

HEALTH-CHEM DIAGNOSTICS, LLC

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ONE STEP AFP (ALPHAFETOPROTEIN) DUAL VALUE TEST™ For Hepatocellular Carcinoma

INTENDED USE

The HCD **One Step AFP (Alphafetoprotein) Dual Value Test™** for hepatocellular carcinoma is a quick performing immunoassay designed for the semi-quantitative determination of human a-Fetoprotein (aFP) in blood/serum/plasma. It is intended for professional use as an aid in diagnosis and monitoring of hepatocellular carcinoma.

SUMMARY

Hepatocellular carcinoma (HCC) appears to be associated with Hepatitis B and C virus infection and is common in patients with cirrhosis caused by chronic viral Hepatitis (1-4). An effective screening system to detect HCC at an early stage may result in more effective treatment. a-FP is the only established tumor marker with a reasonable specificity for detection of HCC. In addition, a-FP may be increased in non-malignant liver disease (5-6). The normal a-FP cut-off value is ~5ng/ml (7-9). The upper limit of normal a-FP was defined as 20 ng/ml (7-17). With higher than 5ng/ml (7-9). The upper limit of normal a-FP was defined as 20 ng/ml (7-17). With higher than 5ng/ml (7-9). The upper limit of normal a-FP was defined as 20 ng/ml (7-17). With higher than ~20 ng/ml, the likelihood of cirrhotic and HCC being present is 95% (7, 8, 11, 17). The Device A is designed according to two cut-off levels: 5 ng/ml and 20 ng/ml.

For detection of HCC, there are two cut-off levels: 100-120 ng/ml and 400 ng/ml. The first cut-off is based on the optimal value by ROC curve analysis (7,10) with specificity 98.9-100%, sensitivity 55.3-67.3% and accuracy 77.6-83.6%. And the second cut-off level: 400 ng/ml is called the recommended diagnostic level for HCC (7,8,10,12,17) with specificity 100%, sensitivity 47.8-63.7% and accuracy 73.9-83.6%.

The Device B is designed according to two cut-off levels: 100 ng/ml and 400 ng/ml.

PRINCIPLES OF THE PROCEDURE

The membrane in the device was pre-coated with an anti-a-FP capture antibody on the Test zone and goat anti-mouse antibody on the Control zone. During the testing, the serum sample is added to the sample well with the aid of a dropper and allowed to flow up through the device by capillary reaction. The a-FP in the sample reacts with a colored conjugate of a monoclonal a-FP antibody, which was pre-dried on the strip, and an

antibody-antigen complex is formed when a-FP is present in the sample. The mixture then moves upward and the color band will be seen in the Test zone. Inversely, a color band will always appear in the Control zone, indicating the device is functioning correctly. This control band also serves as a reference of the color intensity of the approximate value of a-FP designed. Comparing the intensity of the test band and control band, the range of concentration of a-FP can be determined.

Device A

Conc. of a-FP (ng/ml)	Clinical indication
<5	healthy
5-20	conditional healthy
>20	suggest cirrhosis or HCC

Device B

Conc. of a-FP (ng/ml)	Clinical indication
<100, >20	indicate disease
>100	optimal analysis HCC
>400	recommended HCC

KIT CONTENTS

Each pack contains two devices:

Device A: cut-off: 5 and 20 ng/ml

Device B: cut-off: 110 and 400 ng/ml

Each sealed foil includes:

1 Dropper
1 Desiccant

STORAGE CONDITIONS

The HCD One Step AFP (Alphafetoprotein) Dual Value Test™ for hepatocellular carcinoma test kit may be stored at room temperature (15-28°C) for up to 18 months.

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.

WARNINGS AND PRECAUTIONS

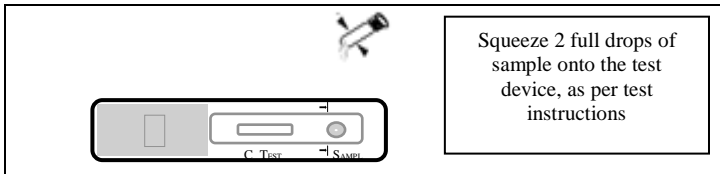
1. For *in-vitro* diagnostic use only.
2. **Avoid splashing or aerosol formation while adding the specimens.**
3. Do not use beyond the expiration date indicated on the label.

4. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single result, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

SPECIMEN COLLECTION AND STORAGE

a-FP is thermolabile. If specimens are not immediately tested, they should be refrigerated at 2-8°C. For storage period more than 3 days, freezing is recommended. For whole blood, an anti-coagulant, such as Heparin or EDTA must be used.

ASSAY PROCEDURE



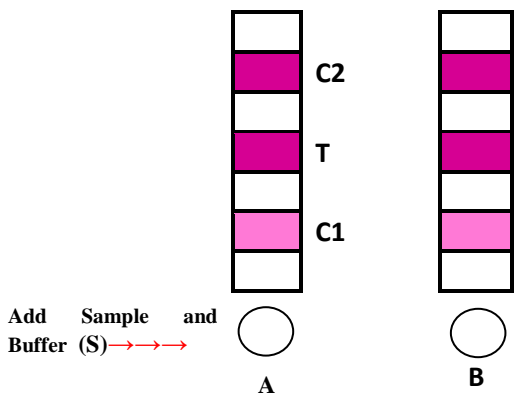
1. Remove the reaction pack from its foil wrapper.
2. Fill the dropper with serum and hold it above the sample well (S) as shown. Add 2 full drops of samples. Allow each drop to soak in before adding the next.
3. Because this test is based on color intensity, it is important to read the results within the time specified, after addition of the sample.

Exact reading time varies from lot to lot and is specified on the Certificate of Analysis provided with each lot.

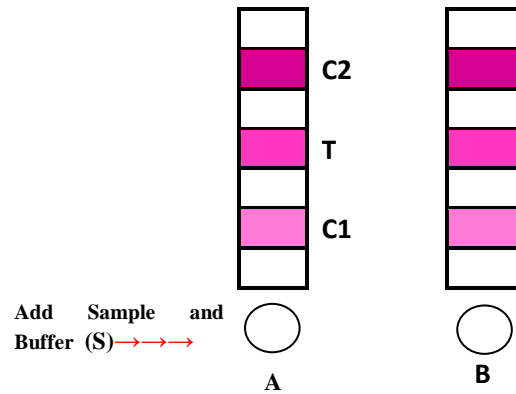
INTERPRETATION OF RESULTS

To determine your result, compare the color intensity, i.e., shade of color, lightness or darkness or color of the Test band "T" to the Control band "C".

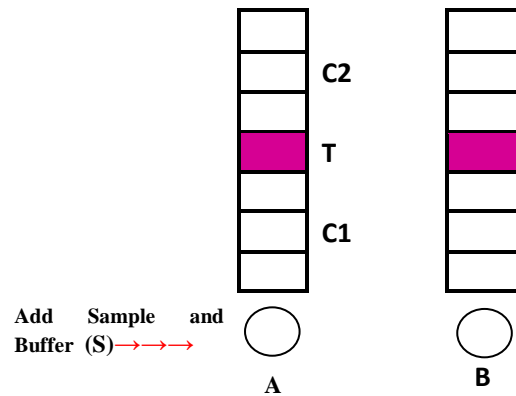
Positive. If the Test band is of equal or greater intensity (equal or darker) than the Control band, this indicates the concentration of a-FP in sample is above the value represented by the Control band.



Negative. If the Test band is of lesser intensity (lighter) than the control band, this means the a-FP of the sample is less than the value represented by the Control band.



Invalid. If no Control band appears within five minutes, the result is Invalid and should be ignored. A visible Control band is needed in all cases to confirm proper test operation. No Control band indicates either the test procedures were not followed correctly, or the test reagents failed. Carefully review the test procedures and retest with a fresh (unused) test device.



Estimation of concentration of a-FP by test

Darkness comparison	a-FP ng/ml
Device A	
T<C1	<5
T=C1	5
C1<T<C2	5-20
T>C2	>20
Device B	
T<C1	<100
T=C1	100
C1<T<C2	100-400
T>C2	>400

PERFORMANCE CHARACTERISTICS

1. **Precision.** Inter assay and intra assay precision tests were run using five <5, five 5-20, five 20-100, five 100-400 and five >400 ng/ml positive serum samples with 100% correct identification.
2. **Accuracy.** A study was performed using 152 serum samples, including 45 of level <5, 21 of 5-20, 16 of 20-100, 42 of 100-400 and 28 of >400 ng/ml. They were assayed using **One Step™ a-FP Test** and a commercially available quantitative ELISA test. The correlation was 98%.

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