510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K083746

B. Purpose for Submission:
New Device Clearance

C. Measurand:
Influenza A and B nucleoprotein antigens

D. Type of Test:
Qualitative, *in vitro* immunochromatographic assay

E. Applicant:
Princeton BioMeditech Corporation

F. Proprietary and Established Names:
BioSign® Flu A+B

G. Regulatory Information:

1) Regulation section:
21CFR 866.3330; Influenza Virus Serological Reagents

2) Classification:
Class I

3) Product Code
GNX, Antigens, CF, including CF controls, Influenza A, B, and C.

4) Panel:
Microbiology (83)

H. Intended Use:

1) Intended use(s):
The BioSign® Flu A+B test is an *in vitro* rapid qualitative test that detects influenza type A and type B nucleoproteins antigens directly from nasal swab, nasopharyngeal swab, and nasalpharyngeal 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.

A negative test result is presumptive and it is recommended these results be confirmed by viral culture.
Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions. The test is intended for professional and laboratory use.

2) Indication(s) for use:
Same as intended use

3) Special condition for use statement(s):
The device is for prescription use only

4) Special instrument Requirements:
NA

I. Device Description:
In vitro immunochromatographic membrane immunoassay

J. Substantial Equivalence Information:

1) Predicate device name(s):
QuickVue Influenza A+B Test by Quidel Corp.

2) Predicate 510(k) number(s): K053146

3) Comparison with predicate:

Table 1: Summary of Device Similarities

Characteristics	BioSign® Flu A+B Test	QuickVue Influenza A+B Test
Device Type		
Technology	Single use, rapid, lateral flow immunoassay	same
In vitro diagnostic device	Yes	Yes
Controls	Yes	Yes
Calibrator	No	No
Intended Use		
Detection of influenza A antigen	Yes	Yes
Detection of influenza B antigen	Yes	Yes
Screening test	No	No
Diagnostic test	Yes	Yes
Strain identification test	No	No
Monitoring therapy	No	No
Acceptable Samples		
Nasal Swab	Yes	Yes
Nasopharyngeal Swab	Yes	Yes
Nasal Aspirates/Washes	Yes	Yes

Reagents/Components Provided		
Nitrocellulose test strip	Yes	Yes
Conjugate reagent	Yes	Yes
Sample Diluent/Negative Control (external)	Yes	Yes
Internal procedural control	Yes	Yes
External positive control	Yes	Yes

K. Standard/Guidance Document Referenced (if applicable):
N/A

L. Test Principle:
The BioSign® Flu A+B test 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 lyophilized 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.

Interpretation of Results
Formation of the Control line serves as an internal control to demonstrate that lyophilized 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. The BioSign® Flu A+B test has two test line indicators, one for influenza A and one for influenza B. The two test line indicators 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.

M. Performance Characteristics (if/when applicable):

1) <u>Analytical performance:</u>

a) ***Precision/Reproducibility:***

The reproducibility study for the 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 test 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).

b) ***Linearity/assay reportable range:*** NA

c) ***Traceability, Stability, Expected values (controls, calibrators, or method):*** NA

d) ***Detection limit:***

The Limit of Detection (LoD) were determined for each of the two strains selected from the influenza type A and type B strains listed in the analytical sensitivity section below. The sensitivity level of each selected viral strain established in the analytical 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

e) ***Analytical reactivity***

The analytical sensitivity (inclusivity) was established for a total of 21 influenza strains: 11 strains of influenza A type and 10 strains of influenza B type. The results are shown in the table 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	B	B/Lee/40	5.00×10^0

A	A/FM/1/47(H1N1)	1.73×10^1	B	B/Allen/45	1.58×10^0
A	A/NWS/33(H1N1)	4.10×10^3	B	B/GL/1739/54	9.95×10^2
A	A/Hong Kong/8/68(H3N2)	8.5×10^2	B	B/Taiwan/2/62	1.58×10^3
A	A/Denver/1/57(H1N1)	7.20×10^0	B	B/Maryland/1/59	1.99×10^1
A	A/Aichi/2/68(H3N2)	9.95×10^0	B	B/Mass/3/66	5.00×10^1
A	A/Port Chalmers/1/73	1.99×10^2	B	B/R22 Barbara	1.6×10^{-1}
A	A/Victoria/3/75(H3N2)	9.95×10^1	B	B/R75	2.94×10^3
A	A/New Jersey/8/76(H1N1)	9.95×10^1	B	B/Russia/69	3.16×10^3
A	A/WS/33(H1N1)	5.00×10^1	B	B/Hong Kong/5/72	2.88×10^1
A	A/Swine/1976/31	1.58×10^2			

NOTE: The performance characteristics of the test with cultured avian influenza A subtype H5N1 virus, or with specimens from human infected with H5N1 or other avian influenza viruses are unknown.

