CANCHECK® - AFP

Rapid test for detection of Alpha Feto Protein in human serum / plasma / whole blood

DEVICE

INTENDED USE

CANCHECK® - AFP is a rapid, qualitative, two site sandwich immunoassay for the detection of Alpha Feto Protein (AFP) levels in human serum / plasma / whole blood.

SUMMARY

AFP is a glycoprotein with a molecular weight of 70kDa. It consists of a single polypeptide chain containing 4% carbohydrate. AFP is synthesized in large quantities during embryonic development by fetal yolk sac and liver. It is one of the major proteins in the fetal circulation. AFP is closely related both genetically and structurally to albumin, having extensive homologies in amino acid sequence. The genes coding for both proteins have been localized to the same area of human genome. As albumin synthesis increases during later fetal development, AFP concentrations in the serum begin to decline. Eighteen months following birth, they finally reach the trace concentrations found in normal adults.

CANCHECK® - AFP is a rapid test for the determination of AFP in human serum/plasma/whole blood.

PRINCIPLE

CANCHECK® - AFP utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. The conjugate pad contains two components - Agglutinating sera for AFP conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test specimen flows through the membrane test assembly of the device, the highly specific Agglutinating sera for AFP-colloidal gold conjugate complexes with the AFP in the specimen and travels on the membrane due to capillary action along with the rabbit globulin - colloidal gold conjugate. This complex moves further on the membrane to the test region (T) where it is immobilized by another specific Agglutinating sera for AFP coated on the membrane leading to formation of a coloured band. A detectable coloured band is formed if AFP level is equal to or higher than 25ng/ml. The absence of this coloured band in the test region indicates a negative test result.

The rabbit globulin - colloidal gold conjugate and unbound complex, if any, move further on the membrane and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membrane at the control region (C), forming a coloured band. The control band formation is based on the 'Rabbit / Agglutinating sera for Rabbit globulin' system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. This control band acts as a procedural control and serves to validate the test results.

NORMAL REFERENCE VALUES

The upper normal limit is approximately 15ng/ml for adults.

REAGENTS AND MATERIALS SUPPLIED

CANCHECK® - AFP kit contains:

A. Individual pouches, each containing :
   1. DEVICE : Membrane assembly pre-dispensed with Agglutinating sera for AFP - colloidal gold conjugate, rabbit globulin- colloidal gold conjugate, Agglutinating sera for AFP and Agglutinating sera for rabbit globulin coated at the respective regions.
   2. PIPETTE : Disposable Plastic Sample Applicator.
   3. Desiccant Pouch.

B. BUF : Sample running buffer in a dropper bottle.

C. Package Insert.

REF	505020010	505020025
Σ	10	25

STORAGE AND STABILITY

The sealed pouches in the test kit & the kit components may be stored between 4°C to 30°C for the duration of shelf life as indicated on the pouch / carton. DO NOT FREEZE. After first opening of the sample running buffer bottle, it can be stored between 4°C to 30ºC for remaining duration of its shelf life.

NOTES

1. Read the instructions carefully before performing the test.
2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
3. Do not use the kit beyond expiry date and do not re-use the test device.

4. Do not intermix reagents from different lots.
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
6. Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infectious material.
7. If desiccant colour at the point of opening the pouch has turned from blue to pink or colourless, another test device must be run.
8. Sample running buffer contains Sodium Azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxide. Flush with large volumes of water to prevent azide build-up in the plumbing.

SPECIMEN COLLECTION AND PREPARATION

1. **CANCHECK® - AFP** uses human serum / plasma / whole blood as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. For whole blood, collect blood with a suitable anticoagulant such as EDTA or Heparin or Oxalate and use the freshly collected blood.
4. Whole blood should be used immediately and should not be frozen.
5. Though fresh specimen is preferable, in case of delay in testing, it may be stored **at 2°C to 8°C for maximum up to 24 hrs.**
6. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.
7. Repeated freezing and thawing of the specimen should be avoided.
8. Do not use turbid, lipaemic and hemolysed serum/plasma.
9. Do not use hemolysed, clotted or contaminated blood specimens.
10. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only should be used for testing.
11. Refrigerated specimens must be brought to room temperature prior to testing.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the kit components of **CANCHECK® - AFP** device to room temperature before testing.
2. Open a foil pouch by tearing along the "notch".
3. Remove the testing device and the sample applicator. ***Once opened, the device must be used immediately.***
4. Label the test device with specimen identity.
5. Place the testing device on a flat horizontal surface.
6. Holding the sample applicator vertically, carefully dispense exactly **two drops** (50µl) of serum / plasma / whole blood into the specimen port 'A'.
7. Add **five drops** of sample running buffer into the buffer port 'B'.
8. At the end of **15 minutes,** read results as follows:

	**Negative Result** Presence of one coloured band at Control (C) region indicates absence of AFP or the concentration of AFP in the specimen is **below** the detection limit of **25 ng/ml.**
	**Positive Result** If the concentration of AFP in the specimen is **above 25 ng/ml**, two coloured bands appear at Test (T) and Control (C) regions. The intensity of test band may be more or less than the control band, depending upon the concentration of AFP in specimen.
 	**Invalid Result** The test is invalid if no band is visible at fifteen minutes. The test should also be considered invalid if only test band appears and no control band appears. Verify the test procedure and repeat the test with a new **CANCHECK® - AFP** device.

