PROSTATE-IC

code XG007

ELISA kit for the assessment of Prostate Specific Antigen (PSA) Immune Complexes (PSA-IgM) in Prostate Cancer (PCa)

Nowadays prostate cancer (PCa) is the most common cancer in male population and for several years a steady increase in the number of deaths caused by PCa has been reported, even in geographic areas where it was not significantly present. PCa mainly affects men aged over 55 years, with five-year survival rate from diagnosis of 70%. More than in other cancer forms a late diagnosis leads to a severe prognosis while the early detection may allow effective therapeutic treatments. Diagnostic tools for PCa detection used in the clinical routine include digital rectal examination, transrectal ultrasonography, determination of serum levels of Prostate Specific Antigen (PSA) and prostate biopsy. The latter remains the most used analysis for diagnostic confirmation of PCa. The PSA assay (including its variants like PSA density, PSA velocity, free/total PSA ratio and age-specific and race-specific reference ranges) was a landmark in PCa diagnosis, but on the other hand increased overdiagnosis because of its poor accuracy. Besides PCa, increased PSA levels are found in patients with benign prostatic hyperplasia (BPH) or bacterial prostatitis as well as after prostatic manipulations and trauma. A PSA level of 4 ng/mL is widely accepted to be a warning signal for neoplastic disease, but a particular attention has to be paid on the PSA range between 4 and 10 ng/mL (grey zone).
PSA-IgM immune complexes which are formed by PSA associated with IgM have shown greater diagnostic potential compared to the PSA [1-6]. Prostate-IC is a highly specific and sensitive ELISA kit for PCa detection designed to measure PSA-IgM in sera.
Clinical studies on male patients with PCa and BPH compared to healthy male donors showed the advantage of Prostate-IC in terms of specificity and sensibility, compared to total PSA (Fig. 2). In the group of healthy donors the PSA-IgM serum levels were always negative (cut off 145.1 AU/mL), while in the group of patients with cancer, PSA-IgM levels were above the cut-off in 40% of cases compared to 22% achieved from PSA analysis (cut off 10 ng/mL). In the group of patients with BPH, PSA-IgM was above the cut-off only in 12% of cases, with higher specificity (88%) compared to that attained with PSA (71%) (Tab. 1).

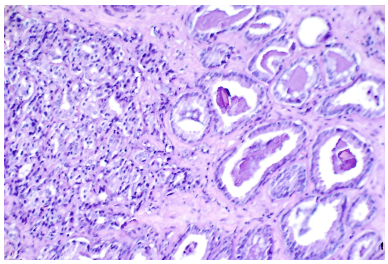

Figure 1: Histological slide showing prostate cancer. Source: Otis Brawley, AV-9612-438.

In the range of PSA levels between 4 and 10 ng/mL, both the positive predictive value (PPV = 77%) and the negative predictive value (NPV = 60%) of PSA-IgM analysis were better than the corresponding values provided by PSA assessment at 4 ng/mL cut off (PPV = 46%, NPV = 20%) and at 10 ng/mL cut off (PPV = 42%, NPV = 48%). Moreover, the combination of PSA-IgM and PSA levels led to the identification of an increased number of patients correctly diagnosed without compromising the specificity index. The results of the study demonstrated that PSA-IgM is a complementary serological marker of PSA for PCa detection suggesting that the approach of assessing PSA-IgM and PSA might be useful in clinical practice.

