P-5713

BioSign® PSA II-WB

Rapid Test for Prostate-Specific Antigen Detection

For *In Vitro* Use

Immunoassay for the Qualitative Detection of Prostate-Specific Antigen in Human Whole Blood, Serum or Plasma

PBM

Catalog No.	BSP-311WB-35	35 Test Kit
	BSP-311WB-10	10 Test Kit

Intended Use

BioSign® PSA II-WB is intended for the qualitative detection of prostate-specific antigen in human whole blood, serum or plasma specimens to aid in the prognosis and management of patients with prostate cancer.

Summary and Principle of Procedure

Prostate-specific antigen (PSA) is an intracellular glycoprotein (34,000 Dalton kallikrein-like protease) synthesized only by the prostate gland.[1-3] PSA, a normal constituent of prostate tissue, is also present in benign hyperplastic and malignant prostatic tissue, in metastatic prostatic carcinoma, and in prostatic fluid and seminal plasma.[4] PSA is distinct from prostatic acid phosphatase (PAP) and is the major protein in seminal plasma.

The concentration of PSA is elevated in the blood of prostate cancer patients due to leakage of the antigen into circulation. The predictive value of blood PSA levels is superior to that of either rectal examination or ultrasound alone.[5-7] Since elevated PSA concentrations are also observed in patients with benign prostatic hypertrophy, measurement of blood PSA concentration is not recommended as a screening procedure for the diagnosis of cancer.[8] PSA is not sufficiently sensitive to detect all prostate cancers, but the combination of measurement of the blood PSA concentration and rectal examination with ultrasonography, performed in patients with abnormal findings, provides a better method of detecting prostate cancer than rectal examination alone.[5-7] PAP, used as a serum marker for the detection of prostate cancer for many years, is now employed only as an adjunct to PSA screening.[6,9] Preoperative PSA levels tend to correspond to the pathological stage; the higher the PSA level, the more advanced the disease and the greater the likelihood of extracapsular disease spread.[10-13] Preoperative PSA alone is not sufficiently accurate to predict pathological stage on an individual basis.[14] In combination with other variables (e.g., PAP, total alkaline phosphatase, carcinoembryonic antigen, creatine kinase isozymes BB, etc.), however, PSA plays an important role in the preoperative assessment of prostatic carcinoma.[11] The most definitive role for PSA is in the post-therapeutic evaluation of prostate cancer patients. PSA is useful in monitoring post-treatment clinical status in patients with stages B2 through D1 prostate cancer.[11-16]

The **BioSign® PSA II-WB** test uses solid-phase immunochromatographic assay technology for the qualitative detection of PSA in human whole blood, serum or plasma. In the test procedure, 45 µL of whole blood sample or 30 µL of serum or plasma sample is dispensed in the Sample well (marked "S" on the device), followed by addition of Developer solution. If PSA is present in the specimen, it will react with the conjugate dye which binds to the capture antibody immobilized on the membrane to generate a colored line at the Test position (marked "T" on the device). A control line should always appear at the Control position (marked "C" on the device), indicating that the test is valid. It has been shown that PSA concentrations in 99% of healthy men were less than 4.0 µg/L.[11,15,17,18] If the concentration of PSA in the sample is 4 µg/L or greater, **BioSign® PSA II-WB** test will yield a positive result, as characterized by a visible pinkish-purple horizontal band at both the Test and Control positions. If a band is present only at the Control position, the result is read as negative, indicating that the PSA concentration is below 4 µg/L. In cases where the serum PSA concentration is less than 4 µg/L, a clinical evaluation should be made using information available from other diagnostic procedures (see above). If no band is present at the Control position, the test should be considered invalid and another test should be run, regardless of the presence or absence of a band at the Test position.

Reagents and Materials Provided

Each **BioSign® PSA II-WB** test kit contains all necessary reagents and materials to perform all the tests.

- **BioSign® PSA II-WB** test device containing a membrane strip coated with anti-PSA antibody and a pad impregnated with anti-PSA antibody-dye conjugate
- Developer solution
- Directions for use

Materials Required But Not Provided

- Vacutainer
- Centrifuge
- Micropipet tip
- Micropipetter

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 separate clean micropipets for different specimens. 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 afterward.
- All patient samples should be handled as if they were capable of transmitting disease. Observe established precautions against microbiological hazard 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 large volumes of water to prevent azide buildup in drains.
- The **BioSign® PSA II-WB** device should remain in its original sealed pouch until ready for use. Do not use the test if the pouch is damaged or the seal is broken.

