the flat surface. Start timing.
9. Read test results at 10-15 minutes. Confirm negative results at 15 minutes.

Nasopharyngeal Wash/Aspirate Sample Procedure (Purchase of BSP-510AS required)

1. Draw nasal wash or nasopharyngeal aspirate sample to the first (lowest) mark of the graduated transfer pipette.
2. Dispense the entire sample in the transfer pipette into the Extraction Well of the test device.
3. Remove the cap from the Extraction Reagent bottle.
4. Using a new transfer pipette, draw Extraction Reagent Solution to the first (lowest) mark.
5. Dispense all of the solution in the transfer pipette into the Extraction Well of the test device.
6. Incubate 1 minute. Re-cap the Extraction Reagent bottle.
7. Raise the test device upright (see picture).
8. Let it stand for 1-2 seconds. Gently tap the device to ensure that the liquid flows into the hole.
9. Lay the device back down onto the flat surface. Start timing.
10. Read test results at 10-15 minutes. Confirm negative results at 15 minutes.

Swab Sample in Transport Media Procedure

To test transport media with a swab sample, remove swab by vigorously rotating the swab in the liquid media (or vortex), then use the media for testing by following the Nasopharyngeal Wash/Aspirate Sample Procedure.

Warning: The performance of the **BioSign® Flu A+B** test has not been evaluated with swab samples collected in transport media.

(continued)

Influenza Type	Viral Strain	$TCID_{50}$/mL	Influenza Type	Viral Strain	$TCID_{50}$/mL
A	A/Victoria/3/75 (H3N2)	9.95×10^{1}	B	B/Allen/45	1.58×10^{0}
A	A/New Jesey/8/76 (H1N1)	9.95×10^{1}	B	B/GL/1739/54	9.95×10^{2}
A	A/WS/33(H1N1)	5.00×10^{1}	B	B/Taiwan/2/62	1.58×10^{3}
A	A/Swine/1976/31	1.58×10^{2}	B	B/Maryland/1/59	1.99×10^{1}
A	2009 H1N1 Clinical Isolate* (Swine Origin Influenza A)	1.00×10^{3}	B	B/Mass/3/66	5.00×10^{1}
A	A/CA/07/2009 (H1N1)	6.15×10^{3}	B	B/R22 Barbara	1.60×10^{-1}
A	A/CA/08/2009 (H1N1)	9.31×10^{3}	B	B/R75	2.94×10^{3}
A	A/NY/18/2009 (H1N1)	2.5×10^{3}	B	B/Russia/69	3.16×10^{3}
A	A/Mexico/ 4108/2009 (H1N1)	8.51×10^{3}	B	B/Hong Kong/5/72	2.88×10^{1}
A	A/CA/07/2009 NYC, X-179A (H1N1)	1.08×10^{3}	B	B/Texas /39/2006**	2.34×10^{4}

*Clinical isolate cultured and titered. Culture confirmed positive for 2009 H1N1 Influenza A strain using proFLU+ Influenza A Subtyping.

**Although this test has been shown to detect these viral strains cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for these viruses have not been established.

Influenza Type	Viral Strain#	EID_{50}/mL	Influenza Type	Viral Strain#	EID_{50}/mL
A	A/Anhui/1/2013 (H7N9)	7.94×10^{6}	A	A/Texas/50/2012	2.03×10^{4}
A	A/Vietnam/1194 /2004 (H5N1)	1.60×10^{6}	A	A/California /07/2009	1.01×10^{6}
A	A/Anhui/01/2005 (H5N1)	1.60×10^{7}	A	A/Washington /24/2012	2.02×10^{4}
A	A/Northern/Pintail/ Washington /40964/2014 (H5N2)	8.04×10^{5}	B	B/Brisbane /60/2008	3.19×10^{6}
A	A/Gyrfalcon /Washington /410886/2014 (H5N8)	2.03×10^{5}	B	B/Montana /05/2012	4.02×10^{5}
A	A/Brisbane /59/2007	1.01×10^{5}	B	B/Wisconsin /1/2010	2.54×10^{3}
A	A/Fujian Gulou /1896/2009	8.06×10^{4}	B	B/Massachusetts /02/2012	1.01×10^{5}
A	A/Perth/16/2009	2.54×10^{5}			

Although this test has been shown to detect these viral strains cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for these viruses have not been established.

