P-5235-C

BioSign™ HIV-1/HIV-2 WB

New One-Step HIV-1/HIV-2 Assay for Human Whole Blood, Serum or Plasma Use

For Professional Use

Immunoassay for the Qualitative Detection of Antibodies to HIV-1/HIV-2 in Human Whole Blood, Serum or Plasma

Catalog No.	BSP-162WB	35 Test Kit
	BSP-162WB-10	10 Test Kit

Intended Use

The BioSign™ HIV-1/HIV-2 WB test is an *in vitro*, qualitative, immunochromatographic assay for the detection of antibodies to human immunodeficiency viruses type 1 and/or type 2 in human whole blood, serum or plasma.

Summary and Principle of Procedure

The etiologic agents of Acquired Immunodeficiency Syndrome (AIDS) are retroviruses known as Human Immunodeficiency Virus Type 1 (HIV-1) and Human Immunodeficiency Virus Type 2 (HIV-2).[1,2]

HIV-1 has been isolated from patients with AIDS and AIDS-related complex, and from healthy persons with high potential risk of developing AIDS.[3,4] Infections with HIV-2 are found primarily in parts of West Africa.[4]

HIV-1 and HIV-2 are similar in their morphology, cell tropism, host interaction and genomic structure.[5]

As determined by serological studies, HIV-1 and HIV-2 have multiple common epitopes in core antigens but much less so in the envelope antigens.[5]

BioSign™ HIV-1/HIV-2 WB is a solid-phase immunochromatographic assay for the qualitative detection of antibodies to HIV-1/HIV-2. In the test procedure, 10 µL of serum or plasma sample or 25 µL of whole blood sample is added to the Sample Well, and followed by 1 drop (40 µL) of Developer Solution in the same well. The result is read in 5 minutes, but within 10 minutes.

Each device has a Reading Window with an upper Control area and a lower Test area and a Sample Well. The presence of antibodies to HIV-1/HIV-2 is indicated by two distinct pink-purple lines in the Reading Window. In the absence of antibodies to HIV-1/HIV-2, only the Control line will appear to indicate the test is negative and to confirm the test was performed correctly and the reagents worked properly. Therefore, two lines—one in the Control area and one in the Test area—indicate a positive result, while one line only in the Control area indicates a negative result.

Reagents

Materials Provided

- Each BioSign™ HIV-1/HIV-2 WB test kit contains enough reagents and materials for 35 tests.
- Each BioSign™ HIV-1/HIV-2 WB test device contains a membrane strip coated with inactive recombinant HIV antigens. The test kit does not contain active virus.
- Each kit contains 2.5 mL of Developer Solution containing 0.05% sodium azide in a dropper bottle.
- Directions for Use

Materials Required but Not Provided

- Vacutainer tubes for whole blood, serum or plasma procedure
- Centrifuge
- Micropipetter (0-200 µL)

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Do not interchange materials from different product lots and do not use beyond the expiration date.
- Use a fresh micropipette or tip for each different specimen. Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterwards.
- All patient samples 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.
- Reagents in this kit contain sodium azide as a preservative, which may react with lead or copper in plumbing to form potentially explosive metal azides. Upon disposal, always flush with a large volume of water to prevent azide buildup in drains.
- The BioSign™ HIV-1/HIV-2 WB 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.

Storage and Stability

The BioSign™ HIV-1/HIV-2 WB test kit should be stored at 2–30°C (36–86°F) in the sealed pouch. The storage conditions and stability dating given were established under these conditions.

Specimen Collection and Preparation

- Collect the blood in a standard tube containing heparin or EDTA as anticoagulant. Guidelines published by NCCLS should be used for collecting, transporting and processing patient samples.

- Heat inactivation of samples may lead to hemolysis or protein denaturation, and therefore should be avoided.
- Turbid serum samples should be centrifuged for 15 minutes at approximately 1,000 rcf.
- Specimens should be run as soon as possible. If specimens are to be stored, the red blood cells should be removed. For short periods, less than 24 hours, the plasma should be refrigerated at 2–8°C. Longer term storage for more than 24 hours should be at temperatures below -20°C. Whole blood samples should not be frozen. It is recommended that fresh sample be used as soon as possible to collect critical patient information. If specimens are to be shipped, they should be packed in compliance with Federal regulations covering the transportation of etiologic agents.
- Bring specimens to room temperature prior to testing. The frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing.

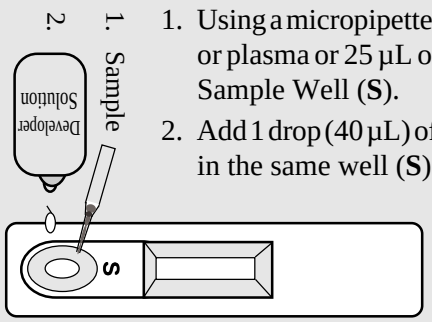
Procedure

Procedural Notes

The instructions below must be followed to achieve optimal test reactivity with blood or serum specimens. Follow the assay procedure and always perform the test under carefully standardized conditions.