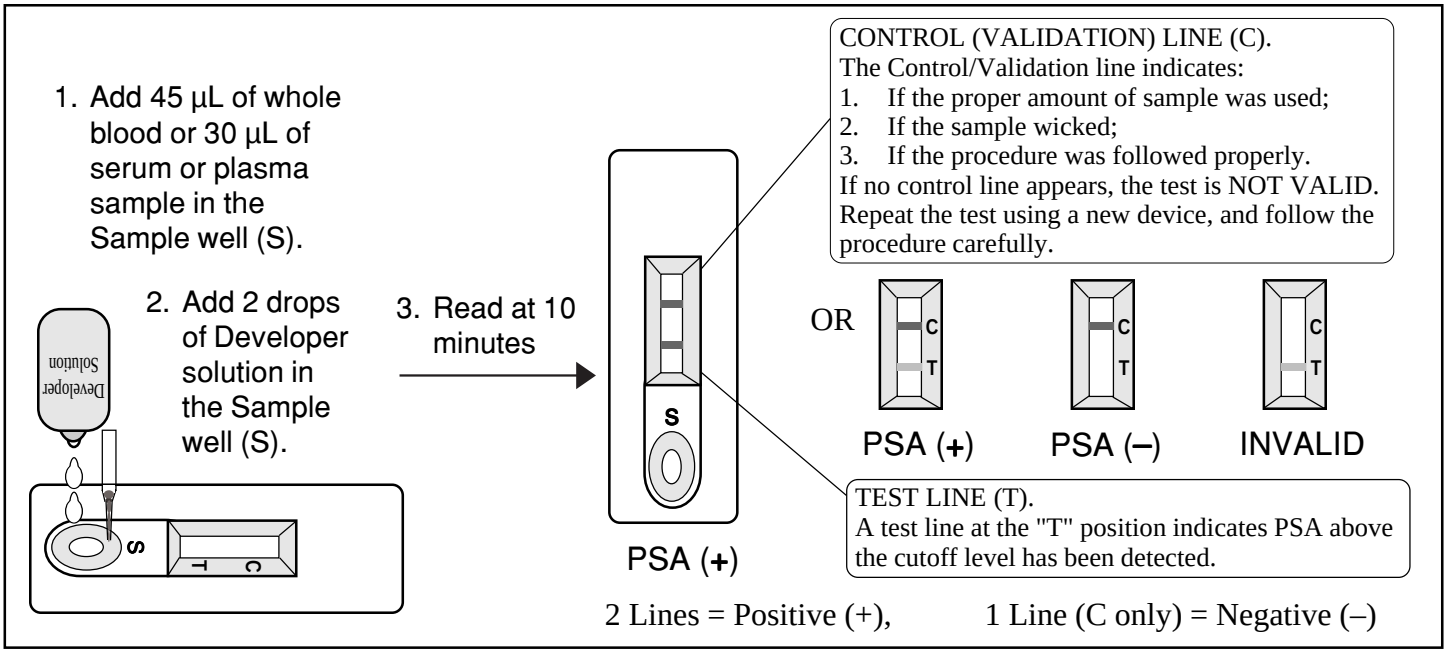
Storage and Stability

The **BioSign® PSA II-WB** test kit is stable until the expiration date printed on the pouch, when stored at 2–30°C (36–86°F) in its sealed pouch.

Specimen Collection and Preparation

The **BioSign® PSA II-WB** test can be performed with whole blood, serum or plasma.

- **For whole blood or plasma samples**: Collect the blood in a standard tube containing heparin or citrate as anticoagulant. Guidelines published by the National Committee for Clinical Laboratory Standards, Inc. should be used for collecting, transporting and processing patient samples.
- **For serum samples**: Collect the blood in a tube without anticoagu-



lant and allow to clot. Remove the serum from the clot as soon as possible to avoid hemolysis. When possible, clear, non-hemolyzed specimens should be used. Specimens containing particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to assaying.