Higher PCa detection specificity

Improved BPH discrimination

Reducing negative prostatic mappings

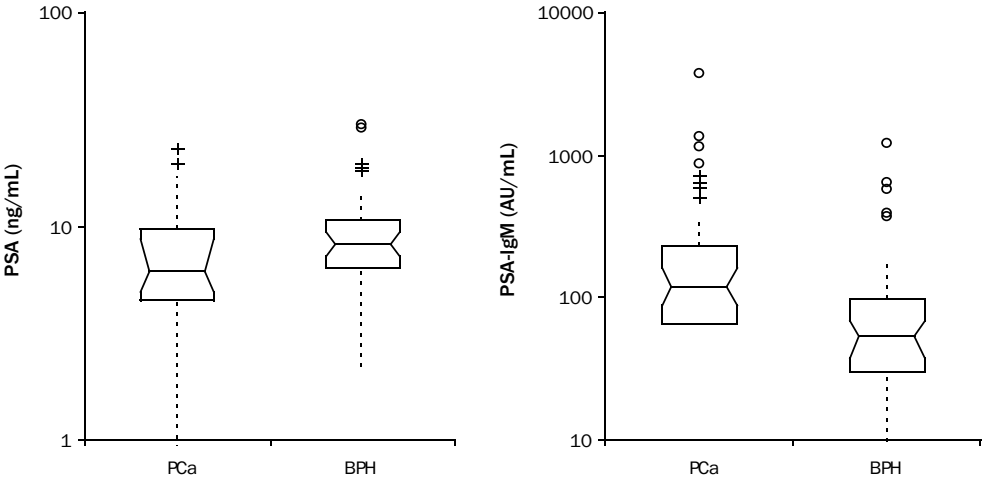

Figure 2: Box-plot for serum levels of PSA and PSA–IgM in the patients with prostate cancer and benign prostatic hyperplasia. The box indicates the lower and upper quartile and the middle line is the median.

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Biomarker	Sens	Spec	PPV	NPV
PSA-IgM (145,1 AU/mL)	40%	88%	77%	60%
PSA (4 ng/mL)	84%	4%	46%	20%
PSA (10 ng/mL)	22%	71%	42%	48%
PSA (10 ng/mL) **o PSA-IgM** (145,1 AU/mL)	60%	63%	61%	62%
PSA (>4 e <10 ng/mL) **e PSA-IgM** (145,1 AU/mL)	43%	88%	76%	55%

Table 1: Comparison of specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) of PSA–IgM and PSA in differentiating patients with prostate cancer (n = 50) from patients with benign prostatic hyperplasia (n = 51).

REFERECES

1. Beneduce *et al.* Annual Congress EAU, Paris, April 5–8, 2006
2. Beneduce *et al.* Cancer Detect Prev 2007, 31 (5):402-7
3. Prayer-Galetti *et al.* Annual Congress SIU, Bologna, June 17- 21, 2006
4. Prayer-Galetti *et al.* Annual Congress SIU, Bari, September 27 - October 1, 2007.
5. Zani *et al.* Annual Congress SIU, Roma, September 22-28, 2008
6. Zani *et al.* Annual Congress SIU, Rimini, October 4-7, 2009

REAGENTS AND MATERIALS PROVIDED

XG007-PL: 96 wells multi-strip Assay-Plate, precoated with affinity purified rabbit anti-PSA.
XG007-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: 10 mL of 5X concentrated Dilution Buffer solution.
XG-WB2: Two tablets of lyophilized Washing Buffer. White powder. 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 XG007-Calibrator with 440 µL of distilled water for each calibrator vial. Dispense 100 µL/well of standard calibrators (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 XG007-Calibrator vial. Dispense 100 µL/well of a 50- or 100-fold diluted sample (in duplicate). Use XG-DB5 dilution buffer as diluent. Also dispense 100 µL/well of XG-DB5 dilution buffer as blank, in duplicate. Incubate 1 hour at room temperature. Wash 6x with XG-WB2 washing buffer (300 µL/well).

Secondary antibody:
Add 100 µL/well of diluted XG-EA enzyme-conjugated secondary antibody solution. Incubate 1 hour at room temperature. Wash six times with XG-WB2 washing buffer (300 µL/well).

TMB Substrate solution:
Apply 100 µL/well of XG-CH3 chromogen solution. Allow color to develop for 10-15 min at room temperature 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 equipped with a 450 nm filter. Stopped reaction should be read within 1 hour.

PROCESSING OF THE RESULTS

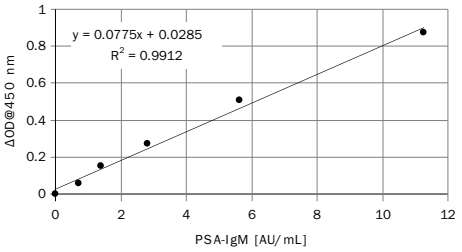

Figure 3A: range of linearity (0 to 11.25 AU/mL) of a typical standard curve for PSA-IgM after 15 minutes of substrate incubation at room temperature and addition of stop solution

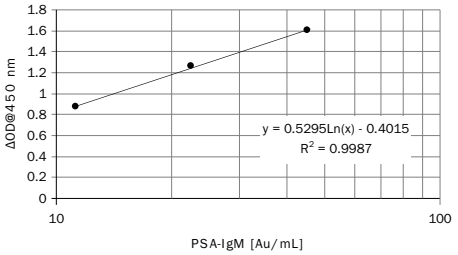

Figura 3B: range of linearity (form 11.25 to 45.0 AU/mL) of a typical semi-logarithmic standard curve for PSA-IgM after 15 minutes of substrate incubation at room temperature and addition of stop solution

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