P-5504-A

BioSign™ CEA

Rapid Test
for Carcino Embryonic Antigen Detection

For *In Vitro* Use

Immunoassay for the Qualitative Detection
of Carcino Embryonic Antigen
in Human Serum

PBM

Catalog No.	BSP-303-35	35 Test Kit
	BSP-303-10	10 Test Kit

Intended Use

BioSign™ CEA qualitatively detects carcino embryonic antigen in human serum to aid in the prognosis and management of cancer patients.

Summary and Principle of Procedure

Carcino embryonic antigen (CEA) is a tumor associated antigen, first described in 1965 by Gold and Freedman[1]. CEA was characterized as a glycoprotein of approximately 200,000 molecular weight[2,3]. Subsequent development of a radioimmunoassay (RIA) made it possible to detect the very low concentrations of CEA in blood, other body fluids and also in normal and diseased tissues[4-6]. The results of clinical studies to date indicate that CEA, although originally thought to be specific for digestive tract cancers, may also be elevated in other malignancies and in some nonmalignant disorders[7-9]. CEA testing can have significant value in the monitoring of patients with diagnosed malignancies in whom changing concentrations of CEA are observed. A persistent elevation in circulating CEA following treatment is strongly indicative of occult metastatic and/or residual disease[10,11]. A persistently rising CEA value may be associated with progressive malignant disease and poor therapeutic response[12]. A declining CEA value is generally indicative of a favorable prognosis and good response to treatment. Patients who have low pre-therapy CEA levels may later show elevations in CEA level as an indication of progressive disease. Clinical relevance of the CEA assay has been shown in the follow-up management of patients with colorectal, breast, lung, prostatic, pancreatic, and ovarian carcinoma[13-18]. Follow-up studies of patients with colorectal, breast and lung carcinoma suggest that the pre-operative CEA level has prognostic significance[19,20].

The **BioSign™ CEA** test uses solid-phase immuno-chromatographic technology for the qualitative detection of CEA in human serum. In the test procedure, 70 µL of sample is dispensed in the Sample well (marked "S" on the device) and allowed to soak in. If CEA 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 99% of the healthy subjects have CEA concentration of less than 5 ng/mL. If the concentration of CEA in the sample is greater than or equal to 5 ng/mL, the **BioSign™ CEA** test will yield a positive result, as characterized by visible pinkish-purple horizontal bands at both the Test and Control position.

Reagents and Materials Provided

Each **BioSign™ CEA** test kit contains enough reagents and materials for 35 tests.

- Each **BioSign™ CEA** test device contains a membrane strip coated with anti-CEA antibody and a pad impregnated with anti-CEA antibody-dye conjugate.
- Directions for use

Materials Required But Not Provided

- Vacutainer tubes
- Centrifuge
- Specimen pipet (70 µL) or micropipet tip
- Micropipetter (0-200 µL range)

Warnings and Precautions

- For *in vitro* diagnostic use.
- Do not use beyond the expiration date.
- 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.
- The **BioSign™ CEA** 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.

