P-5710-A

BioSign™ AFP

Rapid Test for Alpha Feto Protein Detection

For *In Vitro* Use

Immunoassay for the Qualitative Detection of Alpha-fetoprotein (AFP) in Human Serum

PBM

Catalog No.	BSP-302-35	35 Test Kit
	BSP-302-10	10 Test Kit

Intended Use

BioSign™ AFP qualitatively detects alpha-fetoprotein in human serum to aid in the prognosis and management of patients with nonseminomatous testicular cancer.

Summary and Principle of Procedure

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70,000 daltons[1]. Synthesis of AFP occurs primarily in the liver and yolk sac of the fetus. It is secreted into fetal serum, reaching a peak at about 13 weeks gestation and gradually declining thereafter. Elevated serum AFP levels subsequently reappear during pregnancy and in conjuction with several malignant diseases. Alpha-fetoprotein was first described as a human tumor-associated protein in 1964 by Tatarinov[2]. Since then it has been shown that elevation of serum AFP above values typically found in healthy individuals occurs in several malignant diseases[3,4,5,6], most notably nonseminomatous testicular cancer primary hepatocelluar carcinoma. In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stages of disease[7,8]. Elevated AFP levels also have been observed in patients diagnosed as having seminoma with non seminomatous elements but have not been observed in patients with pure seminoma[5,7,9,10].

Greater than 70% of patients with primary hepatocellular carcinoma have been reported to have elevated levels of serum AFP[3,4,11]. Elevated AFP levels have occasionally been found in association with gastrointestinal tract cancers with and without liver metastases[12] and only rarely in other malignancies[3,4]. Serum AFP has been found to be elevated during pregnancy, in diseases such as ataxia telangiectasia, hereditary tyrosinemi, teratocarcinoma and in benign hepatic conditions, such as acute viral hepatitis, chronic active hepatitis and cirrhosis[4,11,13]. Elevation of serum AFP in benign hepatic diseases is usually transient[3].

AFP and human chorionic gonadotropin are important prognostic indicators of survival rate among patients with advanced nonseminomatous germ cell testicular tumors[14]. The usefulness of AFP measurements in the mangement of patients with nonseminomatous testicular cancers has been well documented[5,9,15]. For patients in clinical remission following treatment, AFP levels generally decrease[9]. Post-operative AFP values which fail to return to normal strongly suggest the presence of residual tumor. Tumor recurrence is often accompanied by a rise in AFP before progressive disease is clincally evident[5,7].

BioSign™ AFP test uses solid-phase immunochromatographic technology for the qualitative detection of AFP in human serum. In the test procedure, 30 µL of sample is dispensed in the Sample well (marked "S" on the device) and allowed to soak in. If AFP is present in the serum 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) to indicate that the test is valid. It has been shown that the AFP concentration is less than 7.5 ng/mL in 99% of the healthy subjects. If the concentration of AFP in the sample is 7.5 ng/mL or greater, the **BioSign™ AFP** 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 AFP concentration is less than 7.5 ng/mL. 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™ AFP** test kit contains all necessary reagents to perform the test.