The performance of the BioSign® Flu A+B test was evaluated with human specimens obtained from patients infected with the 2009 H1N1 influenza virus consisting of sixty six (66) frozen clinical nasal and nasopharyngeal swab samples that had previously tested positive for 2009 H1N1 by the 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 tittered specimens and 38% with the lower tittered specimens.

f) Analytical specificity

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 commercially available PCR (not approved by FDA).

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

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

g) Interfering substances

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 performance 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%
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

2) Comparison studies

a. ***Method comparison with predicate device:***
N/A

b. ***Matrix comparison:***
NA

3) Clinical studies

a) Clinical sensitivity:

A prospective clinical study was conducted from January 2007 to March 2008 and during March and April 2009 to determine the performance of the BioSign® Flu A+B test for nasopharyngeal aspirate/wash, 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 to confirm the results of BioSign® Flu A+B. 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.
The combined data from all sites of the prospective study are presented in the tables below. The samples that produced discrepant results between BioSign® Flu A+B and viral culture were further evaluated with an FDA cleared PCR based assay (real time RT-PCR, PCR hereafter). These results are presented in the footnote below each table.

Nasapharyngeal Aspirate Samples

	Reference (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 PCR
** Of 2 discrepant results, 1 was negative by both BioSign and PCR

	Reference (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 PCR
** The discrepant sample was positive by PCR

Nasopharyngeal Swab Samples

	Reference (Virus Culture) Results			
BioSign Flu A+ B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	26	51*	77	Sensitivity: 89.6% 95% CI: 84.0–95.2%
Flu A Negative	3**	171	174	Specificity: 77.0% 95% CI: 74.2–79.8%
Total	29	222	251	

Of 51 discrepant results, 41 were positive by both BioSign and PCR
** Of 3 discrepant results, 1 was negative by both BioSign and PCR

	Reference (Virus Culture) Results			
BioSign Flu A+ B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	33	15*	48	Sensitivity: 86.8% 95% CI: 81.4–92.2%
Flu B Negative	5**	198	203	Specificity: 92.9% 95% CI: 91.2–94.6%
Total	38	213	251	

**Of the 15 discrepant results, 8 were positive by both BioSign and PCR
** Of the 5 discrepant results, 2 were negative by both BioSign and PCR

Nasal Swab Samples

	Reference (Virus Culture) Results			
BioSign Flu A+ B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	33	80*	113	Sensitivity: 91.7% 95% CI: 78.2–97.1%
Flu A Negative	3**	242	245	Specificity: 75.2% 95% CI: 70.2–79.6%
Total	36	322	358	

*Of 80 discrepant results, 65 were positive by both BioSign and PCR
** Of 3 discrepant results, all 3 were positive by PCR

	Reference (Virus Culture) Results			
BioSign Flu A+ B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	14	40*	54	Sensitivity: 82.4% 95% CI: 59.0–93.8%
Flu B Negative	3**	301	304	Specificity: 88.3% 95% CI: 84.4–91.3%
Total	17	341	358	

*Of 40 discrepant results, 18 were positive by both BioSign and PCR
** Of 3 discrepant results, 1 was negative by both BioSign and PCR

As further verification of the PCR test results shown from the samples with discrepant results between BioSign and viral culture, available archived remnant samples from the clinical studies with concordant results were also tested by PCR. The specificity for both Flu A and Flu B was 100%, while the sensitivity for Flu A was 90% and the sensitivity for Flu B was 91.7%.

Archived Samples

Eighty (80) frozen archived samples originally obtained from influenza positive patients visiting Columbia NY Presbyterian Hospital and confirmed as positive for either influenza A or Influenza B by viral culture were tested with BioSign Flu A+B.

The tables below present test results with archived samples.

Aspirate Sample

	Reference (Virus Culture) Results			
BioSign Flu A+ B	Flu A Positive	Flu A Negative	Total	Agreement
Flu A Positive	50	0	50	100%
Flu A Negative	0	30	30	100%
Total	50	30	80	

	Reference (Virus Culture) Results			
BioSign Flu A+ B	Flu B Positive	Flu B Negative	Total	Agreement
Flu B Positive	30	0	30	100%
Flu B Negative	0	50	50	100%
Total	30	50	80	

Swab Sample

T	Reference (Virus Culture) Results			
BioSign Flu A+ B	Flu A Positive	Flu A Negative	Total	Agreement
Flu A Positive	50	0	50	100%
Flu A Negative	0	30	30	100%
Total	50	30	80	

	Reference (Virus Culture) Results			
BioSign Flu A+ B	Flu B Positive	Flu B Negative	Total	Agreement
Flu B Positive	30	0	30	100%
Flu B Negative	0	50	50	100%
Total	30	50	80	

Proposed labeling:

The labeling is sufficient and it satisfies the requirement of 21 CFR Part 809.10.

N. Conclusions

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