# BioSign™ HBsAg

## New One-Step Hepatitis B Assay for Serum or Plasma Use

For Professional Use

**Immunoassay for the Qualitative Detection of Hepatitis B Surface Antigen in Human Serum or Plasma**

***PBM***

| | | |
|---|---|---|
| Catalog No. | BSP-165 | 35 Test Kit |
| | BSP-165-10 | 10 Test Kit |

## Intended Use

The BioSign™ HBsAg test is an *in vitro,* qualitative, one-step immunochromatographic assay for the detection of Hepatitis B virus Surface Antigen in serum or plasma.

## Summary and Principle of Procedure

Viral hepatitis is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis seen in children and adults are caused by hepatitis A virus, hepatitis B virus (HBV), or hepatitis C virus.

Hepatitis B virus was discovered by Blumberg, et al.[1] A complex antigen found on the surface of HBV is called HBsAg. The presence of HBsAg in a serum sample is indicative of an active HBV infection, either acute or chronic. In a typical HBV infection, HBsAg will be detected 2 to 4 weeks before the transminase level becomes abnormal and 3 to 5 weeks before the patient develops symptoms or becomes jaundiced.[2] HBsAg has four principal subtypes: *adw, ayw, ard, and ayr.* Because of antigenic heterogeneity of the *w* determinant, there are 10 major serotypes of HBV.

The BioSign™ HBsAg test uses solid-phase immunochromatographic technology for the qualitative detection of HBsAg in serum or plasma. The test is a two-site immunometric assay in which a combination of monoclonal and polyclonal antibodies is used to selectively detect HBsAg in serum or plasma with a high degree of sensitivity. Each device has a Reading Window with an upper Control area and a lower Test area, and a Sample Well. In the test procedure, 3 to 4 drops (150 µL) of serum or plasma sample is added to the Sample Well and allowed to soak in. The result is read after 5 minutes, but within 10 minutes. If HBsAg is present in the specimen, it will react with the conjugate dye, which binds to the antibody on the membrane to generate a colored line. Presence of two colored lines, one in the test window and the other in the control window, indicates a positive result, while the absence of the line in the test window indicates a negative result.

## Reagents

### Materials Provided

- Each BioSign™ HBsAg test kit contains enough reagents and materials for 35 tests.
- Each BioSign™ HBsAg test device contains a membrane strip coated with anti-HBsAg antibody and a pad impregnated with antibody–dye conjugate in a protein matrix containing 0.1% sodium azide; 35 test devices. The test kit does not contain active or inactive virus.
- Disposable plastic transfer pipettes; 35 each.
- Directions for Use.

### Materials Required but Not Provided

- Vacutainer tubes for either serum or plasma procedure
- Centrifuge

## Precautions

- For *in vitro* diagnostic use only.
- Do not use after the expiration date.
- Use a fresh transfer pipette for each serum or plasma specimen. Do not pipet 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.
- The BioSign™ HBsAg 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™ HBsAg 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 normal laboratory conditions.

## Specimen Collection and Preparation

- The BioSign™ HBsAg test can be performed on serum or plasma only. Sodium citrate, heparin or EDTA may be used as an anticoagulant. Use of other anticoagulants has not been established.
- Remove the serum or plasma from the clot of red cells as soon as possible to avoid hemolysis. Only clear, non-hemolyzed

specimens should be used. Specimens containing any particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation before testing.

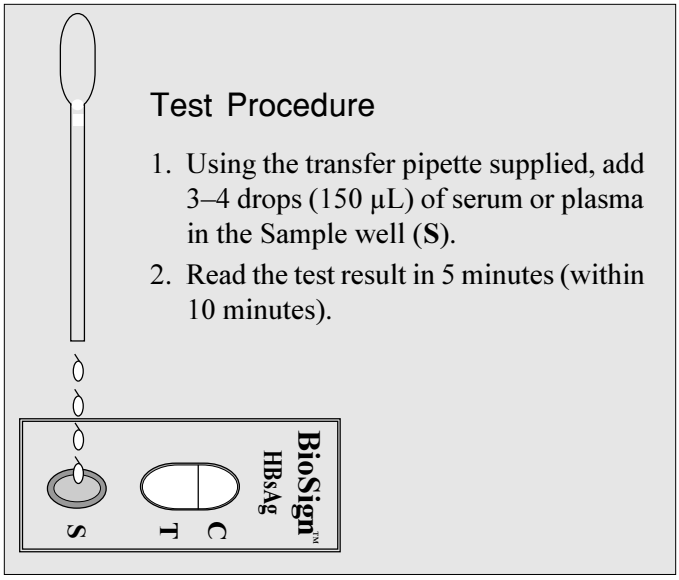
- Testing should be performed as soon as possible after sample collection. Do not leave samples at room temperature for prolonged periods.
- If specimens are to be stored, they should be refrigerated at 2–8°C or frozen. For prolonged storage, samples should be frozen and stored below –20°C. Specimens should not be repeatedly frozen and thawed.
- 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.
- If specimens are to be shipped, they should be packed in compliance with Federal regulations covering the transportation of etiologic agents.

## 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 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 pipette for each specimen.
- Label the device with the patient name or control number.
- When the specimen is dispensed using the transfer pipettes, hold the pipette vertically and add 3 to 4 large drops of the serum or plasma sample.
- 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.




## Interpretation of Results

**Positive:** Two colored lines, one in the upper Control window and one in the lower Test window, indicate that Hepatitis B Surface Antigen has been detected. The test result can be read as soon as a distinctive pink-purple line appears in the Test area.