The performance of **BioSign® Flu A+B** was evaluated with nasal and nasopharyngeal swab samples obtained from patients infected with the 2009 H1N1 influenza virus consisting of sixty six (66) frozen clinical Nasal and Nasopharyngeal samples that had previously tested positive for 2009 H1N1 by FDA-cleared CDC RT-PCR test. The **BioSign® Flu A+B** test detected 71% (47/66) of the CDC RT-PCR test positive specimens. The detection rate was 91% with the higher titered specimens and 38% with the lower titered specimens.

Analytical Specificity

Cross-reactivity

The potential cross-reactivity of the non-influenza respiratory pathogens and other microorganisms with which the majority of the population may be infected was tested using the **BioSign® Flu A+B** test at medically relevant levels, 10^{6} cfu/mL for bacteria and 10^{5} pfu/mL for non-flu viruses. None of the organisms or viruses listed in the table below gave a positive result with **BioSign® Flu A+B** at the tested concentration.

Viruses Tested	
Adenovirus*	Measles**
Human coronavirus**	Human metapneumovirus**
Cytomegalovirus**	Mumps virus**
Enterovirus**	Respiratory syncytial virus; Type B*
Epstein Barr Virus**	Rhinovirus; Type 1A**
Human parainfluenza; Type 1, 2 and 3*	

* In the study the virus was confirmed using FDA approved immuno-fluorecence assay

**In the study the virus was confirmed using commercially available PCR (not approved by FDA).

Bacteria Tested	
Bordetella pertussis	*Mycoplasma pneumoniae*
Chlamydia pneumoniae	*Neisseria meningitides*
Corynebacterium sp.	*Neisseria sp.*
Escherichia coli	*Pseudomonas aeruginosa*
Hemophilus influenzae	*Staphylococcus aureus: Protein A Producer*
Lactobacillus sp.	*Staphylococcus epidermidis*
Legionella sp.	*Streptococcus pneumoniae*
Moraxella catarrhalis	*Streptococcus pyogenes*
Mycobacterium tuberculosis avirulent	*Streptococcus salivarius*

Interference

The interference study was conducted using medically relevant concentrations of the potentially interfering substances listed below with two strains each of influenza type A and type B to assess the potential interference of the substances on the performance of the **BioSign® Flu A+B** test.

The test was conducted by spiking each substance into samples containing the lowest detectable virus level of influenza Type A or Type B for the positive interference testing and into samples without influenza virus for the negative interference testing. Each substance had no inhibitory effect on the **BioSign® Flu A+B** test at the concentration listed in the table below.

Substances Tested	Concentration Tested
Mucin	1 mg/ml
Whole Blood	1%
Phenylephrine	10 mg/mL
Oxymetazoline	10 mg/mL
Sodium Chloride with preservative	20%
Beclomethasone	1 mg/mL
Dexamethasone	1 mg/mL
Flunisolide	1 mg/mL
Triamcinolone	1 mg/mL
Budesonide	1 mg/mL
Mometasone	1 mg/mL
Fluticasone	0.5 mg/mL
Luffa opperculata, sulfur	1%

Prospective Clinical Study from 2007 to 2009 and from 2014 to 2016

Combined prospective clinical data from the 2007 to 2009 study and the 2014 to 2016 CLIA waiver study against the PCR comparator assay are presented in the tables below.

Nasopharyngeal/Nasal Swab Samples (combined): Comparison with PCR

	PCR Results			
BioSign® Flu A+B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	266	27	293	Sensitivity: 91.4% 95% CI: 87.6-94.1%
Flu A Negative	25	598	623	Specificity: 95.7% 95% CI: 93.8-97.0%
Total	291	625	916	

	PCR Results			
BioSign® Flu A+B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	99	33	132	Sensitivity: 87.6% 95% CI: 80.3-92.5%
Flu B Negative	14	770	784	Specificity: 95.9% 95% CI: 94.3-97.1%
Total	113	803	916	

Clinical Study from 2017 to 2018

A supplementary clinical study was conducted to collect additional data for assessing **BioSign® Flu A+B** performance compared against the PCR comparator assay for nasopharyngeal aspirate/wash specimens.

From October 2017 to March 2018, residual nasopharyngeal aspirate/wash samples were sequentially collected from the specimens that were received at a state public health laboratory for influenza confirmation testing. All collected samples were tested with both the **BioSign® Flu A+B** and the PCR comparator assay. The total number of nasopharyngeal aspirate/wash samples tested was 226, of which 147 samples were Flu A positive, 41 were Flu B positive, one sample was both Flu A and Flu B positive, and 37 samples were both Flu A and Flu B negative by the PCR comparator assay. Fifteen (15) percent of the total number of samples were from patients aged 5 and younger, 9% were from patients 6-21 years old, and the remainder were from patients older than 21. Forty-four (44) percent of the total number of patients were male and 54% were female. For five of the samples the gender was not reported.