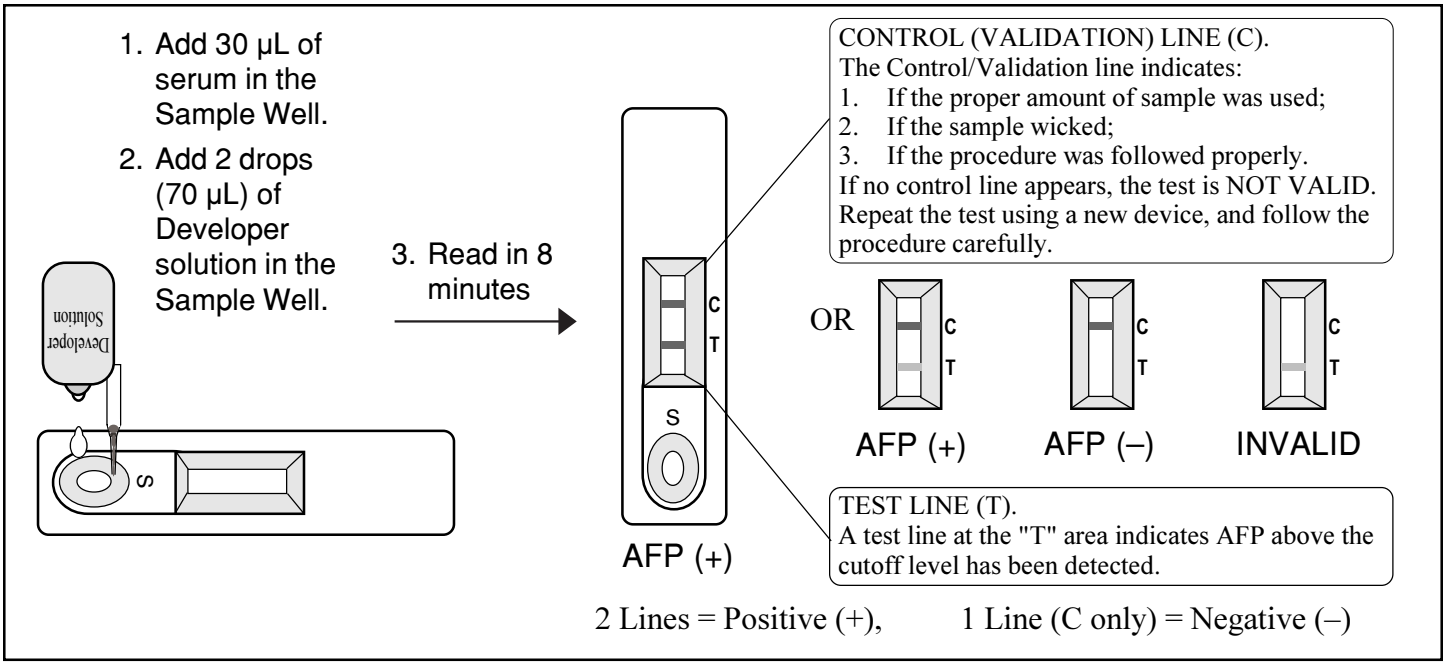
- Each **BioSign™ AFP** test device contains a membrane strip coated with anti-AFP antibody and a pad impregnated with antibody-dye conjugate in a protein matrix containing 0.1% sodium azide.
- Directions for use
- Developer Solution

Materials Required But Not Provided

- Vacutainer tubes for serum procedure.
- Centrifuge
- Specimen pipet (30 µL) or micropipet tip.
- Micropipetter (0-200 µL range).

Warnings and Precautions

- For *in vitro* diagnostic use.
- 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™ AFP** 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™ AFP** 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™ AFP** test can be performed with serum only. Plasma samples should not be used since it has not been validated.

- 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.
- 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. 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, kit reagents or the **BioSign™ AFP** test devices have been stored in the refrigerator, allow them to return to room temperature before testing.
2. Do not open the foil pouch until you are ready to perform the test.
3. Several tests may be run at one time.
4. To avoid cross-contamination, use a clean disposable pipette tip for each specimen.
5. Label the device with the patient name or control number.
6. After testing, dispose of the **BioSign™** device and the specimen dispenser following good laboratory practices. Consider each material that comes in contact with specimen to be potentially infectious.

Test Procedure

1. Using a micropipet, add 30 µL of serum in the Sample Well **(S)**.
2. Add 2 drops (70 µL) of Developer Solution in the Sample Well **(S)**.
3. Read the test result in 8 min.

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 AFP concentration in the sample is greater than or equal to 7.5 ng/mL, which may be associated with the presence of malignant disease or progressive malignant disease and poor therapeutic response.

Note:

- The test result can be read as soon as a distinct pink-purple color band appears at the Test position (**T**).

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 AFP concentration in the sample is below 7.5 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**) within 8 minutes, the test is invalid, and the sample should be run again with a new **BioSign™ AFP** test device.

Limitations

1. The **BioSign™ AFP** test is not recommended as a screening procedure to detect cancer in the general population; however, use of the AFP test in the prognosis and management of cancer patients has been widely accepted. The test results should not be interpreted as absolute evidence for the presence or absence of malignant disease.
2. 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 because the AFP levels may be elevated in patients who are smoking.

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 AFP and can not be used for comparison with patient results.

Performance Characteristics

Assay Precision

Assays were carried out to determine the reproducibility using replicates of at least 20 tests in three different runs for each of three lots.

Samples		< 7.5 µg/L	> 7.5 µg/L
Number of replicates		60	60
Assay results	+	0	60
	–	60	0

Inter-laboratory Precision

Inter-laboratory precision was evaluated in three different laboratories using three different samples. Assays were carried out in three different runs for each of the three lots.