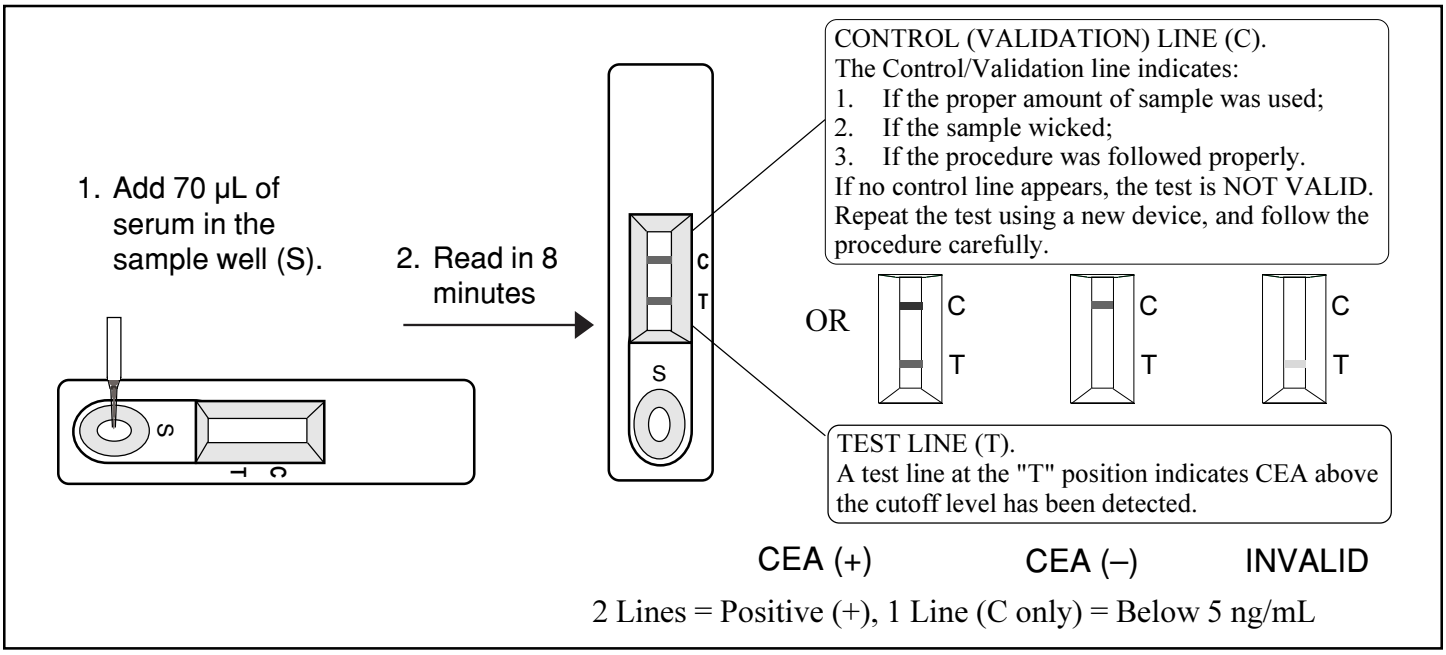
Storage and Stability

The **BioSign™ CEA** 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™ CEA** test must 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™ CEA** devices have been stored in a 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™ CEA** 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 70 µL of serum in the Sample well **(S).**
2. Read the test result in 8 min. Do not read after 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 CEA concentration in the sample is greater than or equal to 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**) and at the Control position (C)..

Negative

Only one colored band in the Control area (**C**), with the absence of a distinct colored band on the Test area (**T**) other than the normal faint background color indicates that the CEA concentration in the sample is below 5 ng/mL.

Invalid

A distinctive colored band in the Control area (**C**) should always appear. If no pink band is present in the Control area (**C**) within 8 minutes, the test is invalid, and the sample should be run again with a new **BioSign™ CEA** test device.

Limitations

1. The **BioSign™ CEA** test is not recommended as a screening procedure to detect cancer in the general population; however, use of the CEA 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. The CEA 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 test results. A quality control test should be performed at regular intervals, and before using a new kit with patient specimens. QC specimens should also be

run whenever there is any question concerning the validity of results. 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 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 CEA and can not be used for comparison with test results.

Performance Characteristics

Assay Precision

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

Samples		< 5 ng/mL	> 5 ng/mL
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		< 5 ng/mL	> 5 ng/mL
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 320 patients were tested for CEA using the **BioSign™ CEA** test and a commercially available EIA test.

The agreement between the two systems with patient samples was 95%. The **BioSign™ CEA** test demonstrated a relative specificity of 95.3% and relative sensitivity of 94.3% when compared with the reference test, as shown below.

		BioSign™ CEA		
		+	–	Total
EIA	> 5 ng/mL	83	5	88
	< 5 ng/mL	11	221	232
Total		94	226	320

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 does not interfere with the test results.

Detection Limit

The minimum detection limit of **BioSign™ CEA** has been shown to be 5 ng/mL CEA in 8 minutes. High dose hook effect has not been observed up to 50,000 ng/mL CEA.

