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One-Step AFP Card Test (α- Fetoprotein)

INTENDED USE

The One-Step AFP Test is a colloidal gold-antibody complex immunoassay for qualitative determination of human alpha-fetoprotein (AFP) in serum or plasma. It is intended for professional use as an aid in the diagnosis of hepatoma, ovarian, testicular, and presacral teratocarcinomas and for the detection of AFP in the serum of patients with various malignancies including those of primitive gut origin and as an aid in the detection of fetal open neural tube defects (NTD).

INTRODUCTION

AFP was first found in the sera of human fetuses in molecular weights ranging from 67,000 to 74,000 daltons. The variations in weight are due to methods of analysis and the different degrees of glycosylation of protein. [1-4]

AFP is a single-chain protein showing close sequence homology with serum albumin.[5] It is suggested to play a role in transporting polyunsaturated fatty acids to developing and malignant cells because of its high affinity to these substances.[12] Studies also indicate that AFP may function as an important in vivo immunoregulator that acts through T cells.[13, 14]

Synthesis of AFP in the human yolk sac ceases between the tenth and twelfth weeks of gestation. The major portion of the protein is then produced by fetal hepatocytes.[6] The upper limit for normal sera is about 9 ng/ml, while levels above 175 ng/ml are highly suspicious of hepatocellular carcinoma. While 82% of patients with clinically verified tumors have higher amounts, 98% of patients with metastatic lever disease have below 175 ng/ml.[7] It has been shown that the elevation of serum AFP in benign hepatic diseases is usually transient.[8]

Many studies confirm the presence of AFP in the early stages of open NTD.[9-11] In these cases, AFP is thought to leak directly into the amniotic fluid and subsequently enter maternal circulation, thus producing abnormally elevated levels of maternal serum AFP.

PRINCIPLE OF THE TEST

The One-Step AFP Test is an immunochromatographic assay, which utilizes a unique combination of monoclonal and polyclonal antibodies to selectively identify AFP in serum or plasma specimens with a high degree of sensitivity. Elevated levels of AFP are detected in ten minutes or less.

Serum specimen migrates through the absorbent device and mixes with labeled antibody-dye conjugate in the test membrane. AFP antigen present in the specimen binds to the labeled conjugate to form an antibody-antigen complex. In the test zone, anti-AFP antibody binds to the antibody-antigen complex causing a pink-rose test band to appear in the test zone "T". The test band indicates that the AFP level in the sample specimen is at or above the detection sensitivity of the test. In the control zone "C" unbound sample-dye conjugate binds to reagents producing a pink-rose color control band. The control band appears when the test is conducted correctly and the reagents are functioning properly.

MATERIALS AND REAGENTS PROVIDED

1. Test device containing an absorbent device with an antibody coated membrane and a pad treated with mouse polyclonal IgG-dye conjugate in a protein matrix containing sodium azide.
2. Sample dropper
3. Product package insert

MATERIALS REQUIRED, BUT NOT PROVIDED

Specimen collection container and clock or timer.

STORAGE

Store the test kit at 2 - 8°C; do not freeze. Prior to use, bring test components to room temperature. The testing device may be stored at room temperature (below 28°C), however the control must be refrigerated at 2 - 8°C.

WARNINGS AND PRECAUTIONS

This kit contains no infectious reagents, however proper precautions should always be taken when handling patient specimens.

1. Preclude any pipetting by mouth.
2. Do not allow smoking or eating where specimen and reagents are being handled.
3. Wear disposable gloves while handling kit reagents or specimens. Wash hands thoroughly afterwards.
4. Avoid splashing or aerosol formation.
5. Clean up spills thoroughly using an appropriate intermediate to-high level disinfectant.
6. Decontaminate and dispose of all specimens and potentially contaminated materials as if they were infectious.
7. Do not use reagents after the expiration date.
8. For *in vitro* diagnostic use only.

QUALITY CONTROL

An internal procedural control has been incorporated into the test to ensure proper kit performance and reliability.

The 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 expected results should be obtained when using the control.

SPECIMEN COLLECTION AND PREPARATION

Collect blood aseptically by venipuncture into a clean tube without anticoagulants. Permit blood to clot for twenty to thirty minutes at room temperature. Centrifuge to obtain clear serum and transfer serum into a clean plastic or glass tube. The test may be performed using human serum or plasma.

If specimens are not immediately tested they should be refrigerated at 2 - 8°C. For storage periods greater than three days, freezing is recommended. If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

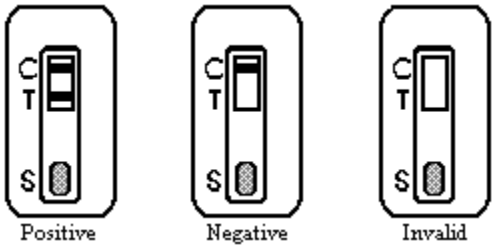
PROCEDURE

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

1. Open a foil pouch by tearing along the splice and remove the test cassette and sample dropper.
2. Holding the dropper vertically, add four full drops of sample specimen without air bubbles to the sample well "S" of the test device.
3. Read the result at ten minutes.

IMPORTANT: Disregard changes in the test band after reading the result at ten minutes. Once activated, reagents in the device membrane will continue to react beyond the cutoff point for the assay.

INTERPRETATION OF RESULTS



Positive:

Two pink-rose bands appear in the result window, one in the control zone "C" and one in test zone "T". The pink-rose test band indicates AFP is present in the sample at or above the 20 ng/ml detection cutoff.

Negative:

One pink-rose control band appears in the control zone "C" with no test band. The AFP level of the specimen is below the 20 ng/ml detection cutoff of the test.

Invalid:

If no rose-pink color band is visible in the control zone "C", the test result is invalid. Retest the specimen using a new test device.

LIMITATIONS

1. The test is limited to the detection AFP in serum, plasma, or recalcified plasma.
2. The test is for *in vitro* diagnostic use only.
3. Although the test is very accurate in detecting elevated AFP levels, a low incidence of false results may occur.
4. The test is a qualitative screening assay and is not suggested for quantitative AFP determination.
5. 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

1. Sensitivity:
   The analytical sensitivity of the One-Step AFP Test is 20 ng/ml.
2. Accuracy:
   A study was performed using ninety-six positive and negative serum specimens. Each specimen was assayed with the One-Step AFP Test and a commercially available AFP test according to the respective package insert instructions.

Correlation Study: ONE STEP / Commercial Test

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

Relative sensitivity: 100%
Relative specificity: 98.3%

The data demonstrates an excellent correlation between the two tests. The clinical significance of the two tests is comparable.

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