

HEALTH-CHEM DIAGNOSTICS, LLC

3341 S.W. 15th Street – Pompano Beach, FL 33069 - USA – Phone: (954) 979-3845 – Fax: (954) 979-7997 Website:
www.healthchemdiagnostics.com

ONE STEP AFP (ALPHAFETOPROTEIN) TEST™

INTENDED USE

The HCD **One Step AFP (Alphafetoprotein) Test™** is a rapid immunochromatographic assay for the qualitative determination of human alphafetoprotein (AFP) in serum or plasma. It is intended for professional use as an aid in screening, diagnosis and monitoring of primary hepatocellular (liver) carcinoma and non-seminomatous testicular cancer.

SUMMARY

Alphafetoprotein (AFP) is a single-chain glycoprotein with a molecular weight between 67,000 and 74,000 daltons, depending upon the degree of glycosylation.¹⁻⁴ The molecule shows a close sequence homology with serum albumin.⁵ AFP is believed to play a role in the transport of polyunsaturated fatty acids to developing and malignant cells.⁶ Studies also indicate that AFP may function as an important *in vivo* immunoregulator that acts through T cells.⁷⁻⁸

AFP is normally synthesized in the human yolk sac until the tenth to twelfth week of gestation; thereafter, it is primarily produced by fetal hepatocytes.⁹ Many studies have confirmed that abnormally elevated levels of maternal serum AFP are associated with open neural tube defects (NTD) in the fetus.¹⁰⁻¹² In these cases, AFP is believed to leak directly into the amniotic fluid and subsequently into the maternal circulation.

The upper limit for normal sera is about 9 ng/ml. Elevation of serum AFP to an abnormally high value occurs in several malignant diseases, most notably in cases of primary hepatocellular carcinoma and non-seminomatous testicular cancer. Serum AFP elevation has also been observed in some patients with cirrhosis and hepatitis. It has been reported that 82% of patients with clinically verified tumors have serum AFP concentrations above 175 ng/ml, while 98% of patients with metastatic liver disease have levels below 175 ng/ml.¹³ Elevation of serum AFP concentration in cases of benign hepatic disease is usually transient.¹⁴

PRINCIPLES OF THE PROCEDURE

The HCD **One Step AFP Test™** is a chromatographic immunoassay which utilizes monoclonal and polyclonal antibodies to selectively detect AFP in serum or plasma with a high degree of sensitivity. In ten minutes, elevated levels of AFP as low as 20 ng/ml can be detected.

In the test procedure, the strip is dipped into a serum or plasma specimen. The specimen migrates through the absorbent test strip and mixes with a labeled antibody-dye conjugate in the test membrane. If AFP is present in the specimen, it binds to the labeled conjugate to form a labeled antibody-antigen complex. The mixture then migrates along the strip to the test zone, which is pre-coated with anti-AFP antibody. The antibody in the test zone binds to the labeled antibody-antigen complex, causing a red-pink band to appear. The formation of this test band indicates that the AFP concentration in the patient specimen was at or above 20 ng/ml.

The mixture continues to migrate along the strip to the control zone. Unbound conjugate binds to the reagents in the control zone, producing a pink-red band. The appearance of this control band confirms that the device is functioning properly.

REAGENTS AND MATERIALS SUPPLIED

1. **25 One Step AFP Test™** strips: an absorbent test strip consisting of an antibody coated membrane and a pad treated with a protein matrix of polyclonal IgG-dye conjugate and 0.1% sodium azide.
2. Positive Control, 1.0 ml
3. Negative Control, 1.0 ml
4. Reaction tubes, 25
5. Reaction tube holder
6. Sample droppers, 25
7. Test Instructions

MATERIALS REQUIRED BUT NOT PROVIDED

1. A clock or timer
2. Sample collection containers
3. Centrifuge capable of 1000 x g (for centrifuging whole blood specimens)

STORAGE CONDITIONS

Store the kit refrigerated between 2 and 8°C upon receipt and when not in use. Prior to use, bring the needed kit components to room temperature. Do not freeze.

QUALITY CONTROL

The daily use of a control is recommended to verify proper kit performance. Quality control samples should be tested according to quality control requirements established by the testing laboratory.

Use the control in the same manner as a specimen by following the test procedure. The controls should be brought to room temperature before use. The expected results should be obtained when using the controls.

WARNINGS AND PRECAUTIONS

1. Wear gloves while handling specimens.
2. Dispose of gloves and specimens using good microbiological practices.
3. For *in vitro* diagnostic use only.
4. Do not use test kit after the expiration date indicated on the label.

SPECIMEN COLLECTION AND STORAGE

1. Serum is obtained following regular clinical procedures.
2. If specimen is not tested the same day as collection, it should be sealed and refrigerated. If not tested within three days of collection, the specimen should be frozen. Frozen specimens should not be thawed and refrozen.

- Specimens containing precipitate may yield inconsistent results. Such specimens must be clarified prior to testing.