- Heat inactivation of samples may lead to hemolysis or protein denaturation, and therefore should be avoided.
- Turbid samples should be centrifuged for 15 minutes at approximately 1,000 relative centrifugal force (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. Long term storage for more than 24 hours should be at a temperature below –20°C. Whole blood samples should not be frozen. Specimens should not be repeatedly frozen and thawed.
- Bring specimens to room temperature prior to testing. 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

1. If specimens, the **BioSign® PSA II-WB** test devices or Developer solution have been stored in a refrigerator, allow them to return to room temperature before testing. Do not open the foil pouch until you are ready to perform the test.
2. Several tests may be run at one time.
3. To avoid cross-contamination, use a clean disposable pipette tip for each specimen.
4. When specimen is dispensed using a micropipetter, make certain that it is dispensed all at once on the upper part of the Sample well (**S**). Avoid adding the sample specimen drop-by-drop on the lower part of the Sample well (**S**).
5. To add Developer solution, hold the dropper bottle in a vertical position above the Sample well (S) and dispense 2 drops into the well.
6. After testing, dispose of the **BioSign® PSA II-WB** device and the specimen dispenser following good laboratory practice. Consider each material that comes in contact with the specimen to be potentially infectious.
7. Open the foil pouch, remove the **BioSign® PSA II-WB** device, and lay the device on a level surface.
8. Label the device with the patient's name or control number.

Test Procedure

1. Add 45 µL of whole blood or 30 µL of serum or plasma in the Sample well (**S**).
2. Add 2 drops of Developer solution in the same well (**S**).
3. Read the test result in 10 minutes.

Interpretation of Results

Positive

The presence of two colored bands, one each at the Test position (**T**) and at the Control position (**C**), indicates that the PSA concentration in the sample is 4 µg/L or greater, which may indicate the presence of malignant disease or benign prostatic hypertrophy.

Negative

Only one colored band at the Control position (**C**), with the absence of a distinct colored band at the Test position (**T**) other than the normal faint background color indicates that the PSA concentration in the sample is below 4 µg/L.

Invalid

A distinctive colored band at the Control position (**C**) should always appear. If no pink band is present at the Control position (**C**), the test is invalid, and the sample should be run again with a new **BioSign® PSA II-WB** test device.

Limitations

1. The **BioSign® PSA II-WB** test should not be used either as a screening assay or for diagnosis. The test results should not be interpreted as absolute evidence for the presence or absence of malignant disease. Less than 1% of healthy individuals have elevated PSA, but a significant number of patients with benign prostatic hypertrophy (more than 15%) have elevated PSA. Even if the test result is positive, careful clinical evaluation should be made in conjunction with other information available from other medical testing and diagnostic procedures.

2. Because the expression of PSA requires testosterone or dihydrotestosterone, PSA levels may be unreliable in patients who receive hormone therapy.[19] Prostate gland manipulation may lead to elevated PSA for 24 to 48 hours.
3. Additionally, elevated PSA concentrations may be observed in the serum obtained from patients with other adjacent genitourinary tissues.
4. Appropriate timed paired specimens may be used to monitor post-treatment clinical status in patients with stage B2 through stage D1.[11-16] Significant changes in the intensity of the test band may occur during the timed period. However, predictions of disease removal or recurrence should not be based solely on the test results obtained from the timed paired specimens.

Quality Control

- **Quality Control:** A quality control test using commercially available Positive and Negative controls should be performed as a part of good testing practice, to confirm the expected QC results, to confirm the validity of the assay, and to assure the accuracy of patient results. A quality control test should be performed at regular intervals, and 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 QC results. QC specimens should also be run whenever there is any question concerning the validity of results obtained. Upon confirmation of the expected results, the kit is ready to use with patient specimens. Control standards are not provided with this kit. For information about obtaining the controls, contact PBM's Technical Services for assistance.
- **Procedural Control:** A colored band at the Control position (C) can be considered an internal procedural control. If the test has been performed correctly and the device is working properly, a distinct colored band at the Control position 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. The Control band is not an internal reference for PSA and can not be used for comparison with patient results.