- If specimens, kit reagents or BioSign™ devices have been stored in the refrigerator, allow them to warm to room temperature before testing.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.
- To avoid cross-contamination, use a new disposable micropipette tip for each specimen.
- To avoid contamination, do not touch the tip of the dropper bottle containing Developer Solution with your hands or to the device.
- Label the device with the patient name or control number.
- When the specimen is dispensed using a micropipetter, allow the tip of the micropipette to touch lightly to the pad underneath the Sample Well (**S**) and dispense the specimen by pressing the micropipette lever.
- To add Developer solution, hold the dropper bottle in a vertical position above the Sample Well and dispense 1 full drop into the well.
- After testing, dispose of the BioSign™ device and the specimen dispenser following good laboratory practices. Consider each material that comes into contact with specimen to be potentially infectious.

Test Procedure



1. Using a micropipette, add 10 µL of serum or plasma or 25 µL of whole blood in the Sample Well (**S**).
2. Add 1 drop (40 µL) of Developer solution in the same well (**S**).
3. Read the test result in 5 minutes (within 10 minutes).

Interpretation of Results

Positive: Two colored lines, one in the Test area and one in the Control area, indicate that antibodies against HIV-1 and/or HIV-2 virus have been detected.

***Note**: The test result can be read as soon as a distinct pink-purple line appears in the Reading Window. The Test line will appear before the Control line in most strong positive cases. The Test line may appear after the Control line in weak positive cases, and the Control line may be darker than the test line. The three possible positive cases, therefore, are:*

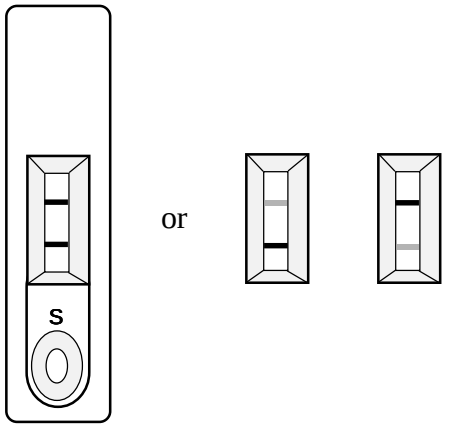
a. Two strong colored lines, one in the Test area (T) and one in the Control (C) area.

b. One strong colored line in the Test area (T) and one light colored line in the Control area (C).

c. One light colored line in the Test area (T) and one strong colored line in the Control area (C).

Negative: Only one colored line in the Control area (C), with no distinct colored line in the Test area other than the normal faint background color, indicates that antibodies against HIV-1 and HIV-2 virus have not been detected.

Invalid: A distinctive colored line in the Control area should always appear. The test is invalid and should be repeated with a new BioSign™ HIV-1/HIV-2 WB test if no line forms in the Control area.



2 Lines = **Positive** (+)

1 Line = **Negative** (–)

Limitations

- The assay must be performed in strict accordance with these instructions to obtain accurate, reproducible results.
- Although a positive result may indicate infection with HIV-1 or HIV-2 virus, a diagnosis of AIDS can only be made on clinical grounds, if an individual meets the case definition for AIDS established by the Centers for Disease Control.[6] For repeatedly reactive specimens, more specific supplemental tests must be performed. Immunochromatographic testing alone cannot be used to diagnose AIDS even if the antibodies against HIV-1/HIV-2 are present in a patient sample.
- A negative result at any time does not preclude the possibility of HIV-1/HIV-2 infection.

User Quality Control

Control standards are not supplied with this kit; however, it is recommended that a control be tested as good laboratory testing practice. Before using a new kit with patient specimens, positive and negative controls should be tested to confirm the test procedure, and to verify the tests produce the expected Q.C. results. Controls should also be run anytime there is any question concerning the validity of results obtained.

Each BioSign™ HIV-1/HIV-2 WB test device has built-in control. The Control line is an internal process (positive) control. A distinctive reddish-purple Control line should always appear if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents are working.

In addition, if the test has been performed correctly and the BioSign™ device is working properly, the background in the Reading Window will clear and provide a distinct result. This may be considered the internal negative process control. If the Reading Window does not clear, the test is considered invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.

The positive and negative process controls contained in each BioSign™ test device satisfy the requirements of testing a positive control and a negative control on a daily basis.

Expected Values

No standards for performance have yet been established for HIV rapid assays. The BioSign™ HIV-1/HIV-2 WB test was tested with commercially available panels comprising early seroconversions and several dilutions of later seroconversions, in comparison with a widely recognized, commercially available ELISA HIV-1/HIV-2 assay and another commercially available rapid assay, as shown in the following section. Early seroconversion samples are from patients with recent infection, and the HIV antibody level is expected to be low in these cases. Later seroconversions may exhibit much higher levels of antibody in the blood, so dilutions of these samples are made to further examine assay sensitivity. All samples detected as positive by the ELISA assay were detected by BioSign™ HIV-1/HIV-2 WB as positive, including dilutions and early seroconversion samples. No cross reactivity or interference was detected from other antigens, lipemic, or icteric sample.