References

1. Gold, P. and Freedman, S.O., Demonstration of Tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorbtion. J. Exp. Med. 121:439, 1965.
2. Krupey, J., Gold, P., and Freedman, S.O., Physiochemical studies of the carcinoembryonic antigens of the human digestive system. J Exp. Med. 183:387, 1968.
3. Krupey, J., Wilson, T., Freedman, S.O., et al., The preparation of purified carcinoembryonic antigen of the human digestive system from the large quantities of tumor tissue. Immunochem. 9:617, 1972.
4. Zamcheck, N., Carcinoembryonic antigen: Quantitative variations in circulating levels in benign and malignant digestive tract disease. Adv. Intern. Med. 19:413, 1974.
5. Go, V.L.W., Ammon, H.V., Holtermuller, K.H., et al., Quantifi cation of carcinoembryonic antigen-like activities in normal human gastrointestinal secretions. Cancer 36: 2346, 1975.
6. Khoo, S.K., Warner, N.L., Lie, J.T., et al., Carcinoembryonic antigen activity of tissue extracts. A quantitative study of malignant and benign neoplasmas, cirrhotic liver, normal adult and fetal organs. Int. J. Cancer 11:681, 1973.
7. Steward, A.M., Nixon, D., Zamcheck, N., et al., Carcinoembryonic antigen in breast cancer patients. Serum levels and disease progress. Cancer 33:1246, 1974.
8. Oehr, P., Schlosser, T., and Adolphs, H.D., Applicability of an enzymatic test for the determination of CEA in serum and CEA-like products in urine or patients with bladder cancer. Tumor Diagnostik 1:P40, 1980..
9. Reynoso, G., Chu, T.M., Holyoke, D. et al., Carcinoembryonic antigen in patients with different cancers. J. Am. Med Assoc. 220:361, 1972.
10. Zamcheck, N., CEA in diagnosis, prognosis, detection of recurrence and evaluation of therapy of colorectal cancer, p. 64, Symposium on Clincal Application of CEA and Other Anitgenic Markers Assays, Nice, France, October 1977. Amsterdam, Oxford, Medica.
11. Martin, E.W., Cooperman, M., et al., A Retrospective and Prospective Study of Serial CEA Determinations in the Early Detection of Recurrent Colon Cancer, Am. J. Surgery, 137:167, 1979.
12. Skarin, A.T., Delwich, R., Zamcheck, N., et al., Carcinoembryonic antigen: clinical correlation with chemo therapy for metastatic gastrointestinal cancer. Cancer 33:1239, 1974.
13. Lokich, J.J, Zamcheck, N., and Lowenstein, M., Sequential Carcinoembryonic Antigen Levels in the therapy of metastatic breast cancer. Annals of Internal Medicine 89:902, 1978.
14. Falkson, H.C., Falkson, G., et al., Carcinoembryonic Antigen as a Marker in Patients with Breast Cancer Receiving Postsurgical Adjuvant Chemotherapy. Cancer 49:1859, 1982.
15. Wanebo, H.J., Cancer Trends: The Role of CEA in Managing Colorectal Cancer. Virginia Medical 110:103, 1983.
16. Zamcheck, N., and Martin, E.W., Factors Controlling the Circulating CEA Levels in Pancreatic Cancer: Some Clinical Correlations. Cancer 47: 1620, 1981.

17. Alsabte, E.A. and Kamel, A., Carcinoembryonic Antigen (CEA) in Patients with Malignant and Non-Malignant and Non-Malignant Disease. Neo-plasma 26: 603, 1979.
18. Khoo, S.K., Whitaker, S., et al., Predictive Value of Serial Carcinoembryonic Antigen Levels in Long-Term Follow-Up of Ovarian Cancer. Cancer 43:448, 1978.
19. Staab, H.J., Anderer, F.A., et al., Prognostic Value of Preopera tive Serum CEA Level Compared to Clinical Staging. In Colorectal Carcinoma. Br. J. Cancer 44:652, 1981.
20. Wanebo. H.S., Rao, B., et al., Preoperative Carcinoembryonic Antigen Level as a Prognostic Indicator in Colorectal Cancer. N.E.J.M. 299:448, 1978.

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