Out of the 226 samples tested, there were no invalid **BioSign® Flu A+B** test results.

Performance of the **BioSign® Flu A+B** against the PCR comparator assay for all nasopharyngeal aspirate/wash samples collected in this clinical study are presented in the tables below.

Nasopharyngeal Aspirate/Wash Samples: Comparison with PCR

	PCR Results			
BioSign® Flu A+B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	126	0	126	Sensitivity: 85.1% 95% CI: 78.5-90.0%
Flu A Negative	22	78	100	Specificity: 100.0% 95% CI: 95.3-100.0%
Total	148	78	226	

	PCR Results			
BioSign® Flu A+B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	36	1	37	Sensitivity: 85.7% 95% CI: 72.2-93.3%
Flu B Negative	6	183	189	Specificity: 99.5% 95% CI: 97.0-99.9%
Total	42	184	226	

Reproducibility

The reproducibility study for **BioSign® Flu A+B** test was conducted at two physicians' offices and one laboratory using a panel of 90 coded specimens for each site. Testing was performed by two personnel for five days at each site. The panel consists of coded samples of high negative, low positive and moderate positive specimens for each of influenza A and B. For influenza A and B positive samples, A/PR/8/34 (H1N1) and B/Maryland/1/59 were used. The low positive was the LOD level of each strain. Each specimen level was tested in triplicate every day per operator. Each operator conducted the tests using the coded samples following the test protocol given in the package insert as if they are testing patient sample including the sample extraction step.

The results obtained at each site agreed 100% with the expected results. No differences were observed within run (15 replicates), between runs (five different days), or between sites (two POL sites and one lab).

Analytical Sensitivity

Limit of Detection (LOD)

The LODs were determined for each of the two strains selected from the influenza type A and type B strains listed in the analytical inclusivity (sensitivity) section below. The sensitivity level of each selected viral strain established in the analytical inclusivity (sensitivity) study was tested 60 times to confirm the sensitivity level as LOD level, which gives 95% detection rate.

All four viral strains tested were detected 96.7% of the time in 60 replicates.

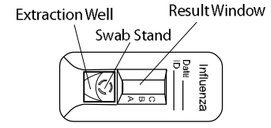
Influenza Type	Viral Strain	$TCID_{50}$/mL	#Positive/ #Total	% Positive
A	A/PR/8/34(H1N1)	1.05×10^{2}	58/60	96.7%
A	A/Victoria/3/75(H3N2)	9.95×10^{1}	58/60	96.7%
B	B/Taiwan/2/62	1.58×10^{3}	58/60	96.7%
B	B/Maryland/1/59	1.99×10^{1}	58/60	96.7%

Analytical Inclusivity

The analytical inclusivity (sensitivity) was established for a total of 49 influenza strains: 34 strains of influenza A type and 15 strains of influenza B type. The results are shown in the tables below.

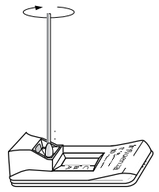
Influenza Type	Viral Strain	$TCID_{50}$/mL	Influenza Type	Viral Strain	$TCID_{50}$/mL
A	A/PR/8/34 (H1N1)	1.05×10^{2}	A	A/Virginia/ATCC2 /2009(H1N1)	2.32×10^{3}
A	A/FM/1/47 (H1N1)	1.73×10^{1}	A	A/Virginia/ATCC3 /2009(H1N1)	5.00×10^{4}
A	A/NWS/33 (H1N1)	4.10×10^{3}	A	A/Indiana/ 10/2011(H3N2)v** ana/10/2011(H3N2)v**	2.34×10^{3}
A	A/Hong Kong/8/ 68 (H3N2)	8.50×10^{2}	A	A/Indiana/08/2011 (H3N2)v**	2.87×10^{6}
A	A/Denver/1/57 (H1N1)	7.20×10^{0}	A	A/Minnesota/11/2010 (H3N2)v**	2.13×10^{6}
A	A/Aichi/2/68 (H3N2)	9.95×10^{0}	A	A/Minnesota/11/ 2010X-203 (H3N2)v**	2.28×10^{3}
A	A/Port Chalmers/1/73	1.99×10^{2}	B	B/Lee/40	5.00×10^{0}

SWAB SAMPLE PROCEDURE



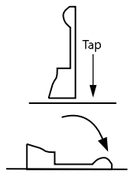
1. 

