COLON-IC

code XG006

ELISA Kit for the detection of Carcinoembryonic Antigen (CEA) Immune Complexes (CEA-IgM) in Colon Rectal Cancer (CRC)

Colorectal Carcinoma (CRC) ranks as the third most commonly diagnosed cancer. It represents approximately 11% of all new cases of cancer. The lifetime risk of developing colorectal cancer has been estimated to be approximately 6%, while the estimated lifetime risk of colorectal cancer death is approximately 2.6%. Colorectal cancer affects men and women with nearly equal frequency and is the third most common cancer in both men (after lung and prostate cancer) and women (after lung and breast cancer) (1). Survival of patients with colorectal cancer depends to a large extent on the stage of disease at diagnosis. Thus, in patients with localized disease, 5-year survival is approximately 90%, whereas in patients with regional spread of disease, 5-year survival decreases to approximately 60%. In patients with distant metastases, 5-year survival is less than 10%. There is now substantial evidence that reductions in colorectal cancer mortality can be achieved through detection and treatment of early-stage cancers. Unfortunately, only a minority of colorectal cancers are discovered when they are still localized. An adequate screening strategy can not be followed due to the lack of effective, low cost and non-invasive diagnosis tests (2).
Carcinoembryonic antigen (CEA) is an extensively characterized tumor-associated molecule. At present there is no role for serum CEA assessment as screening tool or diagnostic marker for early cancer detection, due to the very low sensitivity of this test. In fact, free CEA (fCEA) levels are elevated in a wide number of cases only in the latest phases of cancer progression (i.e. Stage III and IV) (3). In CRC patients CEA can be detected bound to IgM immunoglobulins, as CEA Immune Complexes (CEA-IgM), while is undetectable in healthy controls (4). Colon-IC is a highly specific and sensitive ELISA assay for CRC detection designed to measure CEA-IgM in patients sera. CEA-IgM levels are significantly elevated in particular in a high number of cases in early staging (Stage I) colorectal cancer patient sera, resulting in a higher sensitivity than that provided by the analysis of serum free CEA (fCEA) levels (29% vs 8% respectively) (figure 2). Furthermore, combined determination of the two forms of circulating CEA significantly increases the efficiency for discriminating CRC from normal individuals (5).

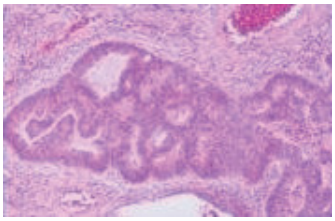

Figure 1: Histological appearance of Colorectal Carcinoma (CRC).

Table 1 shows a comparison of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) between CEA-IgM, fCEA and combined biomarkers levels in patients with cancer and normal controls.

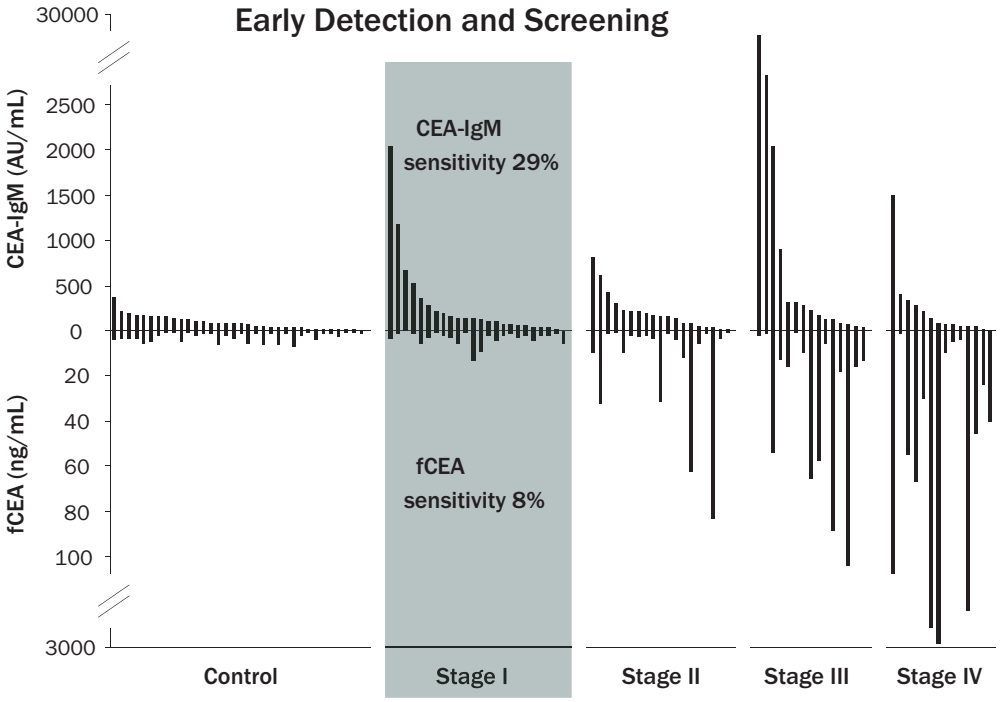

Figure 2: Comparison of serum levels of CEA-IgM and fCEA according to CRC staging.