PERFORMANCE CHARACTERISTICS

The detection limit of **CANCHECK® - AFP** is upto 25 ng/ml.

In an in-house study, the performance of **CANCHECK® - AFP** was evaluated using a panel of specimens of positive (of varying reactivity) and negative sera in comparison with commercially available ELISA kit. 100% correlation with ELISA was observed.

The results of the evaluation are as follows:

SPECIMEN DATA	Total	CANCHECK® - AFP	Commercially Available ELISA
Total number of specimen tested	205	205	205
Number of Positive serum/plasma specimens	23	23	23
Number of Negative serum/plasma specimens	130	129	130
Number of Negative whole blood specimens	52	52	52

Based on this evaluation:
Sensitivity of **CANCHECK® - AFP** - 100%.
Specificity of **CANCHECK® - AFP** - 98.9%.

LIMITATIONS OF THE TEST

1. During foetal life, AFP is synthesized in the gastrointestinal tract, the liver and the yolk sac of the foetus. AFP reaches the maternal serum via the trans-placental route. Maternal serum AFP is first detectable (~5ng/ml) at about 12 weeks' gestation. The concentration increases to about 15% per week at about 25 week at approximately 250ng/ml. The concentration then subsequently declines slowly until term. After birth, the maternal serum AFP level rapidly decreases to less than 2ng/ml.
2. Elevated AFP levels can be found in patients with primary hepatoma and yolk sac-derived germ cell tumours. AFP is the most useful serum marker for the diagnosis and treatment of hepatocellular carcinoma (HCC). Approximately 50% of patients with primary liver cancer have serum AFP elevations greater than 1000ng/ml. AFP is elevated in more than 70% of patients with nonseminomatous cancer. However, as stated earlier, AFP is also transiently elevated in pregnancy. Some benign liver diseases like hepatitis and cirrhosis also cause AFP elevation. Most of the patients with these benign diseases have AFP levels lower than 200 ng/ml.
3. AFP levels greater than 1000 ng/ml are indicative of cancer. At these levels of AFP, about one half of HCC may be detected. However, because the serum levels of AFP correlate with the size of the tumour, detection of HCC at the earlier stages, i.e., when the tumour is small enough to be resectable (<5cm), is more useful than when the tumour is large.
4. In order to detect small tumours, the cut off point of 10-20 ng/ml has been recommended, but at this level, hepatitis and cirrhosis must be considered as possible causes of elevation. For optimum sensitivity and specificity, the detection limit of **CANCHECK® - AFP** is calibrated to 25 ng/ml.
5. AFP is also useful for determining prognosis and in the monitoring of therapy for HCC. The level of AFP is the prognostic indicator for survival. Elevated AFP levels (>10 ng/ml) as well as serum bilirubin levels (>2 mg/ml) are associated with shorter survival time. A significant increase in AFP levels in patients considered free of metastatic tumour may indicate the development of metastasis.
6. AFP level is a good indicator for monitoring therapy and the change in clinical status of a patient. Elevated AFP levels after surgery may indicate incomplete removal of the tumour or the presence of metastasis. Falling or rising AFP levels after therapy may determine the success or the failure of the treatment regimen.
7. Tests for both AFP and hCG help in reducing the clinical staging errors in patients with some testicular tumours and aid in the differential diagnosis of various germ cell tumours. AFP is not elevated in seminomas. The use of AFP combined with hCG is highly predictive for recurrence of testicular cancer.
8. Interferences due to heterophile antibodies, Rheumatoid Factors and other nonanalyte substances in patient's serum, capable of binding antibodies multivalently and providing erroneous analyte detection in immunoassays, has been reported in various studies. Though **CANCHECK® - AFP** uses sufficient amounts of blocking reagents to inhibit the majority of this interference; nevertheless, some samples with high titres may still express clinically important assay interference. Both laboratory professionals and clinicians must be vigilant to this possibility of antibody interference. Results that appear to be internally inconsistent or incompatible with the clinical presentation should invoke suspicion of the presence of an endogenous artifact and lead to appropriate in vitro investigative action.
9. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
10. **CANCHECK® - AFP** should only be used as a screening test in clinically suspected cases only, and its results should be confirmed by a quantitative method before taking clinical decisions.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. Clinical Laboratory Diagnostics, Use and Assessment of Clinical Laboratory Results, Edited by Lothar Thomas, First Edition, TH Books Verlagsgesellschaft mBH, Frankfurt, Germany, 1998: 941-942.
2. Teitz Textbook of Clinical Chemistry. Second Edition, WB Saunders Publishing, 1994, 913-914.
3. Data on file: Zephyr Biomedicals.

SYMBOL KEYS

	Temperature Limitation		Manufacturer	DEVICE	Device		Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.
	Use by		Consult Instructions for use	PIPETTE	Disposable Plastic Sample Applicator		
	Date of Manufacture	REF	Catalogue Number	BUF	Sample Running Buffer		
LOT	Batch Number / Lot Number	IVD	*In vitro* Diagnostic Medical Device		This side up		
	Contains sufficient for <n> tests		Do not reuse		Do not use if package is damaged		

Manufactured by:

Zephyr Biomedicals

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