

Hepa AFP-IC

XG005

ELISA Kit for the detection of Alpha-Fetoprotein (AFP) Immune Complexes in Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies in the world and its incidence, even though with geographical differences, is increasing (1). For diagnosing HCC at an early stage, at risk patients should follow surveillance programs consisting in the periodical screening of the liver by ultrasound imaging and determination of serum alpha-fetoprotein (AFP) levels. Measurement of serum level of AFP provides an indication for the diagnosis and management of HCC. At a cut off level of 20 ng/mL, AFP shows 60-80% sensitivity but unfortunately this value drastically diminishes in small HCC, lowering to 40%. Only small localized HCC can be surgically treated, with good chances of prolonged survival after resection. Adoption of higher AFP cut off value to discriminate HCC from non malignant liver diseases, such as chronic hepatitis or cirrhosis, declines dramatically

assay sensitivity to 5-15% (2). Hepa AFP-IC is an ELISA assay to assess the presence of AFP as circulating immune complexes (IC) in the blood of at risk patients. AFP immune complexes occurs in patients affected by liver diseases, with serum levels increasing according to the severity of liver injury (3).

Detection of complexed AFP (AFP-IC) has been demonstrated to be more sensitive than free AFP (fAFP) in the identification of HCC affected patients. Furthermore, the detection of AFP-IC in parallel with fAFP significantly increases the efficiency for discriminating HCC from cirrhosis and HCC from chronic hepatitis (3).

Data derived from a total of 200 sera indicated that in terms of sensitivity AFP-IC is detected above the cut off level in 30 out of 50 HCC patients (60%). fAFP is elevated only in 44% (23/50) of HCC patients. fAFP levels are above the cut off in 18% (9/50)

and 2% (1/50) of cirrhosis and chronic hepatitis, respectively, compared to 28% (14/50) and 26% (13/50) of positive patients for AFP-IC in the same groups (FIG.1). Co-determination of both markers improves the indexes of diagnostic accuracy for liver cancer diagnosis (3). 82% (41/50) of HCC patients are positive for at least one marker, and detection of both markers in parallel significantly increases the efficiency for discriminating HCC from cirrhosis (combination 71% vs. 66% and 63%, AFP-IC and fAFP respectively) and HCC from chronic hepatitis (combination 78% vs. 67% and 71%, AFP-IC and fAFP respectively) (Tab.1) (3).

Hepa AFP-IC allows to monitor patients detecting not free, but complexed AFP, with a significant increase in the sensitivity of AFP-based screening protocols.