Performance Characteristics

BioSign™ HIV-1/HIV-2 WB, a commercially available, widely used quantitative ELISA (Enzyme-Linked Immunosorbent Assay), and another commercially available rapid assay were used to test a total of 58 difficult seroconversion samples. All tests were performed by properly trained users in random order according to the instructions given in the package inserts. The samples include early seroconversions and dilutions of later seroconversions to compare assay sensitivity, and hemolyzed samples and samples positive for other antigens (but negative for HIV-1/HIV-2) to examine assay specificity. The results, shown in Table 1 below, illustrate the excellent agreement between the BioSign™ HIV-1/HIV-2 WB test and the reference ELISA. All samples detected as positive by the reference ELISA were also detected as positive by BioSign™ HIV-1/HIV-2 WB. Results from tests with two recognized, difficult panels of sera from Boston Biomedica (Boston, MA, USA) further illustrate the excellent performance of BioSign™ HIV-1/HIV-2 WB.

Table 1.

	Sample	ELISA (Signal/Cut-off)	Other Rapid Assay	BioSign™ HIV-1/HIV-2
1	Negative	0.1	–	–
2	”	0.2	–	–
3	Patient B, 1/2 dil.	9.5	+++	++++
4	” , 1/4	8.45	+++	++++
5	” , 1/8	6.25	+++	++++
6	” , 1/16	5.85	++	++++
7	” , 1/32	4.89	+	++++
8	” , 1/64	3.56	+/–	++++
9	” , 1/128	1.75	+/–	+++
10	” , 1/256	1	+/– –	++
11	Patient A, 1/2 dil.	8.69	+++	++++
12	” , 1/4	6.85	+++	++++
13	” , 1/8	4.75	++	++++
14	” , 1/16	3.36	+	++++
15	” , 1/32	2.45	+	+++
16	” , 1/64	1.95	+/–	+++
17	” , 1/128	1	+/– –	+++
18	” , 1/256	0.65	–	++
19	HIV-2	No Data	+++	+
20	Hemolyzed sample	0.15	–	–
21	Negative	0.1	–	–
22	”	0.12	–	–
23	”	0.3	–	–
24	HIV-1	5.15	+++	++
25	”	3.61	+++	++
26	”	3.83	+++	++
27	Hemolyzed sample	0.3	–	–
28	”	0.09	–	–
29	”	0.1	–	–
30	”	0.14	–	–
31	HIV-1	3.42	++	+++
32	”	5.82	++	++
33	”	4.32	+++	++
34	Negative	0.2	–	–
35	”	0.1	–	–
36	”	0.09	–	–
37	”	0.05	–	–
38	”	0.07	–	–

(continued)

Table 1. (continued)

Sample		ELISA (Signal/Cut-off)	Other Rapid Assay	BioSign™ HIV-1/HIV-2
39	BBI panel Q1	ND	–	–
40	” Q2	”	–	–
41	” Q3	”	+/– –	+/–
42	” Q4	”	+/–	+
43	” Q5	”	+	++
44	” Q6	”	+	+++
45	” Q7	”	+	++++
46	BBI panel R1	ND	–	–
47	” R2	”	+/– –	–
48	” R3	”	+/–	+
49	” R4	”	+	++
50	” R5	”	+	+++
51	” R6	”	+	+++
52	HIV-2	”	+/–	+
53	HBV positive	”	–	–
54	HTLV-1 positive	”	–	–
55	Rheumatoid factor	”	–	–
56	SLE	”	–	–
57	Lipemic	”	–	–
58	Icteric	”	–	–

In another study, 612 blind clinical samples were assayed for antibodies to HIV-1/HIV-2 virus with BioSign™ HIV-1/HIV-2 WB and with a commercially available test. The overall accuracy was 99.0% (606/612). The BioSign™ HIV-1/HIV-2 WB test demonstrated a relative sensitivity of 98.9% (437/442) and relative specificity of 100% (169/170) when compared with the reference test (see Table 2 below).

Table 2

BioSign™ HIV-1/HIV-2 WB vs. Reference Test

		BioSign™ HIV-1/HIV-2 WB		
		Positive	Negative	Total
Reference Test	Positive (+)	437	5	442
	Negative (-)	1	169	170
	Total	438	174	612

These data demonstrate the excellent correlation between BioSign™ HIV-1/HIV-2 WB and the Reference test.

In a correlation study between whole blood and plasma, 98 whole blood clinical samples were tested on the BioSign™ HIV-1/HIV-2 WB assay using the whole blood protocol described above. The plasma from these samples was then isolated and tested using the plasma protocol. The agreement was 100% (98/98). The data are presented in Table 3.

Table 3

Comparison of Whole Blood vs. Plasma Results

		Whole blood result		
		Positive	Negative	Total
Plasma result	Positive (+)	15	0	15
	Negative (–)	0	83	83
	Total	15	83	98

Proficiency Evaluation

An intra-laboratory reproducibility study or test proficiency evaluation was performed using 3 lots of devices at 3 locations for a total of 90 tests. At each location, 5 positive and 5 negative samples were used for testing of the 3 lots. The results obtained at each site agreed 100% with the expected results. An intra-assay study was conducted using 3 lots in 3-day testing for both negative and positive results. The results obtained agreed 100% with expected results.

References

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