***Note**: 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 window (T) and one in the control (C) window.

b. One strong colored line in the test window (T) and one light colored line in the control window (C).

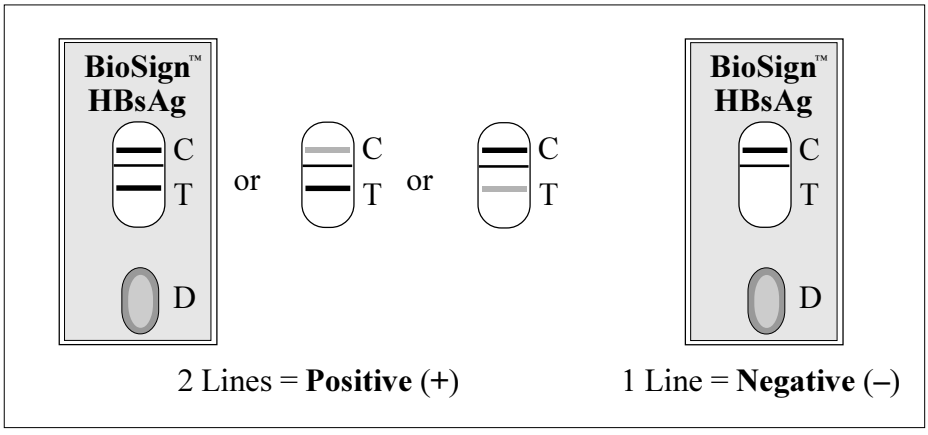
c. One light colored line in the test window (T) and one strong colored line in the control window (C).

**Negative:** Only one colored line in the Control window (C), with no distinct colored line in the Test window other than the normal faint background color, indicates that Hepatitis B Surface Antigen has not been detected.

**Invalid**: A distinctive colored line should always appear in the Control window. If no line forms in the Control window after 5 minutes, the test is invalid and should be repeated with a new BioSign™ HBsAg test.

## Limitations

- The assay must be performed in strict accordance with these instructions to obtain accurate, reproducible results.
- The BioSign™ HBsAg test is for *in vitro* diagnostic use only.
- This test is designed for use with serum or plasma samples only. Use of other body fluids, including whole blood, urine, or saliva, has not been established.



- This test will indicate only the presence or absence of HBsAg in the specimen, and should not be used as the only basis for the diagnosis of Hepatitis viral infection. As with all diagnostic tests, results must be considered with other clinical information available to the physician.
- BioSign™ HBsAg cannot detect extremely low concentrations of HBsAg in specimens. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is required. A negative result at any time does not preclude the possibility of Hepatitis B infection.

## User Quality Control

A quality control check should be made before using a new kit of BioSign™ HBsAg using commercially available control sera. A quality control test using positive and negative control standards should be performed as part of good testing practice and to confirm the expected Q.C. results. The positive control will produce a moderate positive result. The negative control will yield a negative result (control line only). Upon confirmation of the expected results, the kit is ready for use with patient specimens. For information about the commercial controls and other assistance, contact PBM's Technical Services.

A colored line in the control window (C) can be considered an internal procedural control. If the test has been performed correctly and the device is working properly, a distinct colored line will always appear. If a test result is not clear, a new test should be performed. If the problem persists, contact PBM's Technical Services for assistance.

## Expected Values

Commercially available enzyme immunoassays (EIA) and radioimmunoassays (RIA) are most commonly used to detect HBsAg. The correlation between these two systems is generally 99%. HBsAg levels below 5 ng/mL, and as low as 0.5–1 ng/mL, need to be detected for best clinical utility.

## Performance Characteristics

BioSign™ HBsAg was tested with serially diluted control sera, in 3 replicates, and found to detect HBsAg levels of 5 ng/mL in serum in 5 minutes. Levels as low as 0.5–1 ng/mL were visible in 10 minutes.

BioSign™ HBsAg detects all 10 major serotypes of HBV. Cross reactivity with Hepatitis A and Hepatitis C was not observed up to 0.5 ng/mL.

BioSign™ HBsAg was compared with a commercially available EIA using a total of 203 random clinical samples. Overall agreement of 98.5% was found between the two methods (see Table 1).

Table 1.

BioSign™ HBsAg vs. Reference Test

| | | **BioSign™ HBsAg** | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Reference Test | Positive (+) | 116 | 3 | 119 |
| | Negative (-) | 0 | 84 | 84 |
| | Total | 116 | 87 | 203 |

These data demonstrate the excellent correlation between BioSign™ HBsAg and the Reference test.

### 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


1. Blumberg, B.S., et al. The Discovery of Australian Antigen and its Relation to Viral Hepatitis. Virol. 1971;7:223.
2. Swenson, P.D. Hepatitis Viruses. In Manual of Clinical Microbiology, Washington, D.C., pp. 959, 1991.





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Princeton BioMeditech Corporation
P.O. Box 7139, Princeton, New Jersey 08543-7139 U.S.A.
4242 U.S. Route 1, Monmouth Junction, New Jersey 08852-1905 U.S.A.
Tel: (908) 274 - 1000
Fax: (908) 274 - 1010

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 | – | – |

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 | HILV-1 positive | ” | – | – |
| 55 | Rheumatoid factor | ” | – | – |
| 56 | SLE | ” | – | – |
| 57 | Lipemic | ” | – | – |
| 58 | Icteric | ” | – | – |



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Princeton BioMeditech Corporation
P.O. Box 7139, Princeton, New Jersey 08543-7139 U.S.A.
4242 U.S. Route 1, Monmouth Junction, New Jersey 08852-1905 U.S.A.
Tel: (908) 274 - 1000
Fax: (908) 274 - 1010