Tear the tab off the Extraction Reagent Capsule and dispense entire contents into the Extraction Well.

2. 
Insert the specimen swab in the Swab Stand.
- **Spin** swab 3 times to mix the specimen.
- **Let stand** 1 minute.
- **Spin** swab 3 times again.

3. 
Discard the swab.

Raise the device upright and **let stand** 1–2 seconds.

4. 

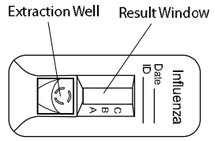
Gently **tap** device to ensure the liquid flows into the hole.

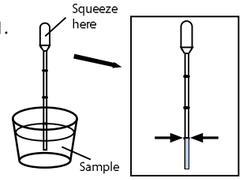
Then, lay the device back down.

Start timing.

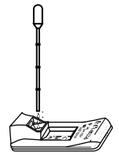
5. Read test results at 10–15 minutes.
Confirm negative results at 15 minutes.

NASOPHARYNGEAL WASH/ASPIRATES SAMPLE PROCEDURE (PURCHASE OF BSP-510AS REQUIRED)



1. 

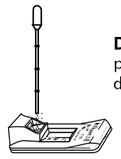
Draw nasal wash or nasal aspirate sample to the **first (lowest) mark** of the graduated transfer pipette.

2. 
Dispense the entire sample in the transfer pipette into the Extraction Well of the test device.

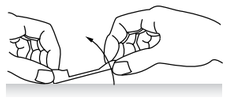
3. 

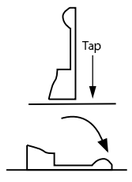
Remove the cap from the Extraction Reagent bottle.

Using a new transfer pipette, draw Extraction Reagent Solution to the **first (lowest) mark.**

4. 
Dispense all of the solution in the transfer pipette into the Extraction Well of the test device.

5. **Let stand** 1 minute.
Re-cap the Extraction Reagent bottle.

6. 
Raise the device upright and **let stand** 1–2 seconds.

7. 

Gently **tap** device to ensure the liquid flows into the hole.

Then, lay the device back down.

Start timing.

8. Read test results at 10–15 minutes.
Confirm negative results at 15 minutes.

Interpretation of Results

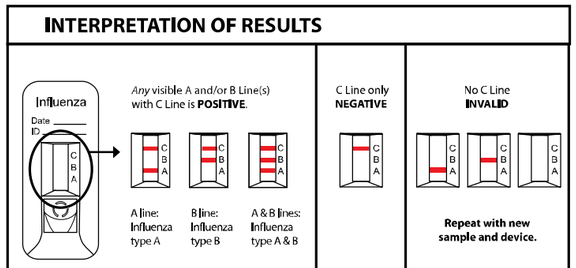
Positive: A reddish purple Control line (C position) and a reddish purple Test line (A or B position) indicate that Influenza A or B antigen has been detected. Lines at the A and C positions indicate the presence of Influenza type A viral antigen, and lines at the B and C positions indicate the presence of Influenza type B viral antigen in the specimen. A positive result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype. Determination of a positive result can be made as soon as both a visible Test line (either A or B) and Control line appear.

Note: *The Test line (reddish purple line) may vary in shade and intensity (light or dark, weak or strong) depending on the concentration of antigen detected. The intensity of the Control line should not be compared to that of the Test line for the interpretation of the test result. Even a light or faint Test line must be interpreted as a positive result.*

Negative: Only a reddish purple Control line (C position), with no Test line at the A or B position, indicates that Influenza A or B antigen has not been detected. A negative result does not exclude influenza viral infection. **Determination of negative results should not be made before 15 min.**

Invalid: A reddish purple line should always appear at the Control line position (C). If a line does not form at the Control line position in 15 minutes, the test result is invalid and the test should be repeated with a new **BioSign® Flu A+B** test device.

NOTE: Co-infection with Influenza A and B is rare. BioSign® Flu A+B "dual positive" clinical specimens (Influenza A and Influenza B positive) should be re-tested. Repeatable influenza A and B "dual positive" results should be confirmed by cell culture or PCR testing before reporting results.