Expected Values

1. The relative distribution of PSA concentrations in healthy subjects, patients with prostatic carcinoma, and patients with nonmalignant diseases is presented below.[17] These data were obtained using a quantitative EIA.

% Distribution of PSA

	< 4 µg/L	≥ 4 µg/L
Healthy Subjects		
Men < 40 yrs	100	0
Men > 40 yrs	97	3
Women	100	0
Cancerous Diseases		
Prostate cancer		
Stage A	37	63
Stage B	29	71
Stage C	19	81
Stage D	12	88
Gastrointestinal	95	5
Genitourinary	98	2
Mammary	99	1
Pulmonary	95	5
Renal	96	4
Other	95	5
Non-cancerous Diseases		
Benign Prostate	80	20
Hypertrophy—Miscellaneous	93	7
Genitourinary—Other	98	2

2. The primary clinical use of PSA is to monitor the response to therapy and the clinical status of prostate cancer patients. Significant changes in the test line intensity or in the timing of the test line appearance will occur using the **BioSign® PSA II-WB** test kit. For example, following radical prostatectomy, PSA levels in cancer-free patients should drop to undetectable levels.[11] Even in a patient without other indications of disease, an elevated PSA level is a clear indication of persistent or recurring cancer.[6] PSA levels are also useful in identifying those patients who may benefit from early adjuvant therapy.[10] In patients undergoing radiation therapy for prostate cancer, the elevalted level of PSA predicts treatment failure[13-16]; since increasing PSA values after radiation therapy correlate with residual cancer and progression to metastatic disease.[20] Low levels of PSA are more difficult to interpret, however, because they can be low following radiation therapy even in the presence of residual disease.[21]

Performance Characteristics

Assay Precision

Assays were carried out to determine the reproducibility using replicates of at least 20 tests in three different runs using three different lots. Results with whole blood and serum samples were identical.

Samples		< 4 µg/L	≥ 4 µg/L
Number of replicates		60	66
Assay results	+	0	66
	–	60	0

Inter-laboratory Precision

Inter-laboratory precision was evaluated in three different laboratories using three different samples. The study was performed with whole blood samples and again with serum samples, giving identical results. Assays were carried out in three different runs using three different lots. The results are shown below.

Samples		< 4 µg/L	≥ 4 µg/L
Assay results:			
Laboratory A	+	0	20
	–	20	0
Laboratory B	+	0	20
	–	20	0
Laboratory C	+	0	20
	–	20	0
TOTAL	+	0	60
	–	60	0

Comparative Clinical Testing Results

Clinical specimens from 869 patients (320 whole blood samples and 549 serum samples) were tested for PSA using the **BioSign® PSA II-WB** test and a commercially available EIA test. The clinical study was performed separately with whole blood samples and with serum samples.

Blood samples were collected from 320 volunteers. Each sample was divided into two parts and one part was centrifuged to obtain plasma. Plasma was tested by EIA and whole blood was tested by **BioSign® PSA II-WB**. The results were compared.

The agreement between the two systems with whole blood samples was 96.3%. The **BioSign® PSA II-WB** test demonstrated a relative specificity of 96.2% (228/237) and relative sensitivity of 96.4% (80/83) when compared with the reference test, as shown next.

		BioSign® PSA II-WB (whole blood)		
		+	–	Total
EIA	≥ 4 µg/L	80	3	83
	< 4 µg/L	9	228	237
Total		89	231	320

The agreement between the two systems with serum samples was 96.9%. The **BioSign® PSA II-WB** test demonstrated a relative specificity of 96.7% (414/428) and relative sensitivity of 97.5% (118/121) when compared with the reference test, as shown below.

		BioSign® PSA II-WB (serum)		
		+	–	Total
EIA	≥ 4 µg/L	118	3	121
	< 4 µg/L	14	414	428
Total		132	417	549

Interfering Substances

Hemoglobin (3 g/L), bilirubin (200 mg/L) and lipemic samples, as indicated by triglyceride (30 g/L), do not interfere with the test results. High protein concentration (100 g/L) also do not interfere with the test results.

Detection Limit

The minimum detection limit of **BioSign PSA II-WB** has been shown to be 4 µg/L PSA at 10 minutes. High dose hook effect is not observed up to 50,000 µg/L PSA.

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