Samples		< 7.5 µg/L	> 7.5 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 310 patients were tested for AFP using the **BioSign™ AFP** test and a commercially available EIA test.

The **BioSign™ AFP** test demonstrated a relative specificity of 94.8% and relative sensitivity of 96.3% when compared with the reference test, as shown below.

		BioSign™ AFP +	**BioSign™ AFP** –	Total
EIA	> 7.5 µg/L	78	3	81
	< 7.5 µg/L	12	217	229
Total		90	220	310

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™ AFP has been shown to be 7.5 µg/L AFP in 8 minutes. High dose hook effect was not observed up to 50,000 µg/L AFP.

References

1. Bergstrand, C.G. and Czar, B. 1956. Demonstration of a new protein fraction in serum from human fetus. Scand. J. Clin. & Lab. Invest. 8:174.
2. Tartarinov, Y.S. 1964 Detection of embryospecific alpha-globulin in the blood sera of patients of patient with primary liver tumor. Vopr. Med. Khim. 10:90-91.
3. Silver, H.K.B., Gold, P., Feder, S., Freedman, S.O. and Shuster, J. 1973. Radioimmunoassay for human alpha-fetoprotein. Proc. Nat. Acad. Sci., U.S.A.

4. Waldman, T.A., Mclntire, K.R. 1974. The use of a radioim munoassay for alpha-fetoprotein in the diagnosis of malig nancy. Cancer 34: 1510-1515.
5. Kohn, J., Orr, A.H., McElwain, T.J., Bentall, M. and Peckham, M.J. 1976. Serum alpha-fetoprotein in patients with testicular tumours. Lancet 2:433-436.
6. Abelev, G.I 1971. Alpha-feroprotein in ontogenensis and its association with malignant tumors. Adv. Cancer Res. *14*:295-358.
7. Scardino, P.T., Cox, H.D., Waldmann, T.A., McIntire, K.R., Mittenmeyer, B. and Javadpour, N. 1977. The value of serum tumor markers in the staging and prognosis of germ cell tumors of the testis. J.Urol. *118*:994-999.
8. Bosl, G.J., Lange, P.H., Fraley, E.E., Goldman, A., Nochomovitz, L.E., Rosal, J., Waldmann, T.A., Johnson, K. and Kennedy, B.J. 1981. Human chorionic gonadotro pin and alpha-fetoprotein in the staging of nonseminomatous testicular cancer. Cancer *47*:328-332.
9. Lange, P.H., McIntire, K.R., Waldmann, T.A., Hakala, T.R. and Fraley, E.E. 1976. Serum alpha-fetoprotein and human chorionic gonadotropin in the diagnosis and management of nonseminomatous germ-cell testicualar cancer. Medical Intelligence 295: 1237-1240.
10. Javadpour, N., McIntire, K.R and Waldmann, T.A. 1978. Human chorionic gonadotropin(HCG) and alpha-fetoproteiin (AFP) in sera and tumor cells of patients with testicular seminoma. Cancer 42:2768-2772.
11. Wespic, H.T. 1981. Alpha-fetoprotein: its quantitation and relationship to neoplastic disease, ppp. 115-129 In Alpha-fetoprotein, Laboratory Procedures and Clinical Applications, Kirkpatrick, A. and Nakamura, R. (eds.), Masson Publishing, New York, 1981.
12. McIntire, K.R., Waldmann, T.A., Moertel, C.G. and Go, V.L.W., 1975. Serum alpha-fetoprotein in patients with neoplasms of the gastrointestinal tract. Cancer Res. 35:991-996.
13. Chen, D.-S. and Sung, J.-L. 1979. Relationship of Hepatitis B Surface Antigen to serum alpha-fetoprotein in nonmalignant diseases of the liver. Cancer *44*: 984-992.
14. Report from the Medical Research Council Working Party on Testicular Tumours 1985. Prognostic factors in advanced non-seminomatous germ-Cell testicular tumours: results of a multicenter study. Lancet *I*:8-11.
15. Perlin, E., Engeler, J.E., Edson, M., Karp, D., McIntire, K.R and Waldmann, T.A. 1976. The value of serial measurement of both human chorionic gonadotropin and alpha-fetoprotein for monitoring germinal cell tumors. Cancer *37*:215-219.

Printed in U.S.A.
P-5710-A 0502BL

PBM

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: (732) 274–1000
Fax: (732) 274–1010
Internet E-mail: support@pbmc.com
World Wide Web: http://www.pbmc.com