Limitations

- A negative test result does not exclude infection with influenza A or B. Therefore, the results obtained with the **BioSign® Flu A+B** should be used in conjunction with clinical findings to make an accurate diagnosis. Additional testing is required to differentiate any specific influenza A and B subtypes or strains, in consultation with state or local public health departments.
- This test detects both viable (live) and non-viable influenza A and B. Test performance depends on the amount of virus (antigen) in the specimen and may or may not correlate with cell culture results performed on the same specimen.
- **BioSign® Flu A+B** uses highly target specific monoclonal antibodies. As in most immunoassays, it may fail to detect, or detect with less sensitivity, influenza A viruses that have undergone minor amino acid changes in the target epitope region.
- Performance of the **BioSign® Flu A+B** has not been established for monitoring antiviral treatment of influenza.
- Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing specimens from adults will result in lower sensitivity than testing specimens from children.
- Positive and negative predictive values are highly dependent on prevalence. 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.
- Individuals who received nasally administered influenza A vaccine may produce positive test results for up to three days after vaccination.
- The performance of this assay has not been evaluated for use in patients without signs and symptoms of respiratory infection.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this test has not been evaluated for sample types other than those specified in the Intended Use.
- The performance of this test has not been evaluated for immunocompromised individuals.
- The **BioSign® Flu A+B** test can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

User Quality Control

Internal Quality Control:

Each **BioSign® Flu A+B** test device has built-in controls. The Control line at the C position can be considered as an internal positive procedural control; i.e., a proper amount of sample was used, sample was properly added to the Extraction Well, sample migrated properly, and the reagent system worked properly. A distinct reddish-purple Control line should always appear if the test has been performed correctly. If the Control line does not appear, the test result is invalid and a new test should be performed. If the problem persists, contact PBM at 800-726-2670 or 732-274-1000 for technical assistance. A clear background in the Test Result Window is considered an internal negative procedural control. If the test is performed correctly and the **BioSign® Flu A+B** test device is working properly, the background in the Test Result Window will be clear, providing a distinct result.

External Quality Control:

Good laboratory practice includes the use of external controls to ensure proper kit performance. It is recommended that external control testing be performed with each new operator and before using a new lot or shipment of **BioSign® Flu A+B** kits to confirm the expected Q.C. results, using the external controls provided in the kit. The frequency of additional Q.C. tests should be determined according to your laboratory's standard Q.C. procedures and local, State and Federal regulations or accreditation requirements. Upon confirmation of the expected results, the kit is ready for use with patient specimens. If external controls do not perform as expected, do not use the test results. Repeat the tests or contact PBM Technical Assistance. The built-in reddish purple Control line indicates only the integrity of the test device and proper fluid flow.

The **BioSign® Flu A+B** kit contains two control swabs. Test the control swabs in the same manner as patient specimens. When the positive control is tested, reddish purple lines appear at the C, A and B positions. When the negative control is tested, a reddish purple line appears at the C position only.

If the controls do not perform as expected, do not report patient results.

The use of positive and negative controls from other commercial kits has not been established with **BioSign® Flu A+B** test.

Expected Values

The prevalence of influenza varies every year and the rate of positives in influenza testing varies depending on many factors, including the specimen collection method, the test method used, the disease prevalence, and the geographic location. The expected values based on **BioSign® Flu A+B** results were 30.3% for influenza A and 13.8% for influenza B during the 2007-2009 prospective clinical study, and were 33.6% for influenza A and 9.8% for influenza B during the 2014-2016 prospective clinical study.

Performance Characteristics

Clinical Performance

Prospective Clinical Study from 2007 to 2009

A prospective clinical study was conducted from January 2007 to March 2008 and during March and April 2009 to determine the performance of **BioSign® Flu A+B** for nasopharyngeal aspirate, nasopharyngeal swab, and nasal swab specimens.

The samples were collected at 5 sites in the USA from patients who visited physicians' offices and clinics with signs and symptoms of respiratory infection during the study period. All collected samples were tested with **BioSign® Flu A+B**, and were cultured. The culture was initially used as the comparator method. The samples that produced discrepant results between **BioSign® Flu A+B** and viral culture were further analyzed with an FDA-cleared real time RT-PCR Flu A and B assay (PCR comparator assay hereafter).

The total number of patients tested was 862, of which 30% were 5 and younger, 38% were 6-21 years old, and the rest were older than 21. Forty-eight (48) percent were male and 52% were female. A total of 253 nasopharyngeal aspirate specimens and 609 nasopharyngeal swab or nasal swab specimens were included in the performance analyses below.

Nasopharyngeal Aspirate Samples: Comparison with Viral Culture

	Virus Culture Results			
BioSign® Flu A+B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	41	30*	71	Sensitivity: 95.3% 95% CI: 92.1-98.5%
Flu A Negative	2**	180	182	Specificity: 85.7% 95% CI: 83.3-88.1%
Total	43	210	253	

*Of 30 discrepant results, 22 were positive by both **BioSign®** and the PCR comparator assay.