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BIOMARKER	Sens	Spec	PPV	NPV	Effic
CEA-IgM: cut-off 200AU/mL					
CRC vs ctrl	38%		93%	42%	56%
CRC (I) vs ctrl	29%		78%	65%	67%
CRC (II) vs ctrl	37%	94%	78%	73%	74%
CRC (III) vs ctrl	53%		80%	82%	82%
CRC (IV) vs ctrl	36%		71	78%	77%
free CEA: cut-off 5ng/mL					
CRC vs ctrl	43%			45%	61%
CRC (I) vs ctrl	8%			61%	62%
CRC (II) vs ctrl	37%	100%	100%	74%	77%
CRC (III) vs ctrl	73%			89%	92%
CRC (IV) vs ctrl	79%			92%	94%
CEA-IgM: cut-off 200AU/mL & free CEA: cut-off 5ng/mL					
CRC vs ctrl	65%		96%	55%	74%
CRC (I) vs ctrl	38%		82%	68%	71%
CRC (II) vs ctrl	56%	94%	85%	80%	81%
CRC (III) vs ctrl	93%		88%	97%	94%
CRC (IV) vs ctrl	86%		86%	94%	92%

Table 1: Comparison of sensitivity (Sens), specificity (Spec), positive predictive value (PPV) and negative predictive value (NPV) of CEA-IgM, fCEA and combined biomarkers in differentiation of patients with CRC at different stage and healthy subjects as controls (ctrl).

REFERENCES

1. American Cancer Society, Cancer facts and figures, Washington, DC, Am Cancer Soc, 2000
2. Muto T. *et al.* Cancer, 1975, 36:2251-70
3. Fletcher RH. Ann Intern Med, 1986, 104:66-73
4. Castaldi F. *et al.* Int J Biol Markers, 2005, 20:204-8
5. Kojima T. *et al.* Mol Med Rep, 2008, 2:477-80

REAGENTS AND MATERIALS PROVIDED

XG006-PL: 96 wells multi-strip Assay-Plate, pre-coated with affinity purified rabbit anti-CEA
XG006-Calibrator: Two vials of calibrator lyophilized from PBS. White powder. Exact concentration on label. Totally soluble.
XG-EA: 200 µL of Enzyme-conjugated goat anti-human IgM secondary antibody (Green cap) 100-fold concentrate solution in PBS containing 1% BSA.
XG-CH3: 10 mL of TMB (3,3',5,5'-Tetramethylbenzidine) chromogen solution ready to use.
XG-ST3: 10 mL of 1N HCl Stop solution ready to use.
XG-DB5: Concentrated Dilution Buffer solution 5X, 10 mL. Once diluted, the working solution contains 1% BSA and 0.05% Tween 20 in PBS. The solution contains Proclin as preservative.
XG-WB2: Lyophilized Washing Buffer. Two white tablets. Once diluted, the working solution contains 0.05% Tween 20 in PBS. Totally soluble.

MATERIAL AND EQUIPMENT REQUIRED

Precision pipettes with disposable tips
Microplate washer
Microplate readers with a 450 ± 20 or 650 ± 20 nm filter
Distilled or deionized water

BRIEF DESCRIPTION OF PROCEDURE

Calibration curve and samples : Reconstitute the lyophilized XG006-Calibrator with 440 µL of distilled water. Dispense 100 µL/well of standard calibrator (in duplicate), starting from the reconstituted solution and performing in-plate 2-fold serial dilutions in order to obtain a seven-point calibration curve. Use XG-DB5 dilution buffer as diluent. For exact concentration of the reconstituted calibrator please refer to the concentration value (AU/mL) indicated on the XG006-Calibrator vial.

Dispense 100 µL/well of 50- or 100-fold diluted samples. Use XG-DB5 dilution buffer as diluent. Also dispense 100 µL/well of XG-DB5 dilution buffer as blank. Incubate the plate for 1h at room temperature. Wash 6x with XG-WB2 washing buffer.

Secondary antibody: Add 100 µL/well of XG-EA enzyme-conjugated antibody. Incubate 1h at room temperature. Wash 6x with XG-WB2 washing buffer.

TMB Substrate solution: Add 100 µL/well of TMB chromogen solution. Allow color to develop in the dark and measure OD values of each well using an ELISA plate reader with a 650 nm filter or, alternatively, apply 100 µL/well of XG-ST3 Stop Solution and measure OD values of each well using an ELISA plate reader with a 450 nm filter. Stopped reaction should be read within 1 hour

PROCESSING OF THE RESULTS

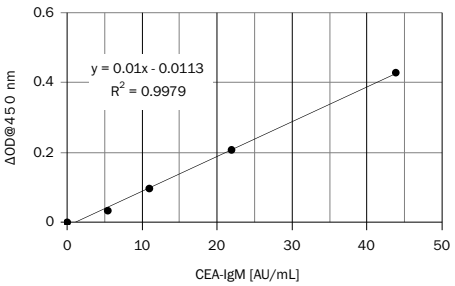

Figure 3A: range of linearity of a typical standard curve for CEA-IgM after 15 minutes of substrate incubation and addition of Stop solution.

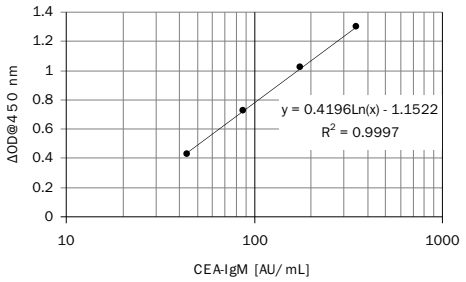

Figure 3B: range of linearity of a typical standard curve for CEA-IgM after 15 minutes of substrate incubation and addition of Stop solution.

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