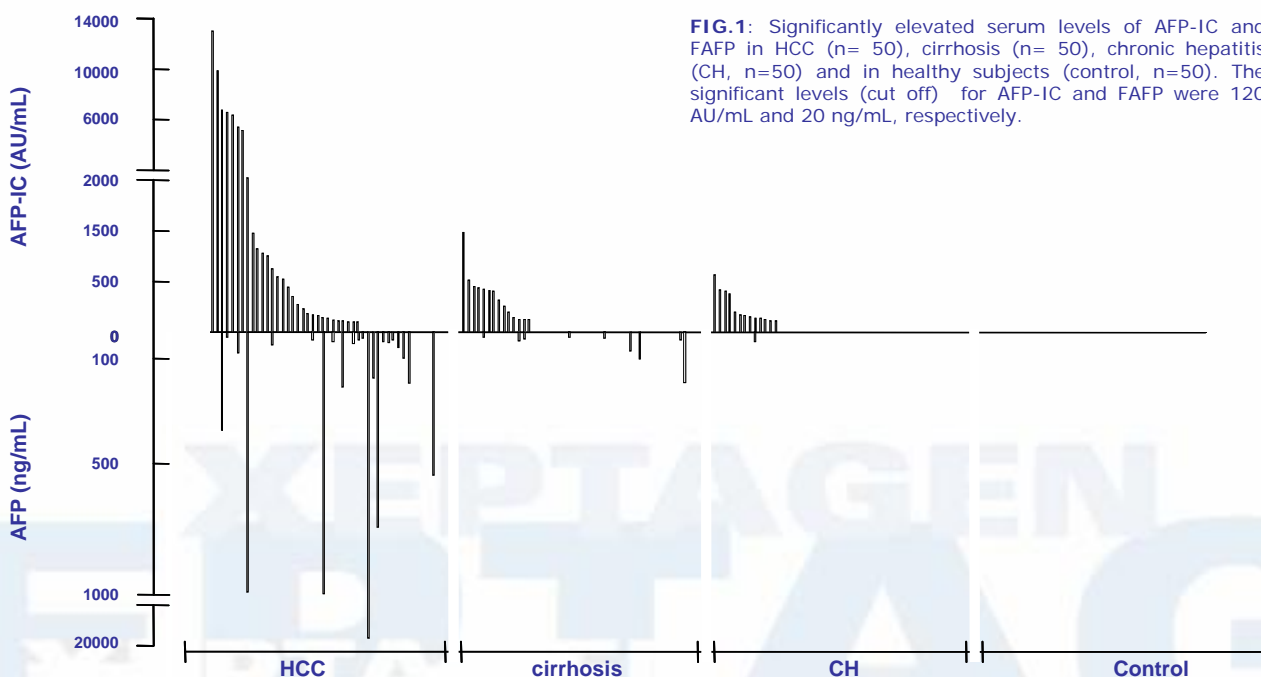


FIG.1: Significantly elevated serum levels of AFP-IC and FAFP in HCC (n= 50), cirrhosis (n= 50), chronic hepatitis (CH, n=50) and in healthy subjects (control, n=50). The significant levels (cut off) for AFP-IC and FAFP were 120 AU/mL and 20 ng/mL, respectively.

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BIOMARKER	Sensitivity	Specificity	PPV	NPV	Efficiency
AFP-IC cff=120 AU/mL					
HCC vs control	60	100	100	71	80
HCC vs cirrhosis		72	68	64	66
HCC vs CH		74	70	65	67
Free AFP cff=20 ng/mL					
HCC vs control	44	100	100	64	72
HCC vs cirrhosis		82	71	59	63
HCC vs CH		98	96	64	71
AFP-IC cff=120 AU/mL & Free AFP cff=20 ng/mL					
HCC vs control	82	100	100	85	91
HCC vs cirrhosis		64	73	77	71
HCC vs CH		80	83	80	78

Tab.1: Comparison of the indexes of diagnostic accuracy for AFP-IC, FAFP and both in combination, in differentiation of patients with HCC from those with cirrhosis, chronic hepatitis and healthy subjects (control). PPV (Positive predictive value) = True Positive (TP) / TP + False Positive (FP); NPV (Negative predictive value) = True Negative (TN) / False Negative (FN) + TN; Efficiency = TP + TN / TP + FP + TN + FN.

References

1. Befeler AS. et al., Gastroenterology, 122:1609-19, 2002.
2. Gebo KA. et al., Hepatology, 36:S84-92, 2002.
3. Beneduce L. et al., International Journal of Biological Markers, 19(2):155-59, 2004.

Specifications

Materials provided:

XG005-PL Multi-strip Assay-Plate: 96 wells pre-coated with affinity purified rabbit anti-AFP.

XG005-IC AFP Immune complexes (AFP-IC)

Standard Solution: human purified AFP-IC standard solution. Red cap.

XG-EA Secondary Antibody: enzyme conjugated secondary antibody. Green cap.

XG-CH4 Chromogen: ABTS (2,2'-AZINO-bis(3-ETHYLBENZOTHAZOLINE - 6 - SULFONIC ACID)).

XG-SB Enzyme substrate solution. Blue cap.

XG-DB Dilution Buffer.

XG-WB Washing buffer.

Equipment required:

Microplate washer and microplate reader

Brief description of procedure

Calibration curve and samples: Dispense 100 µL/well of **XG005-IC** standard solution (starting from 250 AU/mL and performing in-plate 2-fold serial dilutions to a final concentration of 15,6 AU/mL) and of 8-fold diluted samples. Use **XG-DB** dilution buffer as diluent. Also dispense 100 µL/well of dilution buffer as blank. Incubate 1h at room temperature. Wash 6x with **XG-WB** washing buffer.

Secondary antibody: Add 100 µL/well of **XG-EA** enzyme-conjugated antibody. Incubate 1h at room temperature. Wash 6x with **XG-WB** washing buffer.

ABTS substrate solution: Add 150 µL/well of freshly prepared ABTS substrate. Allow colour to develop in the dark at 37° C and then measure OD values of each well using an ELISA plate reader set to 405 nm.

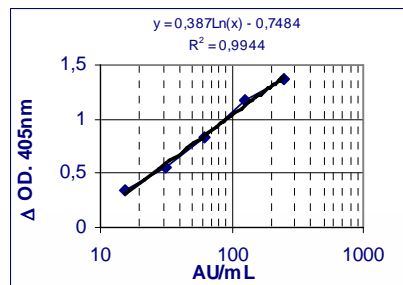


FIG.2: Range of linearity of a typical standard curve for AFP-IC after 20 min of substrate incubation.

Related products:

- **Hepa-Ab**, Antibody for immunohistochemical detection of hepatocellular carcinoma from liver biopsies.
- **Hepa-IC ELISA Kit**, Kit for detection of Squamous Cell Carcinoma Antigen variant (SCCA) Immune Complexes in hepatocellular carcinoma.

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Code XG005 96 well assay

For *in vitro* use only



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