** Of 2 discrepant results, 1 was negative by both **BioSign®** and the PCR comparator assay.

	Virus Culture Results			
BioSign® Flu A+B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	11	6*	17	Sensitivity: 91.6% 95% CI: 83.6-99.6%
Flu B Negative	1**	235	236	Specificity: 97.5% 95% CI: 96.5-98.5%
Total	12	241	253	

*Of 6 discrepant results, all 6 were positive by **BioSign®** and by the PCR comparator assay.

** The discrepant sample was positive by the PCR comparator assay.

Nasopharyngeal/Nasal Swab Samples (combined): Comparison with Viral Culture

	Virus Culture Results			
BioSign® Flu A+B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	59	131*	190	Sensitivity: 90.8% 95% CI: 81.3-95.7%
Flu A Negative	6**	413	419	Specificity: 75.9% 95% CI: 72.2-79.3%
Total	65	544	609	

*Of 131 discrepant results, 107 were positive by both **BioSign®** and the PCR comparator assay.

** Of 3 discrepant results, 1 was negative by both **BioSign®** and the PCR comparator assay.

	Virus Culture Results			
BioSign® Flu A+B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	47	55*	102	Sensitivity: 85.5% 95% CI: 73.8-92.4%
Flu B Negative	8**	499	507	Specificity: 90.1% 95% CI: 87.3-92.3%
Total	55	554	609	

*Of the 55 discrepant results, 27 were positive by both **BioSign®** and the PCR comparator assay.

** Of the 8 discrepant results, 3 were negative by both **BioSign®** and the PCR comparator assay.

Subsequently all available remnant nasopharyngeal swab and nasal swab samples that produced concordant results between **BioSign® Flu A+B** and viral culture (a subset of the concordant nasopharyngeal/nasal swab samples) were also further analyzed with the PCR comparator assay. This subset of concordant samples between **BioSign® Flu A+B** and viral culture includes 46% of all concordant positive samples and 33% of all concordant negative samples for the Flu A analyte, and 23% of all concordant positive samples and 31% of all concordant negative samples for the Flu B analyte.

Performance [3] of the **BioSign® Flu A+B** against the PCR comparator assay for all nasopharyngeal and nasal swab samples are presented in the tables below.

Nasopharyngeal/Nasal Swab Samples (combined): Comparison with PCR

	PCR Results			
BioSign® Flu A+B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	165	25	190	Sensitivity: 92.2% 95% CI: 87.3-95.3%
Flu A Negative	14	405	419	Specificity: 94.2% 95% CI: 91.6-96.0%
Total	179	430	609	

	PCR Results			
BioSign® Flu A+B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	72	30	102	Sensitivity: 90.0% 95% CI: 81.5-94.8%
Flu B Negative	8	499	507	Specificity: 94.3% 95% CI: 92.0-96.0%
Total	80	529	609	

Prospective Clinical Study from 2014 to 2016

An additional prospective clinical study was conducted from December 2014 to May 2016 to evaluate the performance of **BioSign® Flu A+B** for nasopharyngeal and nasal swab specimens when used by operators at CLIA-waived sites. The nasopharyngeal and nasal swab specimens were collected at 7 CLIA waived sites in the USA from patients with signs and symptoms of respiratory infection during the study period. All collected samples were tested with both the **BioSign® Flu A+B** and the PCR comparator assay. The total number of patients tested prospectively in this clinical study was 307, of which 37% were 5 and younger, 50% were 6-21 years old, and the rest were older than 21. Forty-nine (49) percent were male and 51% were female.

The data showing the performance of the **BioSign® Flu A+B** assay against the PCR comparator assay for all the prospectively collected and tested swab samples from 2014 to 2016 are presented in the tables below.

Nasopharyngeal/Nasal Swab Samples (combined): Comparison with PCR

	PCR Results			
BioSign® Flu A+B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	101	2	103	Sensitivity: 90.2% 95% CI: 83.3-94.4%
Flu A Negative	11	193	204	Specificity: 99.0% 95% CI: 96.3-99.7%
Total	112	195	307	

	PCR Results			
BioSign® Flu A+B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	27	3	30	Sensitivity: 81.8% 95% CI: 65.6-91.4%
Flu B Negative	6	271	277	Specificity: 98.9% 95% CI: 96.8-99.6%
Total	33	274	307	