ASSAY PROCEDURE

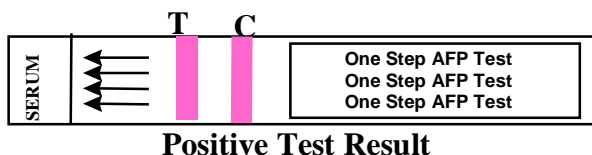
Bring unopened test components and sample specimens to room temperature prior to testing.

- Transfer 4 drops of serum using dropper supplied into a reaction tube.
- Remove the test strip from its foil pouch.
- Place the test strip into the tube with the arrow pointed toward the sample. The sample level should not be higher than the maximum level line marked on the strip.
- Read the results after 10 minutes.

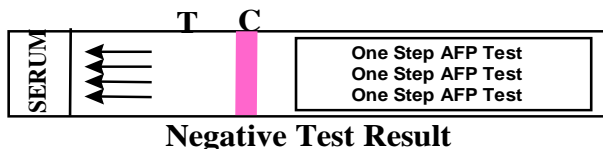
IMPORTANT: Do not interpret the result after more than 10 minutes. Discard the strip after reading and recording the result.

INTERPRETATION OF RESULTS

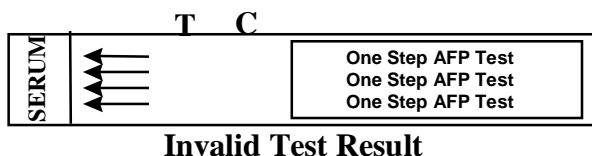
Positive. Two color bands appear: one in the Test zone ("T") and one in the Control zone ("C"). This result indicates that AFP is present in the sample at a concentration equal to or greater than 20 ng/ml.



Negative. One red-pink band appears in the Control zone and no band appears in the Test zone. This result indicates that the AFP concentration in the sample is below the 20 ng/ml detection limit of the test.



Invalid. If no red-pink band appears in the Control zone, the test is invalid. Retest the specimen using a new device.



Note: There is no meaning attributed to the color intensity or width of the lines.

LIMITATIONS OF THE PROCEDURE

- The test is limited to the detection of AFP in serum or plasma.
- The test is for *in vitro* diagnostic use only.
- Although the test is very accurate in detecting elevated AFP levels, a low incidence of false results may occur.
- The test is a qualitative screening assay and is not suggested for quantitative AFP determination.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

- Sensitivity.** The HCD **One Step AFP Test™** has been designed to produce a definitive color band at the Test region when tested with 20 ng/ml or higher AFP.

- Accuracy.** A study was performed using ninety-six positive and negative serum specimens. Each specimen was tested with the **One Step AFP Test™** and a commercially available AFP test according to the respective package insert instructions. The data demonstrate an excellent correlation between the two tests. The clinical significance of the two tests is comparable.

Correlation Study (n = 96)

One Step AFP Test™ / Commercial Test

+/+	+/-
37	1
-/+	-/-
0	58

Relative Sensitivity: 100%

Relative Specificity : 98.3%

BIBLIOGRAPHY

- Bergstrand, C.G., Czar, B., Scand. J. Clin. Lab. Invest., 8:174-179 (1956).
- Halbrecht, I., Klibanski, C., Nature (London), 178: 794-795 (1956).
- Smith, C.J., Kelleher, P.C. Biochim. Biophys. Acta, 605: 1-32 (1980).
- Van Oers, N.S., Cohen, B.L., Murgita, R.A., J. Exp. Med., 170: 811-825 (1989).
- Gibbs, E.M., Zielindki, R., Royd, C., Dugaiczyrk, K., Biochemistry, 26: 1332-1343 (1987).
- Calvo, M., Naval, Lampreave, F., Uriel, J., Pineiro, A. Biochim. Biophys. Acta, 959: 238-246 (1988).
- Murgita, R.A., Tomasi, T.B., J. Exp. Med., 141: 269-286 (1975).
- Peck, A.B., Murgita, R.A., Wigzell, H., J. Immunol., 128: 1134-1140 (1982).
- Gitlin, D., Ann., N.Y. Acad. Sci., 259: 7-16 (1975).
- Polteraner, P., Horak, W., Protides. Biol. Fluids, 27: 297-299 (1979).
- Silver, H.K., Gold, P., Freedman, S.O., Shuster J., Proc. Natl. Acad. Sci. USA, 70: 526-530 (1973).
- Scardino, P.T., Cox, H.D., Waldmann, T.A., McIntire, K.R., Mitemeyer, B., Javadpour, N., J. Urol., 118: 994-999 (1977).
- Bosl, G.J., Lange, P.H., Fraley, E.E., Goldman, A., Nochomovitz, L.E., Rosai, J., Waldmann, T.A., Johnson, K., Kennedy, B.J. Cancer, 47: 328-332 (1981).
- Lange, P.H., McIntire, K.R., Waldmann, T.A., Hakala, T.R., Fraley, E.E., N. Engl. J. Med., 295: 1237-1240 (1976).

Manufactured in the USA by:

HEALTH-CHEM DIAGNOSTICS LLC,
3341 SW 15th STREET, POMPANO BEACH, FL - USA

www.healthchemdiagnostics.com

Certified ISO CMCAS 13485:2003



FM77504 - Quality Award
FDA Registration No.: 1048532